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GEN Bioprocessing

New Antibody Engineering Technologies

More Effective and Targeted Therapeutics Viewed as Anticipated Outcomes

K. John Morrow Jr., Ph.D.

New approaches to antibody engineering were presented at the “European Antibody Congress” held in Geneva, Switzerland last month. At the meeting, companies grappled with long-standing issues of glycosylation, antibody structures, alternative structures, and novel purification approaches.

While the mechanism of tumor-cell destruction by therapeutic antibodies is not completely understood, they appear to function in part by binding to target cells and triggering antibody-dependent cell cytotoxicity or complement-based killing. It has been observed, however, that direct cell killing by cross-linking cellular receptors through antibody bridges is another mechanism that can increase the rate of apoptosis, according to Scott Glaser, Ph.D., director of molecular engineering at Biogen Idec (www.biogenidec.com).

Dr. Glaser and his colleagues reasoned that a multivalent antibody would be more efficient at cross-linking receptor targets, and therefore, the team designed a tetravalent antibody that engaged a target antigen commonly expressed on the surface of leukemia cells.

In order to engineer a satisfactory candidate, it was necessary to build an IgG-like antibody of exceptional stability using engineered single-chain variable fragment domains as building blocks. This decision was driven by the observation that bispecific antibodies, engineered with unstable single-chain antibody components, tend to be susceptible to chemical and physical degradation.

Dr. Glaser and members of his team, including Brian Miller, Steve Demarest, and Alexey Lugovskoy combined three complementary design methods—statistical-computational, structure-based, and knowledge-based—to generate a library of single-chain variable domains from the parent single-chain antibody.

The expression library was then screened in a microplate assay to select those single-chain domains with the highest thermal stability while still retaining binding activity. Iterative rounds of mutagenesis and screening produced an optimal candidate. With these high-stability scFv domains defined, they could be engineered into the tetravalent antibodies that display the expected valency and increased avidity.

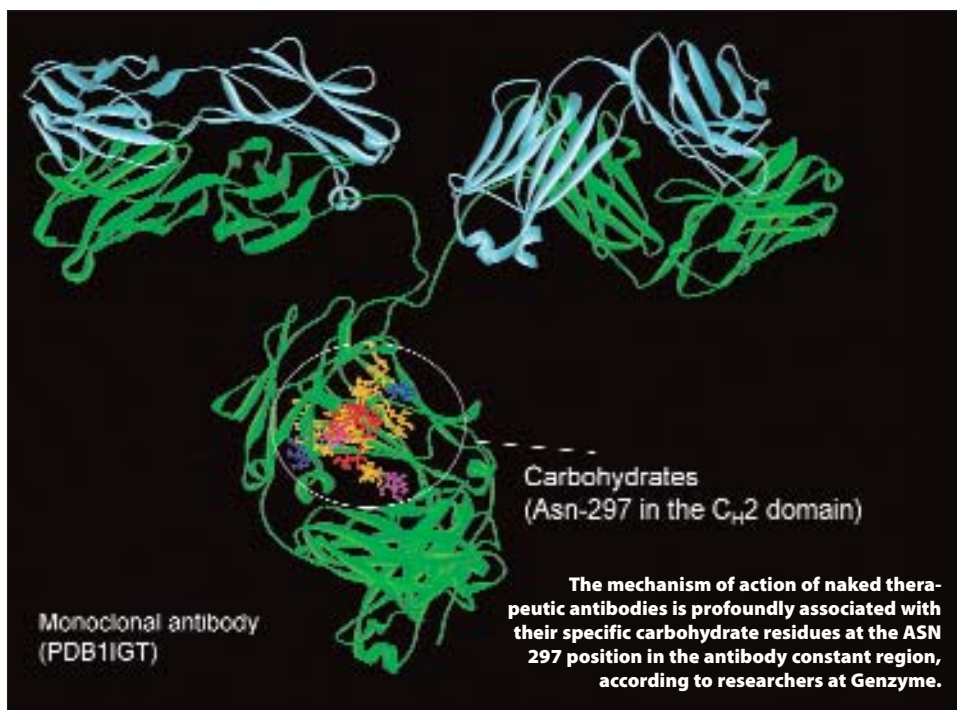
In a series of experiments conducted in the lab of Ann MacLaren, Ph.D., a researcher-scientist at Biogen Idec, the tetravalent antibody was shown to display single-agent apoptotic activity in a cell-culture system that was enhanced by

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cross-linking. As predicted, the induction of apoptosis in these cells marks their eventual demise.

Finally, the tetravalent antibody combined with alemtuzumab (Campath, marketed for the treatment of chronic lymphocytic leukemia) induces a powerful apoptotic response in leukemic cells drawn from patients.

To date, antibody therapies have shown the ability to slow the progress of metastatic cancers, but they do not constitute a cure, and they must be combined with conventional chemotherapy regimens. Tetravalent antibodies, with their ability to bring about cross-linking, will be carefully considered as investigators seek to hone the performance of antibody thera-



peutics and break away from conventional protocols.

“The mechanism of action of naked therapeutic antibodies is profoundly associated with their specific carbohydrate

residues at the ASN 297 position in the antibody constant region,” states Qun Zhou, Ph.D., principal scientist in the department of therapeutic protein research at Genzyme (www.genzyme.com). Dr.

News Bioprocessing Highlights

Athera Contracts Richter-Helm to Produce Cardiovascular Protein Therapeutic

Richter-Helm BioLogics (www.richterhelm.eu) will help Athera Biotechnologies (www.athera.se) with the development and manufacturing of its cardiovascular product. The recombinant protein, Annexin A5, is intended for the treatment of patients with acute coronary syndrome who are at risk for myocardial infarction.

Athera’s candidate reportedly works by preventing plaque rupture and atherothrombosis through binding of the protein, Annexin A5, to endothelium. It was produced using Richter-Helm’s *E. coli*-based expression system.

This agreement could include future large-volume commercial production of the molecule for Athera. Richter-Helm will initiate strain and development of the new process, reaching a 1,000 L production scale.

IntelGenx to Manufacture Hyperlipidemia Treatment for Circ Pharma

IntelGenx (www.intelgenx.com) and Circ Pharma (www.circpharma.com) formed a partnership in which IntelGenx will formulate, manufacture, and supply to Circ, and Circ will develop and commercialize, a novel drug product for the treatment of hyperlipidemia. Under the terms of the agreement, Circ Pharma will fund the

development of the product and IntelGenx will receive royalties from the product’s sales. IntelGenx will use its Versatab technology to formulate the product.

This is the first product in a series of Circ Pharma’s controlled-release lipid-lowering agents specifically designed to target the absorption of a drug in order to reduce the effective dose and potentially lower the side effects.

Lonza Completes Expansion of Chinese API Facility

Lonza (www.lonza.com) recently concluded the first phase of the expansion of its Nansha, China, API plant. Nansha is located in China’s southern Guangdong province, in strategic proximity of Guangzhou and Hong Kong.

The Nansha facility is a fully integrated site with modern utilities, administration, research and development, kilo lab, small-scale production and large-scale production, according to the company. Seventy scientists are supporting the R&D Services, working in a 1,000m² lab area.

Cytovance Biologics Contracted for Production of Selexys’ HPL1 mAb

Selexys (www.selexys.com) selected Cytovance Biologics (www.cytovance.com) to manufacture its

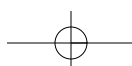
lead drug candidate for Crohn’s disease. The full-service process development and manufacturing contract will be executed in Cytovance’s multiproduct process development and cGMP-production facility in Oklahoma City over the next 15 months.

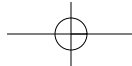
According to Cytovance, its 44,000-square-foot Oklahoma facility meets the latest international regulatory standards and is custom-designed for efficient and cost-effective production.

Novavax Pilot Plant Up and Running

Novavax (www.novavax.com) reported that all of the equipment in its new GMP pilot plant to manufacture pandemic and seasonal influenza vaccine clinical supplies and commercial batches at a 1,000-liter scale is installed and ready for operations supporting scale-up and validation.

The facility is expected to be capable of producing 2–3 million doses of monovalent pandemic influenza vaccine per week at 15 mcg HA/dose (50–75 million doses in six months) once scale-up and validation are complete. Likewise, the facility can support up to 20–25 million doses of trivalent influenza vaccine in six months, according to the company. The facility is GMP compliant and includes a total of 10,000 square feet of production and support space. The facility also includes media and reagent preparation space and





Zhou concurs with Dr. Glaser that widely employed recombinant antibodies, including Rituxin, Campath, and Herceptin, attach to specific tumor antigens and then attract either complement or natural killer cells by way of their carbohydrate residues, which are then able to destroy the targeted cancer cell.

Indeed, experiments have shown that in antibody molecules that have been shorn of their carbohydrates there is no antibody-dependent cell cytotoxicity (ADCC) or binding to the Fc gamma receptor (FcγR), while in the absence of terminal galactose or N-Acetylglucosamine (GlcNAc) there are minor effects on ADCC and FcγR binding.

Addition of a bisecting GlcNAc to antibody molecules lacking this structure resulted in a 20- to 100-fold increase of ADCC. On the other hand, absence of a core fucose moiety brought about by expressing antibodies in engineered cell lines or transgenic systems drove a 50- to 100-fold increase ADCC activity.

There are a number of approaches for production of antibodies that are appropriately glycosylated in a fashion that will optimize their therapeutic potential, including engineering of cell lines, either mammalian, plant, or yeast. Dr. Zhou and his colleagues reasoned that a more rapid and cost-effective approach would be through the use of metabolic engineering, that is, the use of enzyme inhibitors that block steps in the pathway of glycosylation.

It is possible to use inhibitors for enzymes in the glycosylation pathway such as kifunensine to make antibodies with oligomannose-type glycans lacking fucose. Dr. Zhou and his team have observed that treatment of existing antibody-producing cell lines at low concentration results in antibodies with higher affinity for FcγRIIIA and greater ADCC, without affecting antibody production, antigen binding, and antibody pK. When the Genzyme team examined the properties of the antibodies produced through kifunensine treatment, an inverse correlation between fucosylation and ADCC was

demonstrated.

The targeted modification of the antibody carbohydrate structures holds promise for future development; it may allow the design of more effective anticancer treatments and permit the conservation of resources, avoiding treatment of unsuitable patients.

Ligands for Affinity Purifications

“Biopharmaceuticals are a high growth area of therapeutics,” says Frank Detmers,

director of ligand applications at **The Bio Affinity Company** (BAC; www.BAC.nl). “This means the need for faster, purer, cheaper, higher-yield purification technology is imminent.”

BAC specializes in affinity separation for protein-purification technology; however there are a limited number of commercially available affinity ligands. To fill this gap, the company has developed an approach referred to as CaptureSelect® Affinity

Ligands, which takes advantage of the uniqueness of engineered VHH Antibody Fragments.

These are exceptional antibody chains derived from the camelidae (the family comprising llamas, alpacas, and camels). They are noteworthy because they possess a functional antibody fragment consisting of a variable heavy and a constant heavy sequence representing a naturally occurring single-

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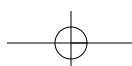
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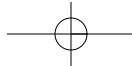
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ADVANCES HIGHLIGHTED IN THIS ARTICLE

- Tetravalent antibodies, with their ability to bring about cross-linking, are being used to hone the performance of antibody therapeutics and break away from conventional protocols.
- Targeted modification of antibody carbohydrate structures allows the design of more effective anticancer treatments.
- Screening for highly specific elution conditions has been shown to facilitate further purification and increase product stability during downstream processing.





Antibody Engineering

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chain antibody. Because they are bereft of much of the accoutrements of the much more common Y-shaped antibody molecule, they can function more effectively as an affinity ligand. Moreover, they can be modified through genetic engineering for a wide range of purposes, with nanomolar affinity and a narrow range of specificity.

The approach followed by BAC in developing new ligands consists of an eight-month program, in which a library of sequences is screened in a yeast display protocol using microwell plates, and binders are set aside, reevaluated, and optimized. This process includes selection for high affinity, stability, and response to specific elution conditions.

The appeal of the technology has allowed BAC to build strategic partnerships with more than 20 companies. An especially valuable tool is antibody ligands that remove albumin and immunoglobulin from human plasma, thus enabling the isolation of proteins present in a billion-fold lower concentration. BAC has developed a panel of ligands for the purification of human serum proteins, including specific immunoglobulins, fibrinogen, transferrin, and albumins. Other proteins present in serum, which can be conveniently purified, include haptoglobin, factor VII, EPO, IFN α -2b, proteins present in amounts as low as 10⁻⁹ M.

"Our CaptureSelect technology lends itself to screening for highly specific elution conditions to facilitate further purification or to increase product stability during downstream processing," Detmers stated.

Quality by Design

"We believe that quality by design is a

BAC's CaptureSelect ligands have been used for a wide variety of purification needs, from monoclonal antibody and virus purification to plasma protein depletion.



revolutionary way of manufacturing that is of great importance for European markets," states Andreas Schneider, director of global sales at **Innovatis** (www.innovatis.com). Quality by design is the concept that quality issues cannot be tested into a product, but should be an integral property, built through a comprehensive scientific understanding of basic manufacturing principles. For global markets, implementing quality by design is the ideal way to standardize production to the rest of the bioprocess manufacturing world.

The FDA's PAT initiative is related to quality by design. It addresses the concept of global harmonization with the goal of expediting the movement of pharmaceuticals to the marketplace through the application of consistent and predictable manufacturing processes. It is intended to foster innovation and drive efficiency in the phar-

maceutical industry based on a set of scientific guidelines supporting originality and the regulatory implementation that will accommodate this inventiveness.

Innovatis' new strategy is intended to overcome fear on the part of manufacturers that new and unique manufacturing and quality-assurance approaches will precipitate a regulatory gridlock.

The quality by design approach dictates precise process control. "For example, in the case of a cell-culture facility, the management and transfer of monitoring data is automated," Schneider continues. "This allows the construction of a historical record, and adjustments are made for every successive run, maximizing efficiency and quality."

The implementation of quality by design is especially germane at the international level, in regions where experience with highly demanding bioprocessing

technology may be limited. The more a process is automated and the more the adjustments to that process are based on scientific data and experience, the easier it is to transfer a particular technology to any place in the world.

In order to implement quality by design, **Innovatis** has taken advantage of the **BiachroMAT CellCount** from **Bayer Technology Services** (www.bayer-technology.com), a fully automated system capable of processing sterile samples directly from a bioreactor, according to the company.

As a quality by design automation platform, **BiachroMAT** integrates multiple biological and chemistry analyzers to enable the measurement of process parameters.

Another **Innovatis** product, the **Cedex** automated cell analyzer, is incorporated as a tool in quality by design. It determines cell concentration and viability during the course of cell cultivation and fermentation processes.

According to Schneider, implementation of quality by design will be more and more essential in the future, most specifically with the rise of individualized therapies and drugs. Relying on continuous improvements based on real-time data, such a strategy is tied into adaptability. The bottom line is that quality by design principles can guide a manufacturing process, reducing time-to-market through approval and release in real time from regulatory authorities

Where Do We Go From Here?


As more and more clinical data is accumulated on the properties of anticancer antibodies, trenchant observations have been made that may lead to more effective and targeted treatment with these agents.

For example, patients possess genetic polymorphisms for the Fc receptor that affect the ability of the antibodies to mobilize ADCC. In the future, patients could be profiled and those for whom the therapy would not be successful can be directed to other treatments.

Moreover, it should be possible to engineer antibodies that can overcome the genetic recalcitrance of nonresponding patients. Management strategies such as quality by design, with its constant monitoring and feedback, should accelerate the discovery process and hasten the development of more effective therapies. **GEN**

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