

Novel Approaches to Engineer Antibodies

Methods Can Identify and Enhance mAbs and Small Molecules

K. John Morrow Jr., Ph.D.

As more experience is gained in the clinic and a large number of antibody drugs are approved, the benefits and limitations of antibodies have become evident. At the recent IBC “Antibody Engineering” conference in San Diego, scientists presented a range of new and recycled options that focus on challenges unimagined a decade ago.

While antibodies are nature’s way of guarding the body against disease, they are less than ideal on a molecular level for fabricating innovative diagnostics and therapeutics. Andreas Plückthun, Ph.D., professor of biochemistry, with his colleagues at the University of Zürich (www.unizh.ch) has extensively characterized alternative scaffolds for engineered antibodies. Their aim is to design a more suitable binding architecture for library construction.

Dr. Plückthun described his strategy to search for antibody-like molecules that can participate in tasks difficult or impossible for a conventional antibody to address. These include construction of multivalent and multispecific molecules, fusion molecules with green fluorescent protein, and molecules with single cysteine residues.

A Potential Antibody Counterpart

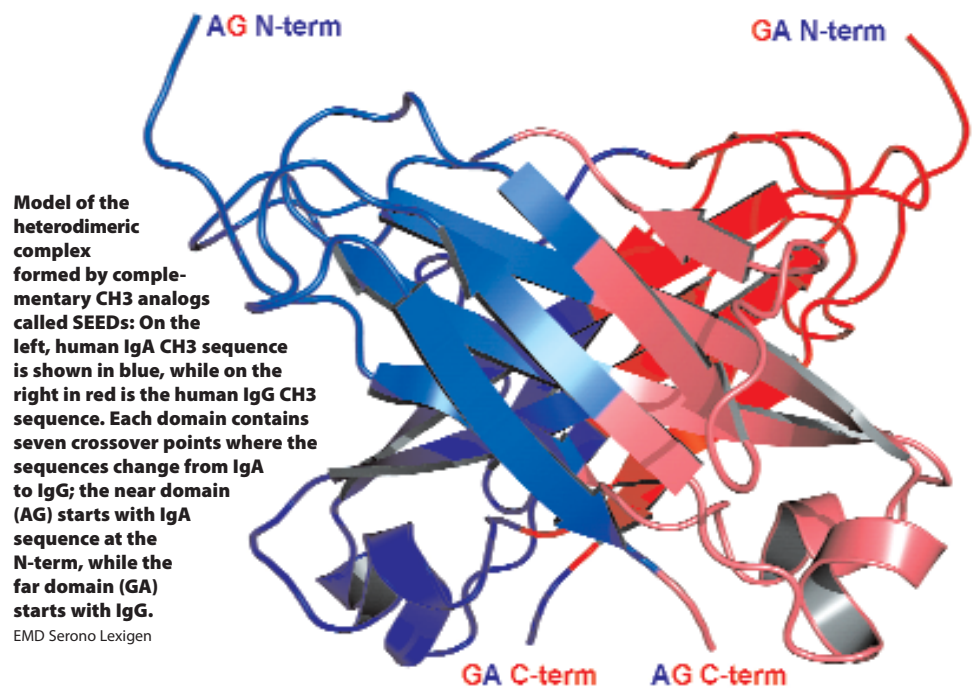
A prime candidate for the role of antibody doppelgänger is the family of ankyrins. They are a widely expressed class of adaptor pro-

teins that mediate specific high-affinity protein-protein interactions in the cell. Ankyrins are copiously varied and have inspired the design of an abundantly diverse synthetic library. Lacking a cysteine in the synthetic library, they function equally well under oxidizing and reducing conditions.

Designed ankyrin repeat proteins, or DARPs, have auspicious biophysical properties. These include ease of expression at high levels of up to 11 grams/L in an *E. coli* system. They are also highly heat-stable and undergo reversible folding. The next task was to engineer a suitable selection system for producing ankyrin-based binder proteins. Both ribosome display and a reengineered phage display system, termed SRP phage display, performed well. The group was then able to select binders from ankyrin libraries with picomolar affinity.

In model experiments it was possible to select ankyrin binders that mimic the HER2neu antibodies such as Genentech’s (www.gene.com) Herceptin performing equivalently in an immunohistochemistry platform. These DARPs also showed efficient tumor targeting in several formats. Moreover, it was possible to generate tri-spe-

cific DARPs against Her2, EGFR, and a human Fc region. Dr. Plückthun cofounded Molecular Partners (www.molecularpartners.com) to commercialize the DARPin technology and explore various therapeutic applications. “DARPs offer a number of advantages over conventional recombinant antibody technologies,” Dr. Plückthun stated. “Because of their small size they localize to tumors efficiently and are stable in vivo and in vitro. They can be used in a variety of platforms, and we have yet to observe toxicity.”



William J. Boyle, Ph.D., president and CSO, Anaptys Biosciences (www.anaptysbio.com), echoed Dr. Plückthun’s caveat that

antibodies derived using conventional methods are not always suitable for commercial and clinical applications. The industry’s knee-jerk response has been to select and optimize existing antibodies through a strategy of redundancy, using resource- and time-intensive approaches to ensure success of its programs.

Reining in Hypermutation

Anaptys’ method, on the other hand, takes advantage of mutational variations in B cells mapped out by Michael Neuberger, Ph.D., and Matthew Scharff, M.D. Over the years, Dr. Neuberger at the Medical Research Council, Cambridge, U.K., and Dr. Scharff at Albert Einstein College of Medicine defined the process of hypermutation and described how the immune system uses it to build high-affinity antibodies in the course of the humoral immune response.

“A major advantage of the Anaptys approach is that it incorporates facile in vitro maturation, which virtually none of the alternative strategies of mAb isolation provide,” according to Dr. Neuberger, who is on the firm’s scientific advisory board. “So, for example, with phage display you simply isolate binders from a large library hoping that some of them are of high affinity. Normally they are not, and so you need to engage a laborious process of individual CDR randomization in the hope of obtaining improved binders.

“But, with hypermutagenesis you don’t need to do any randomization yourself; mutation is going on continuously,” Dr. Neuberger added. “This is, of course, how the natural immune system works.”

Anaptys’ platform uses its library of bivalent, fully human antibody genes that are subjected to selection in a mammalian-based system using FACS to separate the evolving libraries. The activation of the cytidine deaminase enzyme generates additional diversity through the production of somatic point

News Bioprocessing Highlights

Microbix to Manufacture and Further Develop ImaRx Compound

ImaRx Therapeutics (www.imarx.com) and Microbix Biosystems (www.microbix.com) signed a letter of intent related to the manufacture and further development of urokinase. The product is currently marketed under the brand name Abbokinase® as a thrombolytic drug.

ImaRx intends to transfer the manufacturing process and NDA to Microbix to create a new source for urokinase. Microbix has the right to develop the compound for certain indications in catheter clearance and prophylaxis of serious, catheter-related complications such as blood stream infections and venous thrombi. Microbix also expects to investigate the candidates potential in oncology and ophthalmology.

ImaRx will retain its existing drug inventory and exclusive rights to sell the drug as a thrombolytic agent. It will gain access to a long-term drug supply from Microbix.

ImaRx will also pursue development of urokinase for indications other than those signed over to Microbix. Additionally, ImaRx will receive royalties on sales of urokinase for new indications developed by Microbix.

Cobra to Support Production of GenVec’s Late-Stage Cancer Therapy

GenVec (www.genvec.com) entered into a manufacturing development agreement with Cobra Biomanufacturing (www.cobra.bio.com) related to its anticancer agent, TNFerade™. The therapy is being evaluated as a treatment for pancreatic, head and neck, and rectal cancers as well as metastatic melanoma. The most advanced program is a Phase II/III trial in the pancreatic indication.

The agreement will cover technology transfer, scale-up, and validation of the manufacturing process for TNFerade through cGMP consistency lots. These will be produced at Cobra’s facility in Oxford, U.K.

GenVec is developing TNFerade for use in combination with radiation and/or chemotherapy. The treatment is an adenovector that contains the gene for tumor necrosis factor-alpha. After administration, TNFerade reportedly stimulates the production of TNFα in the tumor.

Codexis Opens Fermentation Facility in Budapest

Codexis (www.codexis.com) will be opening Codexis Laboratories Hungary Kft (CLH) in Budapest. The site will concentrate on fermentation-strain development and process technology.

CLH will be an expansion of the company’s existing R&D operations in the U.S., Germany, and Singapore.

Ronen Tchelet, Ph.D., Codexis’ vp and general manager, will head the laboratory. His background is in molecular biology, and he is experienced in strain and technology improvement for industrial fermentation processes.

mutations in the immunoglobulin gene loci.

Dr. Boyle discussed the fact that by transferring the light- and heavy-chain genes into episomes and transfecting the HEK293 cell line, a non B-cell recipient, it was possible to achieve a cell line with a high growth rate and an improved transfection efficiency. The resulting IgG products also contained a transmembrane sequence linking them to the cell surface, allowing high-throughput selection using flow cytometric analysis.

Dr. Boyle and his colleagues performed experiments demonstrating coevolution of light and heavy chains. The actual somatic hypermutational changes observed were the ones predicted to impact the affinity of the resulting antibodies. The data hence suggests that engineered, activation-induced cytidine deaminase is sufficient to direct somatic hypermutation in Anaptys' engineered cell types developed.

Other Roads to High-Affinity Antibodies

Another method to obtain antibodies is from immunized humans or experimental animals. In some cases this is a rapid and efficient method for isolating reagents that are relevant as diagnostic or therapeutic instruments.

Jens Wrammert, Ph.D., a researcher at the Emory Vaccine Center at Emory University (www.vaccines.emory.edu), described his investigation of influenza patients as a source of high-affinity antibodies. Following exposure to an influenza vaccine, B cells differentiate into antibody-secreting cells, producing antibodies against the antigenic determinants. The maximum occurs at seven days post immunization. The robust and transient response is replaced later by a protracted memory B-cell response.

Dr. Wrammert's strategy was to tap into these antigen-specific cells as a source of antibody gene sequences. Antibody-secreting cells identified by flow cytometry with anti-CD38 and anti-CD27 antibodies were isolated. Their immunoglobulin variable genes were randomly cloned and isolated. Using a multiplex PCR-based technique, Dr. Wrammert cloned the variable regions from single antigen-stimulated cells isolated seven days after influenza vaccination and expressed the recombinant mAbs.

With this strategy, he produced over 40 human influenza-specific antibodies. This entire process takes less than 30 days and thus represents a technological advance.

Augmenting Antibody Therapeutics

"At present, therapeutic antibodies have a number of significant limitations," pointed out Thomas Kindt, Ph.D., CSO, InNexus Biotechnology (www.ixsbio.com). "For example, Herceptin targets the HER2neu receptor and is used in breast cancer therapy, but it is only effective in about 25% of patients due to low expression of the protein in cancer cells. Rituxin, an anti-CD20 antibody, gains high initial responses in non-Hodgkin's lymphoma and follicular lymphoma patients, but many relapse, and only 40% responded to treatment overall."

To obtain better performance from ther-

apeutic antibodies, InNexus uses a dynamic cross linking (DXL) technology. In this technique, a peptide integrated into the molecule forces dimerization when the antibody is bound to the target but not when in solution, improving avidity.

Investigators at InNexus converted an anti-CD20 antibody into a DXL reagent, referred to as DXL-625. The modified antibody boasts a prolonged off-rate and could provoke apoptosis in the Ramos cell line, derived from a patient with Burkitt's lymphoma.

The potency improvements of the technology can be applied to inhibiting cell proliferation, stimulating antigen-dependent cell cytotoxicity, complementing dependent cytotoxicity, and triggering apoptosis. Dr. Kindt also argues that DXL modification will be more effective when directed against low-expression biomarkers in a diagnostic platform. This can provide a shortcut to antibody improvement without the laborious procedures associated with various mutagenesis platforms.

Stabilizing Small Molecules

Many small molecules have half lives as short as 15 minutes, which renders them of no value in cancer therapy. Carlos Barbas III, Ph.D., professor of molecular biology and chemistry, and his colleagues at the Scripps Institute (www.scripps.edu) are exploring a strategy for escaping this impasse. Using a platform referred to as catalytic antibodies, Dr. Barbas developed a

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Antibody Engineering

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protocol for covalently coupling small therapeutic molecules to the much larger antibodies. Such molecules are termed CovX bodies.

Dr. Barbas uses a β diketone reactive antibody, which binds the molecule to form a covalent bond. If the potential therapeutic molecule has a ketone group engineered onto its terminus, it forms a tight and permanent link with the antibody, extending its half life. Dr. Barbas' team used a peptidomimetic that targets integrins, critical molecules for tumor cell attachment and performance. The complex, referred to as Ab-SCS-873, was 1,000-fold more

effective in inhibiting tumor cell growth in mice inoculated with the human M21 melanoma.

"The CovX-body manufacturing process is simple and scalable, involving a spontaneous, self-forming reaction, using a binding pocket lysine," Dr. Barbas asserted.

Additional massaging of the peptide molecule and the linker using proprietary technology maximized the pharmacokinetics and activity of the complex. This allowed development of an antidiabetic compound, CVX-096, which provided improved glucose tolerance and reduction in body

Whole Genome Scan

Fine Mapping

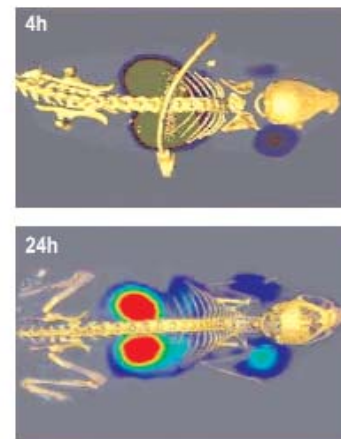
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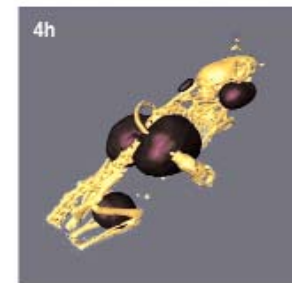
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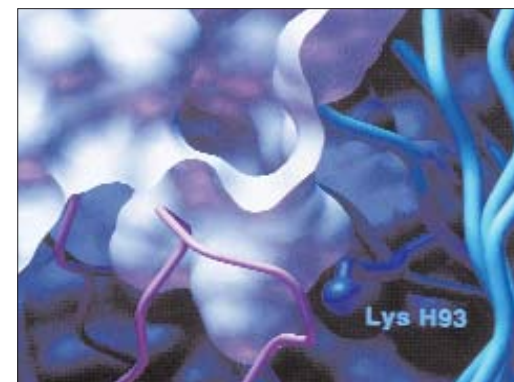


- **DARPin G3 (monomer, no PEG)**
- Labeling with $^{99m}\text{Tc}(\text{CO})_3$
- SKOV3ip Xenograft



(Above) SPECT imaging of xenografted mice with anti-HER2 DARPin
University of Zurich

(Right) At Scripps Institute, a catalytic antibody platform led to a breakthrough class of immunotherapeutics.



weight, no doubt due to its extremely long half-life of 154 hours, and high subcutaneous bioavailability.

SEEDbodies Sprout on Fertile Ground

EMD Serono Lexigen, a division of Merck KGaA (www.merck.de), hosts an extensive program of hybrid protein engineering. Jonathan Davis, Ph.D., senior scientist at the Lexigen Research Center explained the approach that his group pursues. They focus on redesigning Fc domains as heterodimers, with the aim of producing a molecule that outperforms naturally occurring antibodies.

The Lexigen group rearranged DNA sequences coding for the constant heavy regions of IgA and IgG by interchanging the lower portions of the molecules. Since IgA is the major secretory antibody, and IgG is the major circulating antibody, the sequences bear substantial homology but must be substantially modified to dimerize properly.

The reconfigured hybrid molecule is referred to as a SEEDbody (strand exchange engineered domain). SEEDbodies are designed to have structural homology to the Fc of IgG and maintain the structural integrity of the Fc. The platform can be used to produce bi-specific antibodies in which the variable regions have different specificities.

SEEDbodies have a number of properties that make them clinically promising, according to Dr. Davis. They express well in mammalian cells, form heterodimers preferentially, are easily purified with the help of protein A, and possess a stability of at least six months and a pK similar to the native Fc.

New Responses to Old Problems

The advancing wave of new antibody technologies continues to impress. While antibody therapeutics offer unique advantages over small molecules in terms of specificity, fewer unwanted side effects, and much longer half lives in the patient, issues of immunoreactivity continue to pester investigators.

The field has come a long way from the early days of murine antibodies. Even totally human antibodies when dumped into the circulation in large continuing doses, can raise an immune response. Thus it is not surprising that some of the best results in cancer patients have come through the use of antibodies administered to patients with compromised immune systems.

There are a number of strategies under consideration for redesigning epitopes to lower immune responses. Since DARPins and other alternative scaffolds have not yet been evaluated in patient trials, it is too early to know what issues they will present, or how such challenges may be overcome. As the new generations of antibody-like molecules are subjected to clinical evaluation, the next months and years should provide much excitement.

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