

Methods for Maximizing Antibody Yields

New Technologies Could Help Usher in Lower Costs and Increased Availability

K. John Morrow Jr., Ph.D.

Among recombinant proteins, antibodies stand out as the strongest candidates for new therapeutic drugs and represent the majority of submissions to the FDA and other regulatory agencies. Total sales of recombinant antibodies reached \$15.8 billion in 2006 and are projected to go to \$28.6 billion by 2010, according to Rolf Werner, Ph.D., senior vp at Boehringer Ingelheim (www.boehringer-ingelheim.com), who also noted that the biggest selling antibody of 2007 was Remicade, which racked up over \$5 billion in sales worldwide.

The advancement of recombinant protein technology has enabled the humanization of antibody molecules that are well tolerated, safe, and effective. A number of new therapeutic products are in the middle of trials, and although the rate of approvals slowed in 2007, it will probably increase this year.

Yet even in the midst of abundant rewards, antibody therapeutics face enormous scientific, commercial, and legal challenges. The highly competitive environment, the burdensome pricing, lack of tissue penetration, the large upfront investment costs, and the legal thicket of overlapping patents all conspire against new recombinant antibody products. Nonetheless, a large number of antibodies are moving through clinical evaluation (Table).

Much of the current excitement over recombinant antibody proteins is because they are the only therapeutics that extend the life of patients with metastatic disease, even though they do not provide a cure. Much discussion at the recent IBC "Antibody Production and Processing Conference" dealt with advances at both the upstream and downstream ends of the pro-

K. John Morrow Jr., Ph.D., is president of Newport Biotech Consultants. Web: www.newportbiotech.com. E-mail: kjohnmorrowjr@insightbb.com.

duction train, which may, in the long run, help to lower costs and increase availability.

Engineered Cell Lines

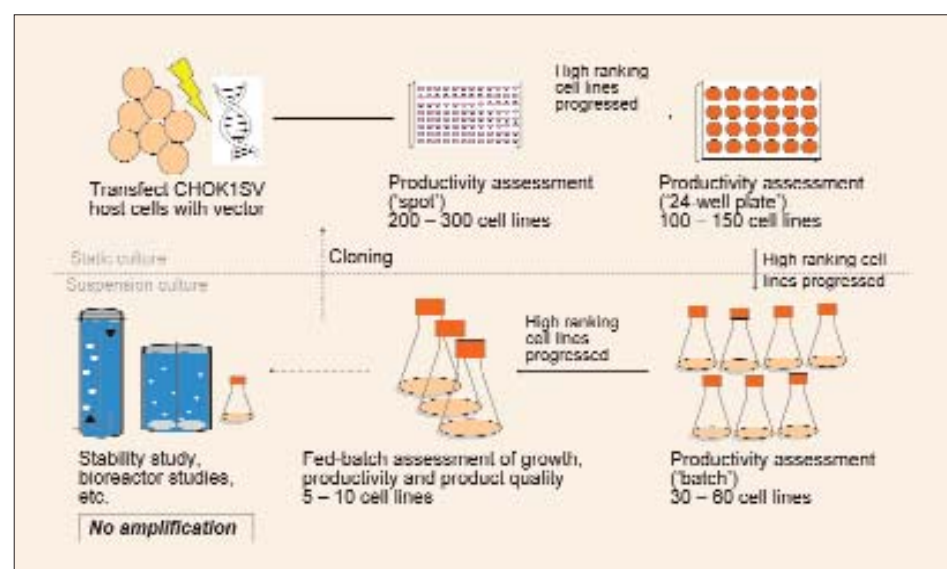
Even superbly engineered proteins can be hobbled without an optimized synthetic system. Because mammalian cells continue to dominate the market as the vehicle of choice for antibody production, the selection of the optimal cell line becomes more and more critical.

Blockbuster drugs require huge amounts of material. Cell line manipulation is a long and arduous process, and errors made early in the selection process can result in irreparable deficiencies further down the development road. So a well-researched and carefully reasoned choice of cell line can result in huge cost savings.

In the early days of recombinant antibody production, murine lymphoid cells and hybridomas were the vehicles of choice. This approach was satisfactory at a time when biotechnologists were content with mg/L of protein yield. Now with yields moving into the multiple grams per liter range, there is pressure to switch to more robust production systems. Unfortunately, the murine lines have subtly different glycosylation patterns and have proven difficult to engineer to higher levels of productivity. For this reason there is great interest in transfected human cell lines and other innovative approaches to cell biosynthesis.

Currently, the two major contenders are CHO cells and Percivia's (www.percivia.com) PER.C6 line. The CHO line has the virtue of being perhaps the most widely

Sartorius Stedim Biotech views disposable components as a viable means of surmounting complex and technically demanding bioprocessing issues.



A Lonza strategy for selection of a GS-CHO cell line using a chemically defined, animal component-free medium

exploited and best understood mammalian cell line in existence but it was never intended to be a protein-production factory. PER.C6, on the other hand, was specifically designed to produce biopharmaceutical products, according to DSM Biologics (www.dsm.com) and Crucell (www.cruccell.com), which formed Percivia to promote the PER.C6 platform.

CHO Line

John Birch, Ph.D., CSO at Lonza (www.lonza.com), discussed his group's efforts to improve protein synthesis capability in the CHO cell line. Manipulation of nutritional conditions, optimization of feeding strategy, and the engineering of the genetic makeup of a particular cell isolate are the critical inputs in maximizing protein production.

In the last 40 years there have been numerous protein-free and serum-free media developed for the CHO line. The Lonza group has focused its efforts on completely protein-free media and feeds for its fed-batch culture systems. As the technology has improved, there have been increases of orders of magnitude, bringing yields in the range of grams per liters. The drivers of these advances are augmentation in cell density and enhancement in per cell protein production. As the technology matured, Lonza realized a 167-fold boost between 1990 and 2008, according to Dr. Birch.

In one series of trials, Lonza scientists isolated a variant, the CHOK1SV cell line, that achieved higher cell concentrations and maintained viability longer than another commonly used line (the CHO DG44) when grown under the same conditions. Dr. Birch strived for a simplified screening system that would allow processing of many clones with different genetic backgrounds.

"We have aimed at a straightforward screening regime," Dr. Birch stated. "We use the GS expression system, which has a high level of selection stringency, and we have started with the CHOK1SV cell line, which has improved growth characteristics."

A particularly favorable property of the cell line is its anchorage-independent growth properties. Ordinarily, CHO cell lines grow attached to the culture vessel, presumably due to the production of adhesive proteins. By selecting for a strain that grows in suspension, the Lonza workers were able to improve performance and save time during the developmental process. Using a relatively simple screening program, the Lonza group is able to routinely generate highly productive cell lines making grams per liter of antibody.

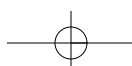
For Dr. Birch, the take-home message is that the optimal approach to improving protein output is based on ratcheting up a cell's capacity for protein synthesis. "To do this you need to understand the biological

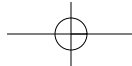
See Antibody Yields on page 38

Some Antibodies under Clinical Development

Target	Company	Project	Phase
CD20	Applied Molecular Evolution (www.ame.biz)	AME-133v ¹	II
	Roche (www.roche.com)	Ocrelizumab ¹	III
	Genmab (www.genmab.com)	Ofatumumab ²	III
	Trubion Pharmaceuticals (www.trubion.com)	TRU-015 ³	II
	Immunomedics (www.immunomedics.com)	IMMU-106 ¹	II
TNF	Applied Molecular Evolution	AME-527 ¹	I
	AstraZeneca (www.astrazeneca.com)	CytoFab ³	II
	UCB Celltech (www.ucb-group.com)	Certolizumab pegol ^{1,3}	Preregistration
	Centocor (www.centocor.com)	Golimumab ²	III
	Advanced Biotherapy	Anti-IFN gamma	I
VEGFR-2	ImClone Systems (www.imclone.com)	IMC-1C11	I
		IMC-1121B	I
EGFR	Merck Research Laboratories (www.merck.com)	Anti-KDR receptor antibody	I
	Genmab	Zalutumumab ²	III
	The Institute of Cancer Research (www.icr.ac.uk)	MAB ICR-62	I
	Life Science Pharmaceuticals	MAB-806 ¹	II
	YM Bioscience (www.ymbiosciences.com)	Nimotuzumab ¹	Registration
	Merck KGaA (www.merck.de)	Matuzumab ¹	II

¹Humanized mAb, ²Human mAb, and ³Fab
Source: Boehringer Ingelheim





Antibody Yields

Continued from page 36

underpinnings of cell performance and perfect your tools for assessing the functional capabilities of the cells," he posited.

You Say You Want a Revolution?

"Cells resistant to high osmolality exhibit increased robustness and stability," said Florence Wu, Ph.D., director, cell sciences, at the PD Direct Bioprocess Services division of Invitrogen (www.invitrogen.com).

To pursue this property, Dr. Wu investigated a gene-

mutation protocol for increasing resistance to high levels of salt added to the culture medium. While high osmolality of the culture medium boosts specific productivity of recombinant proteins, the growth parameters of the cells deteriorate dramatically. So as salt concentrations rise to toxic levels, there will be a point of negative impact on cell performance.

Dr. Wu and her associates reasoned that if it were possible to engineer resistance to high osmolality in cells, then dramatic increases in protein production

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Percivia was formed by DSM Biologics and Crucell to promote the PER.C6 platform for recombinant therapeutic proteins.

could be anticipated. They observed that when they cultured cells continuously in increasing levels of salt, up to 550 mOsm/kg, after 30 days the cells improved in viability and began to proliferate.

Initially it was not clear whether this was the result of gradual physiological adaptation or selection of a preexisting mutant population. Analysis of the growth kinetics of the cultures suggested, but did not prove, that a selection of genetic variants had occurred during this period of continuing growth in the high-salt medium.

Dr. Wu next applied the Invitrogen Revolution™ technology, which operates by interfering with mismatch repair of DNA, increasing mutation rates by as much as a 1,000-fold. Ordinarily, damage to the genetic apparatus is constantly being monitored and repaired, but the Revolution technology interrupts this process, and a torrent of genetic damage accumulates. The classic means of producing genetic variants in cultured cells has been through the use of compounds such as ethyl methane sulfonate, which cause errors during DNA replication.

The company argues that the Revolution system is much more effective than chemical mutagenesis, producing a more diverse and harder spectrum of mutational variants. It is also more rapid, as Revolution-treated cultures produce high osmolality-resistant variants immediately, rather than after a longer lag period as seen in the untreated populations, the firm reports.

Dr. Wu then turned her attention to the CHO DG44 cell line, an important antibody producer. Not only was she able to select a CHO variant with 500 mOsm resistance, but it proved to be stable over at least 75 generations and retained its capacity to produce immunoglobulin.

The PER.C6 Cell Line

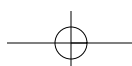
"Percivia's PER.C6® Development Center investigates the use of PER.C6 cells as a technology platform for recombinant therapeutic proteins," said Marco Cacciuttolo, Ph.D., CEO of Percivia. The firm was formed in 2006 to provide technology transfer and technical support to the users of the PER.C6 platform.

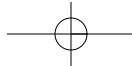
Dr. Cacciuttolo stressed that although PER.C6 has only existed since 2000, it has already shown impressive performance, especially in terms of expression of IgGs and IgMs. "Our titers in fed batch have gone up from 5.8 to 7.8 g/L," he continued. "This achievement for PER.C6 demonstrates the power and robustness of its manufacturing platform. We are confident that we will be able to drive productivity even further."

In fact, PER.C6 researchers designed an ultrahigh-performance production process, named XD™, characterized by a short burst of exponential growth to densities of 150 million cells/mL, resulting in product titers in the range of 15 g/L for an IgG at harvest. "This is unprecedented and it represents a substantial increase over typical yields," added Dr. Cacciuttolo.

Counting on Disposables

"The next breakthrough in disposable processing will come from old and boring enabling technologies," explained Uwe Gottschalk,





Ph.D., vp for purification technologies at Sartorius Stedim Biotech (www.sartorius-stedim.com). Challenges in today's bioprocessing industry are formidable—complex and technically demanding, with ever-increasing regulatory scrutiny. Disposable components are looked upon as a means of resolving many of these issues. In his discussion, Dr. Gottschalk looked at the various steps in the downstream process including major separation, aggregate removal, polishing, and viral removal.

Sartorius Stedim Biotech and other companies involved in filtration technology are focusing on disposable chromatography, that is, products that enable efficient and cost-effective use of resources over a short time frame. Such single-use modules require lower capital investment per device and allow reduced exposure of the separation media to the environment. Examples of disposable chromatographic products include membrane absorbers, monoliths, and prepacked columns.

Although Protein A is the affinity separation medium of choice, selectivity comes at a high price. Protein A ligands for attachment to columns represent a cost contribution of about \$16/g of antibody purified, while the much less specific approach of ion exchange will only add \$2 in cost per gram of antibody purified. Two-column processing is becoming widespread in the industry, including membrane chromatography.

Breaking Bottlenecks

“Another aspect to consider is that when using CHO cells or other mammalian cell systems, current success depends on the particular proficiency of the organization using the system and the particular cell sub-line or strain of the original culture,” Dr. Cacciuttolo said.

Since CHO cells have been in circulation for so long, there are many variants, some separated by thousands of generations. Clearly, the isolates described by Dr. Birch and his team at Lonza are light years away from many of the other CHO cell lines in circulation, and biotechnologists need to be aware of this. For both proprietary and biological reasons it is expected that newer cell lines are much more consistent.

But even assuming consistent advances in antibody production at both the up- and downstream ends, recombinant antibodies will still be vastly more expensive to produce than small molecule therapeutics.

Dr. Werner feels that this could create a stranglehold on the sector, clouding the possibility of commercial success. To avoid such a dismal outcome he recommended a number of promising alternatives to conventional antibody therapeutics that could dramatically lower costs.

These include artificial scaffolds, protein backbones that hold the variable regions and are derived from a variety of naturally occurring protein families. They are noted

for their simple structure and robust behavior and can be engineered to high affinity and specificity. Because they are smaller and less complex than complete, naturally occurring antibody molecules, they could be produced in microbial systems.

Other modifications of the molecules that would extend their half-life or improve their effector functions can be engineered, providing an additional degree of performance with cost-lowering potential.

Moreover, since the antibody-scaffold technology is quite recent, the IP situation is not nearly as crowded as that of recombinant antibodies.

In this latter case, while none of these new antibody substitutes has seen clinical exposure, the next few years will test their value. “This therapeutic success will be crucial in gaining confidence in alternative immunotherapies,” Dr. Werner concluded. **GEN**



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