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The Science & Business of Biopharmaceuticals

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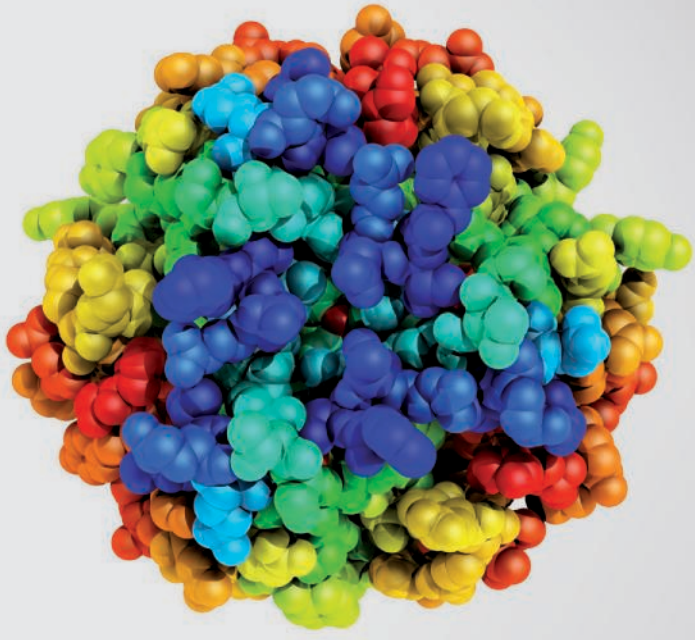

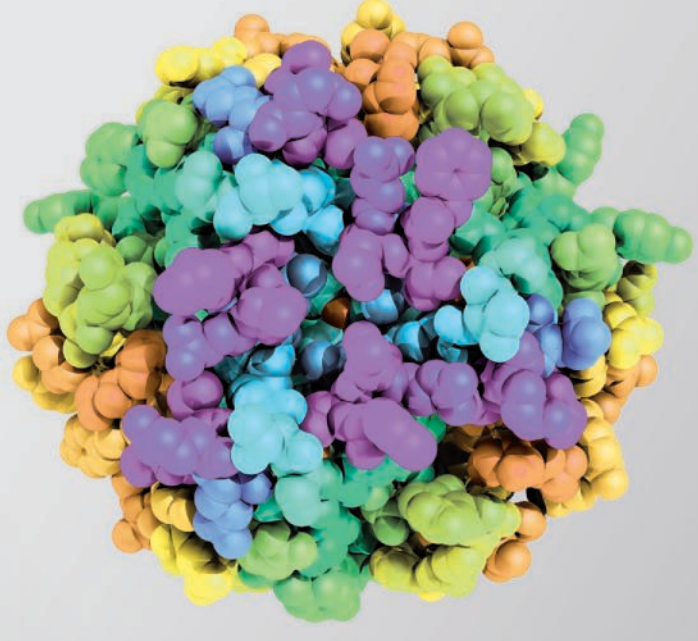

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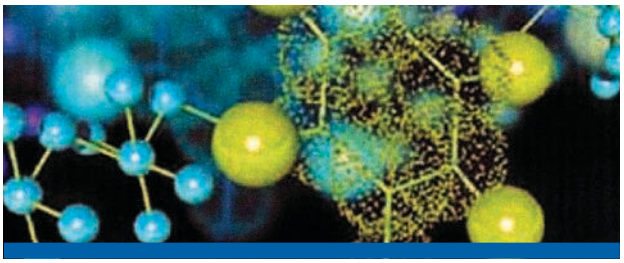

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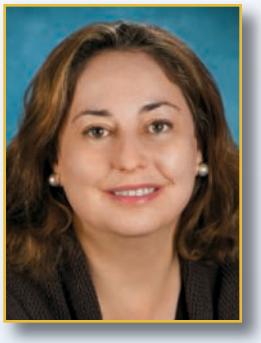

Michelle Hoffman is the editorial director of *BioPharm International*.

At a time when the industry is struggling with innovation, it might do well to learn a lesson from a few great innovators.

Passion, Innovation, and Loss

It's almost inevitable that when people speak of pharmaceutical innovation that someone doesn't ask how pharmaceutical companies can become more like Apple. It's a question that sprung up again with the death last month of Steve Jobs, Apple's iconic founder and innovator-in-chief. What was it about Jobs and the company he founded that nurtured so much innovation and creativity, and can it really be brought into pharmaceutical innovation?

Jobs outlined his personal trajectory in a tale of passion and loss in his 2005 commencement address at Stanford University. His path to Apple started when he dropped out of Reed College in order to be free to take any course that interested him rather than follow a prescribed curriculum leading to a specific major. This in turn led him to a calligraphy course that, he says, informed his ethic of blending aesthetics and technology to create Apple products. But as with many great relationships, what started out with so much generative promise ended in heartbreak—Jobs's specifically, when at the age of 30 he was asked to leave the company he'd founded.

"I'd been rejected," he said, "but I was still in love." That first loss, painful as it may have been also relieved Jobs of the "heaviness of being successful," and replaced it "with the lightness of being a beginner again." Jobs the beginner embarked on yet another creative period that resulted in the formation of Next and Pixar, which ironically, brought him back to Apple and it to him for yet another creative period that yielded iPods, iPhones, and iPads and revolutionized both the computer and the communication industries.

The other great focusing event in Jobs's life, as we all now know, was the imminence of his death from pancreatic cancer. In the face of death said Jobs, "we are already naked," and because of which, he admonished Stanford's class of 2005 not to be "trapped by dogma, which is living with the results of other people's thinking." Rather, Jobs urged the graduates to follow their individual passions.

Jobs died the same week the winners of the 2011 Nobel Prizes were announced, and among those winners was Ralph Steinman, a Rockefeller University professor, who elucidated the biology of dendritic cells. Steinman died the same week as Jobs, just days before the Nobel announcement was made. But in an interview on National Public Radio on Oct. 3, 2011, Adam Steinman, the Nobelist's son talked about his father's passion for his work, and his desire to "instill a sense of excitement and discovery in the next generation."

Reading these stories, one might suggest, as Jobs did in his Stanford address, that it's the threat of loss that should make one insist on following their passions to discovery. But I keep thinking of one my professor's observation that children are the true scholars, because they want to learn everything and are unfettered by dogma. Jobs and Steinman and other great innovators are the ones that, in spite of prevailing dogma, never lose their childlike passion for learning and exploring. So the pharmaceutical industry may be misguided in looking for ways to inspire innovative thinking in its scientists. The real secret might be in not wringing it out of them. ♦

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Microbubbles Deliver Across the Blood–Brain Barrier

Ultrasound-activated microbubbles can be used to transport large molecules across the blood-brain-barrier (BBB) in mice without damage to neural tissue or microvasculature, as presented in the Oct. 4, 2011 issue of the *Proceedings of the National Academy of Sciences*. The tightly regulated BBB presents a challenge for delivery of both small-molecule and large-molecule therapeutics to the central nervous system. The Columbia University authors demonstrated how the barrier could be breached noninvasively using microbubble carriers. Microbubbles are stable bubbles with diameters of less than 10 uM composed of a lipid or polymer shell that incorporates a therapeutic, and a gas core. The microbubbles are delivered systemically, then activated by locally-applied ultrasound pulses that cause release of the therapeutic only at the site of activation. It is thought that the ultrasound causes the bubbles to expand and contract, and thereby forces them through the capillary walls. Calibrating the ultrasound pulses to maximize delivery while minimizing damage to the microvasculature and surrounding neural tissue was the challenge addressed in the study. Four different ultrasound pulse parameters were varied systematically: peak-rarefactional pressure, frequency (within the pulse), pulse length, and pulse repetition frequency. The authors identified a minimum peak-rarefactional pressure required for BBB disruption, and found short pulses delivered in bursts were most effective at depositing fluorescently-tagged molecules in the brain without cellular disruption. Fluorescently-tagged dextran molecules as large as 70 kDa were delivered to the hippocampus in mice using this method. Moreover, the dextran was homogeneously distributed at the activation site, and occasionally uptake into neurons could be visualized. This method offers the potential to deliver large-molecule therapeutics noninvasively to one of the body's most inaccessible compartments.

Source: *J.J. Choi et al., Proc. Natl. Acad. Sci. 108 (40) 16539–6544 (2011).* —Amy Ritter

Market Report from Russia

Win Initiative/Getty Images

Foreign bio/pharmaceutical companies, including many of the leading global players in patented and generic drugs, are starting to invest heavily in Russia under the expectation that the country will remain one of the world's fastest growing drug-manufacturing markets. Bio/pharmaceutical sales are increasing at around three times faster than the country's GDP. By 2016, total revenue in the Russian bio/pharmaceutical market is forecast by market researchers Frost & Sullivan to reach more than $37 billion—that's 2.4 times higher than 2009 levels and equivalent to an annual growth rate of 13.5%.

The Russian government is hoping that investments by multinational drug companies will help modernize drug production in the country. The government wants the Russian bio/pharmaceutical sector to reach Western standards of drug manufacture and innovation, which the industry has a long way to go to achieve.

The latest government initiative has been the launch of a state fund that will invest jointly with foreign sovereign wealth and private equity funds as much as $50 billion in priority areas such as bio/pharmaceuticals and healthcare. This money will supplement 120 billion RUB ($3.7 billion) of state money announced late last year under the country's Pharma 2020 program. Those funds will be allocated to upgrading bio/pharmaceutical manufacturing, including the introduction of new production technologies in more than 160 facilities.

The initial aim behind the Pharma 2020 strategy is to raise the share of domestic production in Russia from a meager 20% of total drug sales to 50%. A separate goal aims to increase domestic production of vital medicines to 90% by the end of the decade.

Prime Minister Vladimir Putin has warned that restrictions will be imposed on multinational manufacturing firms, now comprising most of the top 10 drug suppliers in the country, if they do not set up local production facilities and transfer their technologies into Russia.

"The Pharma 2020 goals can be achieved only if the domestic bio/pharmaceutical industry becomes innovative through partnerships with international bio/pharmaceutical companies and Russian academia," says Oleg Korzinov, director of the Center for Development at the Moscow-based Pharmaceutical Cluster Northern, the country's first innovative pharma cluster.

The drugs of international bio/pharmaceutical companies with a local manufacturing presence are likely to be favored by an expanded state-reimbursement scheme covering high-cost therapies. "A local plant will be the entry ticket into the Russian market and an improved healthcare system," explains Martin Schlow, a Russian pharmaceuticals specialist at the management-consulting firm PricewaterhouseCoopers (PwC).

As a result, there have been a series of recent announcements by top global players, including Novartis, AstraZeneca, GlaxoSmithKline, and Pfizer, about investing in manufacturing and R&D projects in the country.

In addition, leading generic-drug producers such as Dr. Reddy's Laboratories and Teva Pharmaceuticals are strengthening their Russian manufacturing capabilities.

The Russian government initially expects foreign companies to help bolster innovation in drugs but ultimately wants those companies to assist in giving the country a platform for becoming a major producer of APIs.

Establishing such a position will be a big task because currently only 15% of APIs in medicines on the Russian market are made in the country. More important, only 10% of the country's 400 bio/pharmaceutical plants comply with GMP standards.

Many of the multinational companies setting up plants in Russia are using centralized API production. "Due to reasons of economies of scale, all active bio/pharmaceutical ingredients will continue to be manufactured at our large active ingredient plants in Denmark," explains Mike Rulis, head of communications at Novo Nordisk, the Danish insulin maker, which is building an insulin plant in Russia's Kaluga region.

The largest barriers to API production in Russia include a "lack of a long tradition of API exports, absence of binding rules pertaining to approval of the production and the quality control of drugs [and] a low level of training of specialists in international requirements like GMP," says Monika Stefanczyk, head bio/pharmaceutical market analyst at PMR, a market researcher in Kracow, Poland.

Nonetheless, PMR believes that with the aid of the Pharma 2020 program and a new law-making GMP certification that becomes obligatory by 2014, Russia has the potential to become an alternative center to India and China for API production.

Progress will depend on how long Russia takes to establish a thriving bio/pharmaceutical manufacturing market, which currently is relatively small in per capita terms. "The limited affluence of Russian society restricts drug consumption and this is not expected to change in the near future," says Dominika Grzywinska, a Frost & Sullivan research analyst.

—Sean Milmo is a freelance writer based in Essex, UK.

FDA Outlines Strategy to Spur Biomedical Innovation

In response to concerns about the sustainability of US drug-development efforts, FDA has released a report, titled Driving Biomedical Innovation: Initiatives to Improve Products for Patients. The report outlines several steps that FDA will take to spur biomedical innovation and ensure that such innovation can be quickly translated into safe and effective therapies. Input used to compile the report was obtained from a broad spectrum of stakeholders, including members of large pharmaceutical companies, small biotech companies, academic researchers, and patient advocacy groups.

The report outlines major areas in which FDA will be launching initiatives to address feedback obtained from stakeholders. They include:

- Rebuilding FDA's small business outreach services: FDA will establish a FDA Small Business Liaison program and a Young Entrepreneurs program, as well as establish a new partnership with the US Small Business Administration.
- Building infrastructure to drive and support personalized medicine: FDA will invest in regulatory science and clarify agency policies to support the emerging field of personalized medicine.
- Creating a rapid drug-development pathway for important targeted therapies: To clarify the pathway for rapid development of promising therapies, FDA will host a series of scientific meetings intended to achieve a common understanding of steps needed when an investigational drug being studied for a serious disease shows exceptional promise during the early stages of development.
- Harnessing the potential of data mining and information-sharing while protecting patient privacy: FDA is rebuilding its IT and data analytic capabilities and establishing science enclaves that will allow for the analysis of large, complex datasets while preserving patient privacy.
- Training the next generation of innovators: FDA is designing a new Future Innovators Program under which promising candidates will be brought into the agency for training and experience. This program will provide FDA with important outside expertise and perspective while equipping these innovators with highly marketable skills and experience.
- Streamlining and reforming FDA regulations: The agency is reviewing its current regulations to identify burdensome, unclear, obsolete, ineffective, or inefficient regulations.

The report comes at a time when new drug applications have fallen to record lows, despite continuous increases in research and development budgets. FDA hopes that the initiatives outlined in this report will promote innovation, help developers overcome challenges in product development, and speed the delivery of safe and effective treatments to patients. In her introduction to the report, FDA Commissioner Dr. Margaret Hamburg says, "It is important to note that while these are important first steps to address the immediate concerns before us, this is an ongoing initiative at the agency and will be expanded beyond the reforms outlined in this document."

—Amy Ritter

Drug Shortages Create Crisis for Manufacturers, Regulators

Clamor mounts over compromised care and rising costs due to lack of crucial therapies.

The outcry from physicians, pharmacists, and patients over disruptions in the supply of vital medicines to treat cancer, pain, and other serious conditions is drawing attention on Capitol Hill and throughout the healthcare system. Oncologists cannot obtain widely-used chemotherapies to treat seriously ill patients. Surgeons are postponing operations because they lack key anesthetics and pain medications. Doctors are struggling to obtain vital parenterals for premature babies and for patients requiring infusion. And dozens of clinical trials have been halted because investigators don't have adequate supplies of cancer drugs used as components of treatment arms or as controls.

While there always have been periodic drug shortages, the problem has become much more acute in the past two years. The main culprit appears to be drug-manufacturing and supply-chain failings, particularly related to the production of generic sterile injectables. Decisions by firms to exit certain low-profit markets often leave only one or two producers for a widely-used parenteral or other product. Some manufacturers have had to halt production due to problems meeting GMPs. Such developments raise charges that too-strict FDA oversight aggravates the problem—or that industry negligence and business practices put the public health at risk. Another possibility is that policies and practices that drive down reimbursement for old-line generics discourages industry investment in certain therapeutic categories.

Jill Wechsler is *BioPharm International's* Washington editor, Chevy Chase, MD, 301.656.4634, jwechsler@advanstar.com.

These issues made headlines in September as Congressional committees held hearings on the drug shortage crisis and broader pharmaceutical supply-chain problems. An FDA public workshop on Sept. 26, 2011, provided a forum for health professionals, patient advocates, drug distributors and manufacturers to air concerns and propose remedies for short drug supplies. The resulting publicity has boosted Congressional interest in legislation to address pharmaceutical supply-chain issues in general, and the drug shortage situation in particular. FDA has prepared a report analyzing steps it can take to tackle shortages with its current limited authority, and the Government Accountability Office (GAO) is examining the causes and responses to the drug-shortage crisis.

GETTING WORSE

Policymakers may take action because, despite extensive FDA efforts, "drug shortages are getting worse," said Douglas Throckmorton, deputy director of FDA's Center for Drug Evaluation and Research (CDER), at the FDA workshop. The agency recorded 178 drug shortages in the US in 2010, up from 157 in 2009—and much more than the 50–60 per-year range of previous years, reported Edward Cox, coordinator of CDER's drug-shortage program. The problem is even more serious when looking at all drugs and biologics: the University of Utah Drug Information Service recorded 210 shortages this year as of mid-September, slated to surpass last year's total 211 short-supply problems. And because supply problems often last more than a year, some 260 active shortages are in the Utah tracking system.

Moreover, 74% of the shortages reported to FDA last year involved sterile injectables—and almost all of them for "medically necessary" drugs, noted Howard Koh, assistant secretary for health in the US Department of Health and Human Services (HHS), at a September hearing before the House Energy and Commerce Health subcommittee.

Shortages in parenterals have taken a toll on hospitals and clinics: a survey by the American Hospital Association in June 2011, found that virtually all hospitals have experienced at least one

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drug shortage this year, and half report 21 or more supply problems. This builds on a March survey by the Premiere Healthcare Alliance that documented soaring shortages along with exorbitant prices for these products (see the June 2011 Washington Report column, "Safety Concerns and Shortages Challenge Manufacturers").

Koh cited a long list of underlying factors driving the short-supply trend: industry consolidation, limited raw materials, changes to inventory and distribution practices, production delays, increases in demand, and business decisions to close down a manufacturing site. When there are only one or two sources for a crucial drug, explained pharmacist Richard Paoletti of Lancaster (Pennsylvania) General Health, a small shift in production lines, plus just-in-time inventory controls at hospitals, make it hard to buffer the impact.

INFORMATION PLEASE

One response is for manufacturers to inform FDA in advance of supply problems. The agency reports that early warnings helped it head off 38 potential shortages in 2010 and 99 so far this year. When FDA knows of a looming supply interruption, its staff can expedite the review and approval of supplements for new suppliers, alternative production sites and changes in specifications—actions responsible for most (84) shortage preventions this year. Sometimes, FDA can bend the rules to permit continued marketing of a violative product with some corrective action. And the agency has had some success in encouraging other firms to ramp up production or to enter a market through speedy regulatory actions.

FDA also has approved temporary importation of unapproved sources to fill supply gaps, a strategy that helped alleviate shortages this year in several cancer drugs. APP Pharmaceuticals worked with FDA to reduce a shortage of the widely used anesthetic propofol by importing the product from Germany.

Unfortunately, FDA often lacks information on looming shortages because, under current law, only sole-source manufacturers of critical medicines have to notify FDA of plans to discontinue production. Some companies voluntarily inform FDA of problems likely to lead to short supplies, but most do not, and often the information comes in too late for timely resolution.

To remedy this limitation, Congress is looking to enact legislation that would require six-months advance notification of production changes for a broad range of prescription drugs. Rep. Diana DeGette (D-CO) has bipartisan support for legislation in the House, and Sens. Amy Klobuchar (D-MN), and Robert Casey (D-PA) lead the early-notification campaign in the Senate. They propose leeway for manufacturers who show that a manufacturing problem could not be anticipated, as well as penalties for failure to comply. A related proposal requires manufacturers to inform FDA of situations that make a product vulnerable to shortages, such as a single API source, to highlight the need for back-up emergency sources.

Although advance notification may help FDA head off some shortages, the danger is that wider release of such information could lead to hoarding and price gouging. Gray-market profiteers are inundating health professionals with faxes and phone calls offering scarce drugs at huge markups. Rep. Elijah Cummings of Maryland, top Democrat on the House Oversight and Government Reform Committee, launched an investigation of secondary distributors last month, seeking information on sources and profits for certain drugs. His targets include high-priced offers of leukemia treatment cytarabine by Allied Medical Supply (Florida); Superior Medical Supply (Colorado) offers for paclitaxel; Premium Health Services (Maryland) sales of leucovorin; fluorouracil offers from PRN Pharmaceuticals (Maryland); and Reliance Wholesale (Florida) marketing of magnesium sulfate.

To avoid profiteering, FDA wants to be informed by manufacturers of possible shortage situations, but doesn't necessarily want to make that information public right away, noted Sandra Kweder, deputy director of CDER's Office of New Drugs at the House hearing. The agency first wants to assess if the problem is real, its likely impact, and what actions can be taken to mitigate difficulties. "Early notification to FDA is a very useful tool," said Kweder, "but it's different from early publication."

Advance notification is not always possible. FDA officials concede that many manufacturing problems cannot be anticipated (e.g., equipment breakdowns and plant fires, and earthquakes, volcanoes, and other natural disasters) can suddenly disrupt supplies and material transport.

ADDRESSING PRODUCTION PROBLEMS

Manufacturers now support stronger early notification requirements, largely to reduce complaints about industry responsibility for most shortages. FDA reports that most supply problems arise from GMP and product quality failings, along with difficulties obtaining active ingredients or problems at a manufacturing site. Industry consolidation has reduced the number of generic-drug firms making sterile injectables, Cox observed at the FDA workshop, and it takes time for another manufacture to establish a facility to produce these more complex and costly medicines.

Reliance on contract manufacturers also has led to shortages for both brand and generic drugs. Johnson

& Johnson faces serious supply problems for cancer and AIDS treatment Doxil (liposomal doxorubicin) because its contract producer, Boehringer Ingelheim's Ben Venue Laboratories, decided to exit the CMO business.

The strong link between manufacturing issues and shortages raises questions about pharma's commitment to quality production. FDA wants manufacturers to prevent shortages by developing continuity of supply plans, with backup suppliers and alternative production strategies for critical products. Industry has a responsibility, FDA officials assert, to proactively identify and promptly resolve manufacturing problems and to implement quality-by-design strategies to prevent failures.

An alternative view is that FDA creates shortages through overly aggressive enforcement of manufacturing rules. Agency officials maintain that they don't halt production for minor violations, but only for significant problems with drug sterility and contamination. Before requesting a drug recall or seizure, they check to make sure such action won't precipitate a shortage. But strong action is needed when inspectors find glass and metal particles in vials and new impurities and degradants. In some cases, companies have been cited multiple times for violations and still fail to correct manufacturing deficiencies until threatened with total shutdown.

FDA officials also emphasize that they can act fast to help bring online a new producer or new supplier when needed. "We can turn things around in a matter of weeks," Kweder insisted at the House hearing, in response to manufacturer claims that FDA requires two-to-three years to approve a new manufacturing site. "This is not business as usual," she stated.

But stakeholders feel there is more that FDA and other government agencies can do to prevent critical shortages. FDA should revise how it calculates risks and benefits from regulatory action to give greater weight to patient safety issues that arise with shortages. The federal government should establish stockpiles for medically necessary drugs, as done for treatments against bioterrorist attacks and pandemics. And antitrust officials should scrutinize proposed pharma company mergers to assess how the combination would affect limited drug supplies.

At the same time, there's interest in providing more incentives for manufacturers to enter depleted markets. Tax credits or rebates could spur manufacturers to update facilities or launch production of low-profit drugs. Some kind of exclusivity could be offered for new production of a drug in short supply. Or, generic-drug makers might be eligible for reduced user fees on applications to produce hard-to-obtain medicines.

Shortages in controlled substances, such as long-acting painkillers and drugs to treat children with attention deficit hyperactivity disorder, are generating calls for the Drug Enforcement Agency (DEA) to work more closely with FDA and industry to modify limits on active ingredients. Manufacturers receive DEA annual quotas on controlled drug substances, but would like a way to transfer allotments when one company ceases production.

There's also support for more resources for FDA to deal effectively with shortages. FDA formed the drug-shortage program in 1999 to manage anticipated supply disruptions from the Y2K shift to the new millennium. But with only five staffers and limited legal authority, FDA is hamstrung in preventing supply disruptions.

BALANCE NEEDED

Parallel to the shortage crisis, FDA has been campaigning for more authority to control counterfeiters and manage an increasingly global pharma supply chain. A number of bills before Congress would empower FDA to deal more forcefully with illegal imports and to address drug manufacturing problems. The challenge is to ensure that efforts to strengthen FDA clout does not aggravate drug shortages.

At a hearing Sept. 14, before the Senate Health, Education, Labor, and Pensions (HELP) Committee, Deborah Autor, recently named FDA deputy commissioner for global regulatory operations and policy, presented a long list of desired policy changes to promote drug safety and level the playing field between domestic and foreign manufacturers. Autor wants mandatory recall authority for drugs, power to detain and destroy violative imports at the border, and much stiffer penalties for noncompliance. Drug manufacturers and importers would have to register and list manufacturing facilities using identifier numbers, and importers would have to demonstrate that they meet quality standards, instead of FDA proving that they do not. Autor also seeks more authority to enforce track-and-trace standards, which would help hospitals and physicians know whether drugs from unknown or unusual sources are legitimate.

Industry supports many of these changes, but is wary that continued shortages will spur calls for even greater government intervention in the market. The danger is that added rules could make low-profit drug markets less attractive to manufacturers. Yet, patients are waiting: Short supplies of drugs to treat children with leukemia in the US is "shameful," lamented oncologist Len Lichtenfield at the FDA workshop. "Maybe we need more government intervention." ◆

Biosimilars' Technology Needs

Biosimilar manufacturers need better expression systems and analytical tools to compete.

Biosimilars manufacturers recognize that to compete and provide cost-savings relative to reference products, they must be cost-effective. Some improvements for biosimilar manufacturers will include: improved expression systems, higher titers to reduce capacity requirements, and better assay methods to permit process monitoring, and product characterization (1).

TECHNOLOGY NEEDS

Improved expression systems

Biosimilars manufacturers will need to increase expression-system yields to enable use of smaller bioreactors and lower cost facilities. New expression-system technologies, along with large single-use bioreactors for late-stage and commercial manufacturing, may begin to displace stainless steel for commercial manufacture. This shift is particularly true for recombinant monoclonal antibodies (mAbs), where a large number of biosimilar products are in development. mAbs have high repeated dosing requirements, and large amounts of protein in the magnitude of hundreds of kilograms per year often are needed.

New platforms (i.e., new host cells/organisms) and genetic-engineering advances applied to traditional platforms also can offer advantages in product yield, product quality, lower operating, purification, and infrastructure costs (2). Some newer technologies offer shorter times required to go from gene to transformed host cell line/organism to commercial-scale manufacture. Currently, there are just a few major players promoting new expression-system technologies, but there are many advances ready for adoption, adaptation, and further development.

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Higher titers to reduce capacity requirements

The trend in increasing protein expression yields has had broad ramifications for biosimilars. From lowering construction requirements to increasing bottlenecks at the downstream end of production, the effects of upstream advances and the availability of more varied expression systems have created options and problems not previously seen. According to a recent BioPlan Associates analysis, the overall average yield reported for commercial mAb manufacture in 2011 is 2.18 g/L, up from 1.94 g/L in 2009 (see Figure 1) (1). It was not that long ago that mammalian-cell culture commercial production yields were lucky to achieve 1 g/L or greater (1).

The next generation of commercial products will have an even greater average yield. For late-stage clinical manufacture, the overall average in 2011 is 2.68 g/L, up from 1.96 g/L in 2008. Although protein yields over 30 g/L are being reported by expression system developers and early adopters, these yields are the exception, and yields for commercial manufacture in the 5–10 g/L range are more likely in coming years (1).

Analytical technology

Better assay methods to permit process monitoring and product characterization will enable more biosimilar approvals. FDA and other regulatory agencies will largely rely on analytical data to support conclusions that biosimilar candidates are sufficiently similar to their marketed reference products to allow generic-like approvals. As such, regulators are likely to look for better analytic characterization through more combinations of attributes at greater sensitivities with multiple complementary methods. For drug developers, analytical method development and testing will be less expensive and quicker compared to reliance on clinical trials to support approvals.

Progress in the development of bioprocessing-related assays and analytical equipment, however, has not kept up with industry demands.

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Figure 1: Annual change in titre, commercial, and clinical-scale production of monoclonal antibodies, 2008–2011 (data, reference 1).

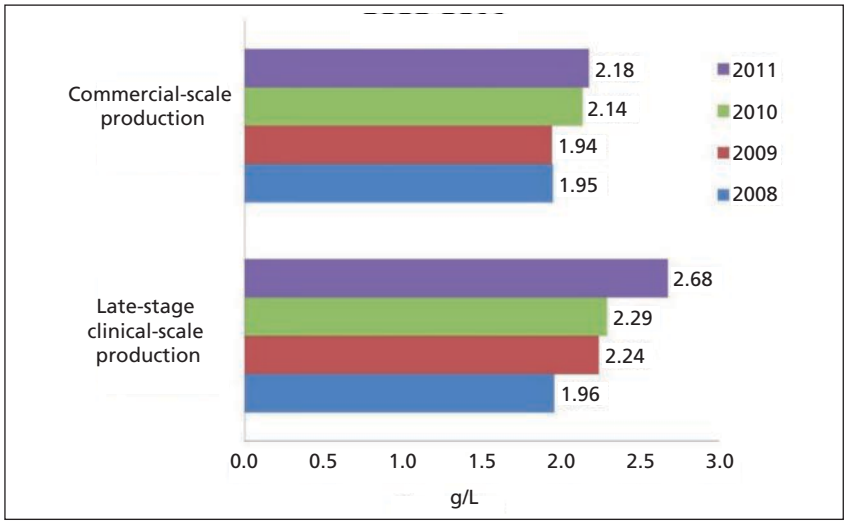


Figure 2: Select new product-development areas of interest (data, reference 1).

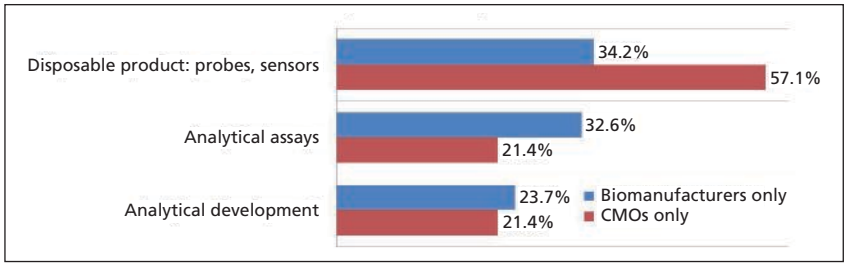
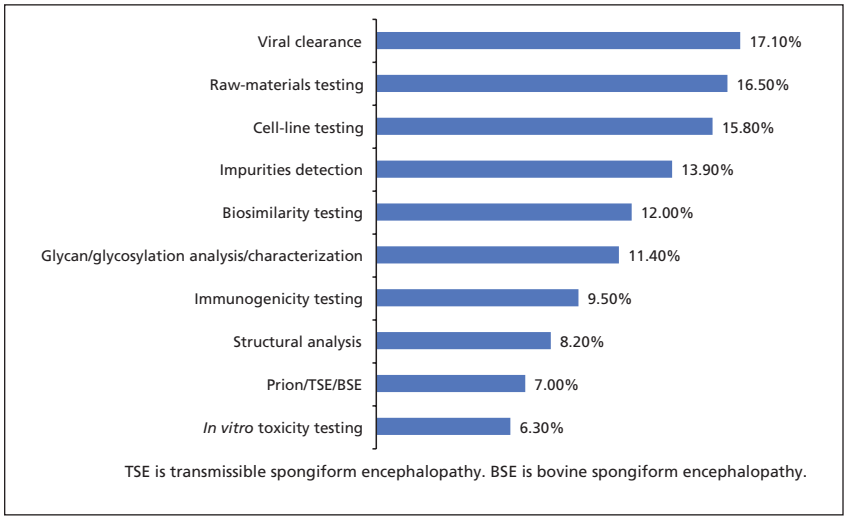


Figure 3: Percentage of all biopharmaceutical Industry vendors actively funding assay-related R&D (data, reference 1).



Better analytical technologies are being sought by the industry for improving productivity, process monitoring, real-time product quality control, process optimization, and analytical characterization for biosimilars. These trends are reflected in a recent BioPlan Associates survey, where more than 32% of biopharmaceutical manufactures and 21% of CMOs expressed demands for improved analytical assays (see Figure 2). The survey showed that, after single-use/disposable bioprocessing equipment, more relevant and cost-effective assays and analytical equipment are strong needs. Areas of interest include better probes and sensors, in-house real-time analyses of process streams, use of biological assays to determine active agent-related quality (e.g., glycosylation variants), and outsourcing of specialized chemical analysis and biological assays.

More than one quarter (26.2%) of respondents cited analytical testing and drug-product releases a major factor likely to constrain their organization's production capacity during the next five years. Respondents identified 26 areas where new and improved testing technologies are required. More than 40%, cited glycosylation and comparability testing (primarily for demonstration of comparability between lots/batches of the "same" product, but also to show similarly for biosimilar products). Also, nearly 40% of all respondents cited the need for better host-cell protein assays, biophysical characterization during process development, and in-process testing and aggregation assays. Figure 3 shows the percentage of vendors funding assay-related R&D.

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Developing and Sustaining a Quality Culture

Employee training—at all levels—is crucial for moving forward with a successful risk- and quality-based manufacturing strategy.

FDA regulatory oversight and enforcement have never been more intense or potentially more costly. According to the business intelligence firm FDAzilla, the agency is on pace in 2011 to break its record for 483s for the third year in a row, issuing well over 10,000 citations a year—that's one every 52 minutes. For biopharmaceutical companies, with their highly complex and expensive operations, the total cost of cGMP compliance continues to constitute a significant percentage of the cost of goods sold.

Meanwhile, a variety of factors have made compliance and quality more challenging. Complex global supply chains increase the likelihood of lapses. Economic pressures to cut costs can result in compromised processes and increased operational as well as quality risk. Conversely, anxiety about regulatory action and inadequate understanding of risk can lead to expensive gold-plating and redundancy in quality and compliance activities. Company growth, the introduction of new products, and entry into new global markets can also attract an increase in regulatory scrutiny. In mergers and acquisitions, achieving consistency in quality and compliance can be especially daunting, particularly when a traditional small-molecule manufacturer acquires a biologics manufacturer or licenses a biologic product, thereby requiring interaction and compliance with a totally different branch of FDA with which it has little or no prior experience.

In the face of these pressures, the business case for optimal quality and compliance is compelling: improved operating performance, greater productivity, less compliance risk, less rework, and fewer interruptions of supply to the market resulting in lost revenue. Yet, in many companies, compliance problems persist, often because the company culture consists more of fighting fires than of thinking right-first-time and maintaining a reliable state of quality and compliance throughout the company. For an organization to do this successfully—and sustainably—this mindset and behavior focused on quality compliance must start at the top and be emulated by individuals at all levels and in all functions within the company. Moreover, in a culture of quality, it is important that employees adopt this mindset, not because they have to, but because they understand the importance and benefits of this thinking and behavior and appreciate the risks of not adopting it.

In a culture of quality, it is important that employees adopt this mindset, not because they have to, but because they understand the importance.

Consider the wildly divergent views of quality that were uncovered when a major biopharmaceuticals manufacturer undertook a comprehensive assessment of quality across a number of its global operations. The company's manufacturing sites, as well as corporate headquarters, were polled on such questions as how well leadership defined and communicated their vision of the desired quality culture, whether management had identified what was required to create and sustain a quality culture, whether the individual had the tools and resources to get his or her job done correctly, and much more. When asked whether the right behaviors were encouraged

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for sustaining a quality culture, a strong majority of respondents at one site responded favorably, yet overall, barely one third of the staff polled at several other sites, including their corporate headquarters, responded positively.

Not every dimension that was assessed turned up such divergence. For example, nearly everyone agreed that they were held accountable for the quality of their work; and by overwhelming majorities throughout the company, respondents agreed that if they observed noncompliant activities they felt comfortable calling it to someone's attention. But in many areas there were wide divergences and in others—like whether the company's people had the skills to do a high quality job—there was low favorability almost across the board. It also emerged that the company was perceived by employees as focusing on short-term fixes in quality issues, and that personal development, teamwork, rewards, and recognition were inadequate for promoting a quality culture. Instead of a single, unified environment, the company had many disparate cultures.

QUALITY CULTURE ASSESSMENT

Transforming organizations to obtain and sustain a quality culture begins with a comprehensive evaluation of the various organizational, procedural, staffing, and other parameters that impact quality within the organization. In other words, all of the activities, attitudes, and interactions that together constitute culture must be considered, including elements such as:

- *Quality/compliance governance structures:* Are there effective mechanisms for such activities as global change management for new product introductions, processes for regulatory changes, pharmacovigilance, product complaints, quality related councils, and material review boards?
- *cGMP compliance activities:* These include batch/lot issuance, batch review and disposition, deviation management, corrective and preventive action, change control, document control, internal auditing/inspection, risk identification/remediation, annual product review and all of the other relevant processes and procedures. Are they uniform, compliant, and effective at each site, across sites, and across the entire organization?
- *Quality metrics:* These include such measures as right first time, cycle time, product complaints, regulatory events, action plan attainment, reportable events, and the like. Quality metrics should be appropriate and provide the basis for effective review of quality performance.
- *Leadership styles and behaviors:* Do leaders take a comprehensive view of quality, communicate that vision effectively throughout the company, and behave in ways that foster and support the efforts of all employees?
- *Human-resource practices:* Are personnel sourced, recruited, hired, and on-boarded in ways that promote a consistent, high-performing quality culture? This can be an even more challenging issue for manufacturers of seasonal vaccines, because they often hire many seasonal workers who may have little long-term allegiance to the company or exposure to the company's values in regard to quality.

Figure 1: Key elements in establishing a sustainable quality culture.

FIGURE COURTESY OF AUTHORS

- *Learning and personal development systems:* How broad, deep and effective is the organization's training program? Are personnel given opportunities for further professional development?
- *Quality behavior reinforcing mechanisms:* Are performance management policies, rewards, and recognition designed to motivate individual employees as well as teams to consistently strive for quality?
- *Quality-related information systems:* These include not only IT and enterprise resource planning systems used for document management, deviation management, change control, and the like, but also the way in which information is shared. Are best practices and lessons learned at one site communicated to the other sites within the company to maintain a uniform, high-quality company culture?
- *Employees' perceptions in relation to quality at the company:* As with the example of the biopharmaceuticals company described above, how do employees react to statements that describe the cultural norms and behaviors of a high performance organization with a strong quality culture?

On the basis of this assessment, it is then possible to characterize the organization's quality culture, or more likely, cultures, as the basis for undertaking transformation.

KEY ELEMENTS OF A SUCCESSFUL TRANSFORMATION

From the assessment there should emerge clear recommendations for improvement, including quick wins, and goals for the short-, medium-, and long-terms. Because organizations differ, the particulars of these recommendations for transforming any given culture will vary. But there are some constants in the execution of such recommendations. A centralized project management framework should be put in place to help guide the planning and successful implementation of the recommendations.

To help drive and sustain changes, a compelling quality culture message must be developed—in effect, internally branding the effort. The message must be clear, relevant, understood by all, and designed to provide a point around which every employee can rally, motivating them to contribute to the effort. Best practices in human resources, organizational development, and leadership should be followed in order to maximize employee engagement, assure effective rewards and recognition, provide timely communication of progress, and institutionalize accomplishments. Finally, appropriate operational-excellence tools should be used to further help assure successful implementation and sustainability of these efforts—as well as to provide the metrics needed to monitor and report progress along the way.

What should the resulting high performing, sustainable quality culture look like? Again, the particulars may vary from company to company, but such cultures should share the following characteristics (see Figure 1):

- Employees at all levels understand the organization's quality objectives, policies, and procedures and their individual roles in helping to achieve them.
- Leadership at all levels is visibly engaged in supporting the development of a quality culture and effectively engages and motivates others to do the same—leading to self-motivated accountability and sustainability.
- Effective communication, enterprise-wide sharing of best practices, engagement of all employees, and rewards and recognition for both teams and individuals maintain the momentum and enthusiasm required for sustainability.
- The organization hires people who possess the quality values, norms and work practices the company desires.
- Staged on-boarding and technical and quality training are deployed at the company, business unit, functional area, and individual levels.
- Consistent and sustainable standards of quality are defined clearly and deployed across the organization in conjunction with quality and compliance systems that enable the organization to achieve those standards.
- The organization distinguishes between people and processes as the root cause of mistakes, and instead of blaming people looks to correct processes.
- Leaders and managers at all levels establish an environment of trust and collaboration in which challenging issues can be raised without fear of reprisal.
- The organization institutionalizes a process for capturing, analyzing, and incorporating lessons learned from past successes and failures.
- As the organization grows and changes, the quality culture is continually monitored and fine-tuned to ensure that it remains effective and sustainable.

The journey from quality culture assessment to transformation to sustainability need not take long or consume massive resources. But it does require the recognition that achieving quality and compliance is not a matter of a discrete, isolated process but of the larger environment in which it takes place. ♦

Focus on Standardization, Quality by Design, and Regulatory GMP

Highlights from the IBC Single-use Applications meeting, the PDA Single-use Workshop, and the Bio-Process Systems Alliance International Single-Use Summit provide insight into current single-use practice.

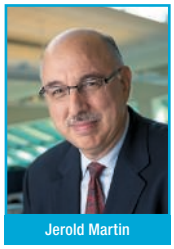
IBC: FOCUS ON STANDARDIZATION

At the IBC Single-Use Applications meeting, held in Boston on Jun. 6–9, 2011, I gave a talk entitled "Standardization of Single-Use…Pros, Cons and Possibilities." The topic of standardization has appeared in many surveys as one of the top 10 needs for expanding single-use implementation. However, there is little definition of what is meant by "standardization," which could be applied to many areas.

One particular area of discussion is the interchangeability of sterile connectors to enable single-use unit systems with sterile connectors from different suppliers to be linked. In preparing my talk, I also discovered that there is already an interchangeable "standard sterile connector" recognized by industry. If you search for this phrase in Google Images, you'll find a photo of a traditional two-way hosebarb fitting.

In my talk, I discussed how standardization of advanced sterile connectors must begin with the end-user's company. Different systems' assemblers and integrators can generally source whatever advanced sterile connector is specified, so the first effort must be for the end-user's company to decide what its preferred sterile connector will be. Since the introduction of the first advanced sterile connector (Pall's Kleenpak), there are now five additional designs on the market from other suppliers, but there is still no industry consensus on which design might become the "standard" of the future. Unlike the easily copied tri-clamp style hygienic flange connector, which is commonly used in stainless-steel and some single-use systems, or the aforementioned hosebarb fitting, many advanced single-use sterile connectors incorporate patented technology and designs. Users calling for the standardization of sterile connectors have yet to address which design should dominate or why the patent-holder should license their design to competitive suppliers. Once bioprocessers agree on a preferred design within their own facilities or companies, once there is consensus on an industry-preferred design (such as happened with tri-clamp style connectors), and once patents expire, options will appear to either "standardize" one of the original sterile connector designs. The alternative is that users will prefer an innovative proprietary design that is superior to current designs, but has yet to be seen. Until then, care has to be taken to avoid inhibiting innovation and it may not be realistic to expect meaningful voluntary standardization from the bioprocessing supply industry.

One area where there is an industry initiative underway to standardize connectors, however, is for polymeric tri-clamp style hygienic flange seal connectors. In response to stimulus letter from the Bio-Process Systems Alliance (BPSA), the American Society of Mechanical Engineers bioprocessing equipment standards group (ASME–BPE) has formed a task force to review the requirements for single-use polymeric tri-clamp style connectors and how the ASME–BPE Standard for stainless steel hygienic flange seal connectors can be modified in response to accomodate their use. Unlike their stainless steel counterparts, single-use polymeric tri-clamp connectors do not require cleanable finishes, crevice-free cleanable seals, or thermal resistance and mechanical strength suitable for steaming in place. They also do not require tolerances that are readily achieved with stainless steel on a lathe, but are difficult to achieve with plastic molding, thus incurring unnecessary mold costs.



Jerold Martin

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Althea Technologies

Expansion and relaxation of the ASME–BPE standard to cover the specific requirements for single-use polymeric tri-clamp style connectors will be a benefit to both suppliers and users.

PDA: FOCUS ON QBD

The PDA held its first Single-Use Workshop in Bethesda on June 22–23, 2011. The main focus was to preview the draft PDA Technical Report on Single-use Manufacturing, which is currently in development, and to solicit attendee feedback. As a member of the PDA Single-use Task Force, I served on the planning committee and as a moderator and presenter on the report for this workshop.

The purpose of the new report is to provide the reader with critical concepts and topics to consider when implementing a single-use manufacturing strategy for drug or vaccine production. The draft report discusses single-use systems that may be in direct or indirect contact with raw materials, intermediates, intermediate products, pharmaceutical drug substances or the drug product. The primary goals for developing a single-use manufacturing strategy are customer-based and focus on patient safety and product availability, as well as product and process understanding and control.

The workshop program focused on QbD principles and other high-level topics to guide users on their initial decision, selection, validation, and implementation of single-use processes. The workshop included several opportunities for Q&A and open discussion, which gave the task force valuable feedback from attendees on the draft report. Key take away points included:

- The report should aim to help end-users move away from 'gut-feeling' to fact-based decisions.
- Control of suppliers will also come under more scrutiny and supplier audits can be expected to increase.
- More detail on materials and manufacturing methods (e.g., films) may be required.
- Partnership between the supplier and end user was stressed; those companies that truly embrace partnership will be the ones most likely to achieve success.
- The industry will expect suppliers to have pharmaceutical standard quality systems in place, particularly with regard to having an appropriate materials change control and change notification program.
- Training in single-use will also be a key requirement/capability from suppliers.
- Clear communication strategies encompassing quality, trust, track record, openness, and security will be in focus.

The target date for the report's publication is by the end of this calendar year. Current activities entail completion of the manuscript incorporating the feedback from the June workshop and final technical review of completed sections. Follow-on workshops are planned for Uppsala, Sweden, on Nov. 28, 2011 and Phoenix, AZ, on Apr. 18–19 2012 (see www.pda.org for details).

REGULATORY GMP FOCUS EXPLAINED AT BPSA CONFERENCE

The third important single-use conference of the summer was the BPSA International Single-use Summit, which was held in Washington, DC, on Jul. 27–29 2011. This inaugural conference for BPSA served as a forum for suppliers and users to highlight the business model for single-use. In addition to business leader speakers, the summit featured J. David Doleski, the consumer safety officer at FDA's Center for Drug Evaluation and Research. Doleski opened with a review of relevant FDA regulations that impact single-use manufacturing, including 21 *CFR* 211.65 on Equipment, 211.94 on Drug Containers, 600.3 on Biological Purity and 600.11 on Biological Control. Each of these has similar statements about assuring that process equipment and containers do not adversely affect the drug or biological product. Additional compendial standards noted were USP General Chapters <87> and <88> on Biological Reactivity, <661> on Plastics, and <381> on Elastomers, as well as relevant FDA guidances, including cGMP for Phase I Investigational Drugs (July 2008) and Container Closure Systems for Packaging Human Drugs and Biologics (May 1999).

Advantages of single-use manufacturing were recognized as:

- Reduced need for cleaning and sterilization systems and validation
- Reduced risk of cross-contamination
- Improved containment
- Potentially greater control over aseptic operations (as facilitated with sterile connectors/disconnectors and tube welders/sealers).

Doleski's talk continued with an excellent overview of topics that should be incorporated in process documentation and FDA filings. Initially highlighted were considerations for vendor partnerships and materials control, included establishment of manufacturing (quality) agreements, vendor audits, notifications of changes in product (materials or design), certificates of analyses, and flow path testing for endotoxin, particulates and bioburden (where necessary). With regard to sanitization (e.g., irradiation for microbial control) or sterilization, bioprocessers should note where sterilization is performed (i.e., contract irradiator) and provide documentation on the sterilization validation method, sterilization records, impact on materials (sup-

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plier validation data) and repeated sterilization where applied (note that single-use systems are generally not suitable for multiple irradiations at doses > 25 kGy).

Extractables and leachables studies are performed to assess the potential impact of leachables on product quality, efficacy, and safety. This can begin with compatibility and extractables data from the supplier, testing with additional model solvents under manufacturing process parameters (e.g., temperature, pH, pressure, and time) where needed, considering the cumulative effect of all manufacturing equipment and conducting further risk assessments to determine if a leachable study is necessary (e.g., for final product formulation).

Process-validation considerations should take into account the full range of the manufacturing process, and incorporate multiple unit operations and actual manufacturing parameters, such as mixing speed and duration of perfusion culture. Sterile-media simulations should be conducted for filling of sterile product (i.e., bulk or unit dosage). Where fluids are stored in single-use containers, validation should include the length of time and temperature range, with assessments of the impact of fluid on materials, and the impact of materials on product, buffers or media, and container integrity (i.e., leakage) after storage. Where bulk fluids are shipped, considerations should include the effect of pressure changes, such as altitude, effect of motion (i.e., acceleration or vibration) and the protection offered by external containers. Other environmental considerations can include light, chemicals, and other mechanical forces that may affect the contained fluid.

Despite commonly cited concerns, leak integrity issues with today's improved biocontainer designs are rare. However, possible issues should be qualified and noted, such as movement or shipping of storage containers, operator error (handling training), improper operation parameters (i.e., tube welding), and exposure to extreme temperature.

Doleski summarized his talk by saying that FDA recognizes the importance of the user's relationship with their single-use equipment suppliers and expects users to work with suppliers to develop knowledge of their single use equipment, understand their product and processes, consider potential issues, conduct a corresponding risk assessment, perform appropriate validation, and establish proper quality systems to maintain a state of control. ◆

CMOs Ratchet up use of Single-Use Devices to Improve Performance

Eric S. Langer

Single-use systems continue to gain traction among biomanufacturers, especially CMOs.

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CMOs are on the leading edge of the growth of single-use devices. This growth is partly driven by their highly competitive business model that requires that they continually drive for efficiency and productivity. As an example, today more than 86% of CMOs are using disposables/single-use devices to create improvements in bioprocessing performance, according BioPlan Associates' 20th annual report (1). The survey asked 352 global biomanufacturers and CMOs which aspects of manufacturing technology have improved performance at biologics facilities over the last year. Among the 15 areas identified, some significant differences were seen between CMOs and biologics developers.

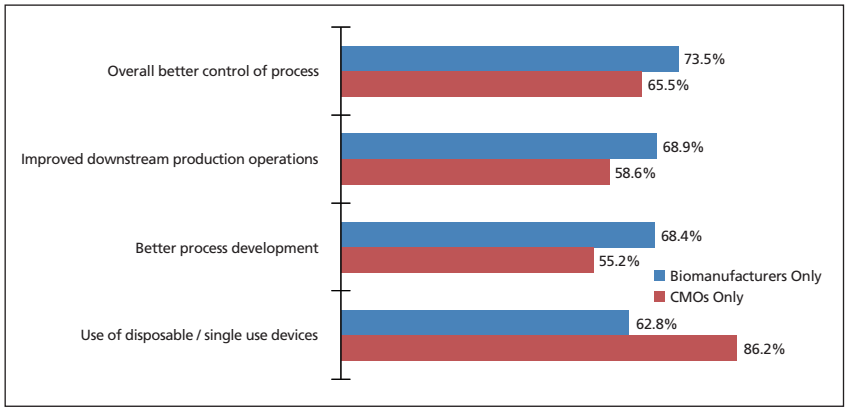
This year, the vast majority of CMOs, 86.2%, pointed to finding "significant" or "some" improvements as a result of single-use devices adoption, far outstripping their next most popular factor, "improved upstream production operations," at 69%. By contrast, 62.8% of product manufacturers reported "significant" or "some" improvements from single-use devices usage—a healthy percentage, but lagging behind factors such as "overall better control of process" (73.5% for biomanufacturers), "improved downstream production operations" (68.9%) and "better process development" (68.4%), among others. The data, released in BioPlan Associates' 8th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production, shows that implementation of single-use devices was one of the few areas where CMOs reported implementing more performance improvements than product manufacturers.

UNITED STATES VERSUS EUROPE

In comparing responses from manufacturers in the US, Western Europe, and the rest of the world (ROW), single-use device adoption was one of the major areas with divergent responses, with more in Europe than the US and ROW reporting improvements from this area. Specifically, 79.2% of Western European respondents signaled improvements derived from single-use devices, second only to "overall better control of process," at 82.8% of European biomanufacturers. By contrast, 62.3% of US respondents and 55.2% of ROW respondents reported improvements from single-use technologies, behind other factors such as improved production operations (upstream and downstream), and process control enhancements. The Europeans' preference for single-use technologies may reflect their customer purchases and/or vendor marketing catching up with those in the US, while much of the ROW still may not be heavily implementing single-use systems, or vendors are simply not yet marketing heavily in these regions.

Taken together, 65.5% of biomanufacturing and CMO respondents pointed to benefits from usage of single-use devices this year, a slight decrease from the 67.2% in 2010 (2). Indeed, this year, reported improvements from single-use devices dropped to fourth on our list of factors, from second last year. This year, as in 2010, "overall better control of

Figure 1: Select factors in biomanufacturing performance creating "significant" or "some" improvements identified by biomanufacturers versus CMOs.



process" was the leading factor creating performance improvements, with 72.6% of respondents (compared with 74.2% in 2010).

WHERE HAVE SINGLE-USE SYSTEMS PROVED BENEFICIAL?

BioPlan evaluated 15 activities manufacturing organizations have implemented over the past year to specifically speed drug-development timelines. The use of more disposables and single-use devices in manufacturing took the top slot, with 40.8% of respondents. Following disposables was the use of more "platform'" downstream processes, at 37.7%. Analysis of those activities showed that there was a cluster of mid-level activities that sped drug-delivery times. These included activities such as using "platform" cell lines, improved in-process monitoring, use of "design of experiment" methods, and use of better chromatography resins. At the bottom of the attributes was "use of different expression systems." However, because expression systems are typically a one time decision, this is not unexpected.

Comparing biomanufacturers to CMOs, BioPlan found that for CMOs the number one activity implemented to speed drug development timelines was the use of more disposables and single-use devices in manufacturing (72% vs. 42% for biomanufacturers).

Trends in the time required for product development and approval are closely related to productivity and cost concerns. This year, the survey showed that acceptance of single-use devices is also being used as a cost-cutting action. Of 212 respondents to this question, 35.4% accepted single-use (disposable) systems into clinical manufacturing operations to cut costs, while 19.3% accepted single-use systems into commercial manufacturing operations for the same reason.

PRIMARY REASONS FOR INCREASING USE OF DISPOSABLES

BioPlan identified reasons for the increasing trend towards use of disposable and single-use system components. Single use disposable systems can provide cost savings, and sometimes these savings can be substantial compared with fixed stainless steel systems, particularly with regard to larger systems. Indeed, as shown above, biomanufacturers are accepting single-use systems as a cost-cutting mechanism, and according to BioPlan's data, almost 58% of respondents indicate that using disposables to lower annual costs is a "very important" attribute.

However, as in previous years, direct cost reductions do not appear to be among the most widespread ("important" or "very important") factors to these decision-makers. As in earlier years, the data indicate that disposable systems users are more concerned about factors that will save time, reduce risks, and accelerate campaign turnaround. Increasingly, they are also interested in reducing capital equipment.

The primary reasons biopharmaceutical developers and CMOs use these products are to:

- Eliminate cleaning requirements (90.2% indicating "important" or "very important")
- Reduce capital investment in facility and equipment (85%)
- Reduce time to get facility up and running (85%)
- Decrease campaign turnaround time (81.7%).

A significant shift is the focus on reducing capital investment in facility and equipment. Despite the loosening of budgetary constraints, end-users are increasingly interested in seeing how disposable systems can help them avoid capital investments in manufacturing.

ARE CRUCIAL FACTORS LEVELING OFF?

The reasons cited by biopharmaceutical manufacturers for increasing their usage of disposables shows relative consistency from past years. For example, a few years ago, "eliminating cleaning requirements" took the top position over "decreasing risk of cross-contamination," as the dominant reason for increased usage. This year, 48.5% of respondents indicated that eliminating cleaning was a "very important" reason, holding consistent over the last few years.

However, many respondents are slowly reducing the impor-

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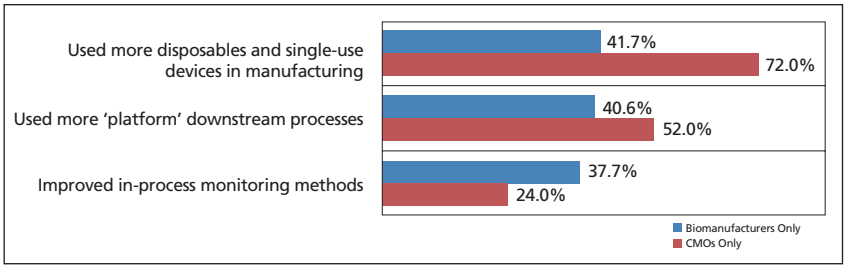
Figure 2: Select activities implemented to speed drug development timelines identified by biomanufacturers versus CMOs.



tance they are putting on individual product attributes. This reduction may reflect a maturation of this segment. As the industry becomes more aware of the attributes and benefits of disposable usage, and as the use of disposables increases, decision-makers' needs and specific reasons for increasing usage continue to level out. For example, when considering all users, "Decreasing risk of cross-contamination" as a "very important" reason saw a decrease this year to 42.4% (down from 50% last year) of users. Much of this stabilization may be the result of greater experience and awareness of these devices.

WHERE DEVELOPERS AND CMOS DIFFER

BioPlan found new distinct perceptions between CMO and biotherapeutic developers when measuring their top reasons for increased use of disposables. According to Dr. Abdul Wajid, senior director of process sciences at XOMA (US) LLC, "Significant differences with biotherapeutic developers reflects CMO business model which has multiple projects pipeline, generic quality documentations enabling them to achieve faster turn around resulting in higher productivity and better use of plant time to maintain business edge," (1).

CMOs in particular can save on facility or campaign costs, which can also reduce operating costs and capital investments. The flexibility and quick turn-around times between process runs can also improve efficiency, which can reduce costs. These savings can be passed on to the client. This is the result of the CMO being able to perform different processes within the same facility. CMOs often use disposables to reduce risks of cross-contamination between campaigns, and are motivated by the need to get a facility or project up and running quickly. By contrast, biotherapeutic manufacturers tend to be more motivated by the need to keep maintenance costs low. If suppliers or associated standard-setting bodies are able develop methods for standardizing disposables, it would enable the use of secondary vendors, which is an increasingly important factor today.

"Very important" reasons for increased use of disposables that are much more applicable for biotherapeutic developers:

- Reduce time to get a facility up and running (44.6% of developers vs. 34.6% of CMOs)
- Reduce water requirements (25% of developers vs. 8% of CMOs)
- Lower annual maintenance (22.2% of developers vs. 12% of CMOs).

"Very important" reasons that are more applicable for CMOs:

- Decrease risk of product cross-contamination (56.0% of CMOs, 39.4% of developers)
- Reduce space requirements (36% of CMOs, 19.7% of developers)
- Faster turnaround time (40% of CMOs, 33.1% of developers).

These crucial reasons are reflected by the recent expansion of the CMC Biologics facility in Copenhagen to include a 2000 L single-use bioreactor. According to Gustavo Mahler, chief operations officer at CMC, the advantages are clear: "Reduced project times, reduced risk of cross-contamination resulting in increased process reliability, and reduced batch manufactur-

Survey methodology

The 2011 Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production in the series of annual evaluations by BioPlan Associates yields a composite view and trend analysis from 352 individuals at biopharmaceutical manufacturers and contract manufacturing organizations (CMOs) in 31 countries. The methodology also encompassed an additional 186 direct suppliers of materials, services and equipment to this industry. This year's survey covers such issues as: new product needs, facility budget changes, current capacity, future capacity constraints, expansions, use of disposables, trends and budgets in disposables, trends in downstream purification, quality management and control, hiring issues, and employment. The quantitative trend analysis provides details and comparisons of production by biotherapeutic developers and CMOs. It also evaluates trends over time, and assesses differences in the world's major markets in the US and Europe.

ing costs that will allow CMC Biologics to be even more competitive," he says (3).

SUMMARY

There are many recognized benefits from the use of disposables, many of which can shorten lead-time to get a facility or campaign up and running. CMOs can be the first to reap these advantages, but all biomanufacturers have seen benefits. The top five reasons most commonly cited by all respondents as "most critical" include:

1. Reduce capital investment in facility and equipment (21% for 2011, up from 14.4% in 2009)
2. Eliminate cleaning requirements (12.6%)
3. Faster campaign turnaround (11.2%, up from 6.9% in 2009)
4. Decrease risk of product cross-contamination (8.4%)
5. Reduce time to get facility up and running (6.3%).

It is clear that single-use devices continue to gain acceptance in the industry, and that their benefits are being increasingly noticed. Over time, we expect biomanufacturers and CMOs to integrate disposables into most processes, especially as downstream single-use solutions and specific devices such as probes and sensors continue to evolve. In addition, as new regions such as China and India gain prominence and begin to produce biologics and biogenerics, the need for consistent quality will likely dictate the greater use of disposables, because they can often be implemented more efficiently and with fewer risks of quality problems (4). As new biomanufacturers enter the industry and new products enter the pipeline, the disposables option will become part of the decision process from the very start.

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Biobetters and the Future Biologics Market

Niall Dinwoodie

Despite their difficult approval pathway, biobetters offer the potential for innovation and decreased healthcare costs.

The development of biological products is now a mature industry and, as befits a mature population, the next generation of products is starting to appear. This next generation fits loosely into the category of follow-on biologics, a title that covers various products that are copies, near-copies, or improvements of original biologic products (see Figure 1). Within this group there appear to be both easy wins and challenging approaches that will require great effort to bring a product to market. However, a detailed analysis of the costs and benefits of follow-on biologics shows how increased investment in development can offer a better marketing opportunity.

BIOSIMILARS

On Mar. 23, 2010, the Biologics Price Competition and Innovation Act (BPCA) became law in the US, the world's single largest pharmaceutical market. As a result of this act, FDA is developing mechanisms for the approval of products known as biosimilars that may be considered "highly similar" to and "interchangeable" with authorized biologics that have lost market protection (see sidebar "Product Protection"). There is a vital distinction between these classes; not all highly similar products will be "interchangeable," but all "interchangeable" products are "highly similar" to the product cited in their application. An interchangeable follow-on biologic can be substituted for the original product by a pharmacist, thus increasing potential market usage. A product that is similar is deemed to have the same clinical effect but must be prescribed in its own right. This distinction is necessary because of the complexity of biological products, the potential for different immune responses, and other side effects which are extremely difficult to characterize through analytical methods or *in vivo* testing. In other strongly regulated markets such as Europe, Australia, Canada, and Japan, the same principles have been enacted for some time (1–4). The European market, in particular, has extensive experience with the two-tier approach to similarity.

IMITATORS

While markets with strong regulatory oversight have been struggling with the definitions and approval routes for biosimilars, markets that are not as well-regulated have seen the introduction of imitator products. These products are intended to mimic originator products, but because of differences in quality and composition, they cannot, in general, be considered similar to the originator (5). Any claim of high similarity by many of these products is likely to be disputed by the more regulated markets (6).

BIOBETTERS

The final class of follow-on biologics is not intended to be similar to the original products; it is are intended to be better. Biobetters, also known as biosuperiors, are improvements on original biological products that provide enhanced safety, efficacy, or dosing regime. As a result of chemical modification, protein fusion, altered

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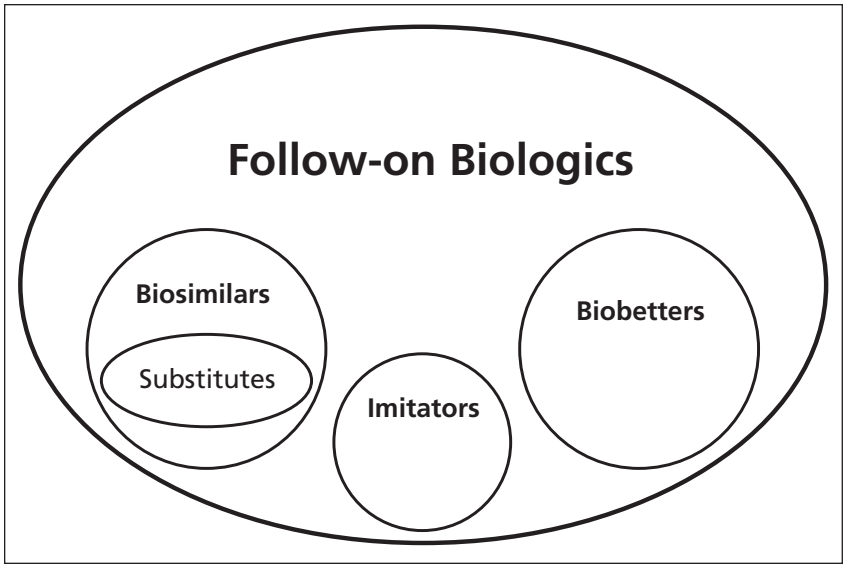
Figure 1: Follow-on biologics fall into the biosimilar (highly similar), imitator, or biobetter classes. Within biosimilars, there is a subset of products that can be substituted for the originator product (i.e., they are interchangeable).



amino acid sequence, or humanization of the glycosylation pattern (see Table I), the biobetter aims for the same target as the original biological, but has its effect on that target for a longer period of time, typically at lower doses and with fewer side effects. To license a biobetter, existing regulatory pathways for a new product must be followed. That is, a unique portfolio of quality, safety, and clinical efficacy data must be generated.

CAN BIOSIMILARS GENERATE PROFITS?

The development costs of a biosimilar product that meets the requirements of a strongly regulated market have been estimated by those developing such products to be $75–250 million and by the Federal Trade Commission to be $100–200 million (7,8). When considering the global registration of a biosimilar, one variable still to be assessed in these estimates is whether clinical trials will be required in each regulatory market. At present, the EMA has requested testing against a locally approved, European-labeled reference product and there are signs that FDA will require testing against a US-approved and labeled reference product. Duplication of the pivotal clinical trials is thus a possibility. In the US, the extent of data required to prove that a product is "interchangeable" is undefined, but it can be expected to be significantly greater than the requirements for a claim of "highly similar" to the reference product. With this high entry cost, a biosimilar will only be a viable prospect when the potential sales of that product exceed $250 million per year (9).

Data from European experience with biosimilars can be examined to investigate the probable returns on the level of investment needed to bring a biosimilar to market. In Europe, a biosimilar is approved through the central process common to all member states, then each country decides whether the product may be substituted for the reference product and at what level that substitution may occur. Table II provides data for biosimilar market penetration in Europe, by country, in the second quarter of 2009. At this time, biosimilar human growth hormones (HGH) had been approved for three years, biosimilar epoetins (EPO) for two years, and biosimilar filgrastims (G-CSF) for less than nine months.

The relatively high penetration of G-CSF in a short period of time suggests that increased familiarity with biosimilars in the European market is improving uptake, but it could also reflect differing substitution concerns. What is noticeable is that the originator product maintains a significant market share in the face of biosimilar competition. There is no evidence of the dramatic erosion of sales seen in the patent cliffs of small-molecule originators.

The second part of the picture for biosimilar sales is the number of entrants to the market and the period over which they launch products. With no equivalent of the Waxman–Hatch 180-day exclusivity period for the first generic competitor, the European experience has been that several biosimilar products enter the market at the same time. While this has in part been influenced by the timing of the development of the European biosimilar guidelines, the situation is likely to be mimicked in the US as guidelines are issued or market exclusivity or patent periods expire. Figure 2 shows the situation in Germany following the approval of the EPO biosimilars Binocrit and Mircera on Sept. 28, 2007, and Silapo and Retacrit on Dec. 18, 2007. Although overall sales value for biosimilar EPO products has increased since 2008, the split between the manufacturers has been maintained.

Based on a European market of approximately $600 million for EPO, extrapolation of these figures would likely give a biosimilar uptake of $185 million, with the single biggest seller obtaining European sales of $100 million. These figures are theoretical; for an example of real sales figures, Sandoz, which has been the leader in the biosimlar business since launching Omnitrope (HGH) in Europe in 2006 and in the US in 2007, achieved combined global sales of the biosimilars Omnitrope, Binocrit (EPO), and Zarzio (G-CSF) of $185 million in 2010 (12).

The key factor in these calculations is the uptake per country, which is governed by the substitution policy. Including the potential US market, which is approximately 1.5 times that of Europe, achieving a sales target of $250 million per year for a biosimilar will only be possible if a product is granted interchangeable status with the originator in the US market. The requirements for interchangeable status have yet to be set but they can be expected to be very challenging.

A BIOBETTER APPROACH

In contrast to the regulations for biosimilars, the route to approval for a biobetter is clear. A full biological licence application (BLA) will be required in the US, and the equivalent procedure will be required in other markets. The cost of bringing a new product through the BLA process has been estimated at $1.24 billion (13). This figure, however, incorporates a 30% success rate for molecules entering the process. With biobetters, it is arguable that the success rate will be significantly higher because the target and efficacy of the originator product are known. Thus, failures during the development period will be due to unwanted side effects, as opposed to lack of efficacy. The average development costs for a biobetter will therefore be closer to the estimated direct costs for a single product of $375 million.

Table I: Routes to biobetters and their supporters.

Improvement	How achieved	Example of companies supporting the approach
Humanization	Use human cell lines to express proteins with human glycosylation patterns	Crucell (PER.C6) Cevec (CAP) Glycotype (GlycoExpress)
Chemical modification	Attachment of polyethelene glycol (PEG) to the protein to extend serum half-life	Amgen's Neulasta is a pegylated form of their own Neupogen
Fusion proteins	Addition of albumin or other protein moieties to extend serum half-life	CoGenensys purchased by Teva in 2008
Altered amino acid sequence	Engineering antibody Fc domains for improved effector function, affnity or half-life	Xenocor Medimmune
	Addition of *C*-terminal peptide to enhance half-life	Prolor Biotech

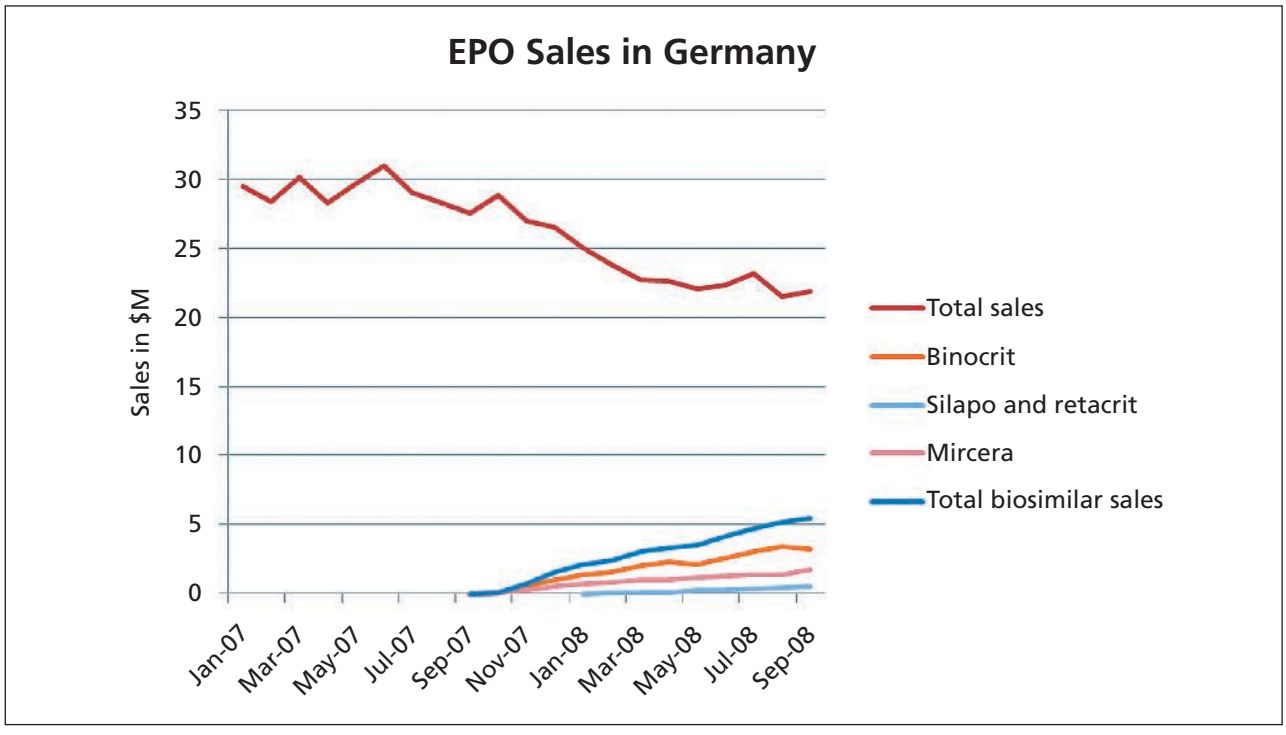
Table II: Market share value for biosimilars in Europe (10). HGF is human growth hormone, EPO is epoetin, and G-CSF is granulocyte colony stimulating factor.

	HGH	EPO	G-CSF
France	12.5%	2.0%	3.8%
Germany	5.1%	52.1%	31.0%
Italy	15.3%	0.2%	18.5%
Spain	1.4%	1.6%	7.1%
United Kingdom	1.0%	0.9%	24.0%

As each modified molecule progresses through the development process, more is learned about the effect of the modifications, and this knowledge can then be applied to the design of studies to test subsequent molecules. As a result, companies can further improve success rates by developing platform technologies. Using consistent platforms also reduces manufacturing design costs and can simplify the analytical characterization program. A typical example of the platform approach is the carboxy terminal peptide attachment developed by Washington University in St Louis, MO. Merck brought a follicle-stimulating hormone (FSH) modified in this way to the European market as Elonva. Prolor Biotech is using the technology as well, to develop modified HGH (Phase I completed), Interferon (preclinical), Factors VII and IX (preclinical), EPO (preclinical) and other therapeutic peptides. The platform approach is also readily applicable to antibodies. MedImmune and Xencor are among those modifying amino acid sequences in the Fc domain to develop biobetter versions of existing monoclonal treatments.

Platform approaches also allow smaller companies to enter the market. By developing and protecting the technology of the modification, such companies can enter partnerships with larger organizations that have the necessary production, distribution,

Figure 2: Epoetin (EPO) sales in Germany following approval of the first biosimilars (data, ref. 11).



and sales capability, rather than funding the full development package themselves.

If the costs of bringing a biobetter to market are two- to fourfold higher than those of a biosimilar, what are the advantages? The most obvious is that the product will be new, and therefore subject to 12 years of market exclusivity in the US. In Europe and other markets, exclusivity is more closely aligned with patent protection. The data from the European experience of biosimilar competition presented above suggest that biosimilar competition will be weak, and thus a long period of exclusivity may not be as necessary for profit generation as would be expected. The benefits to the license holder will be earned by the nature of the product; its improved characteristics must be used to attract patients and increase sales. While the biosimilar aims to take market share by being slightly cheaper than the originator, the biobetter has to gain market share on merit alone. Sales presentation of a newly approved biobetter will thus extol the benefits of the product rather than relying on price alone to drive business.

HEALTHCARE COSTS

What are the potential benefits to patients? The main intention of the BPCIA was to drive down costs of biologics to the healthcare system, recognizing that biologics will form an increasing component of the drug expenditures and have high relative treatment costs compared with traditional small molecules. The Federal Trade Commission report estimated that the introduction of biosimilars represented a potential savings of 10%–30% (8). Given the European experience of biosimilar uptake and the effect of biosimilar introduction on originator price, the overall impact on the drugs bill for a particular indication where biosimilar treatment becomes available is likely to be a 10–20% reduction (8).

A biobetter will command a premium price for its improved characteristics, but the improved dosing regimes common to such products can result in significant cost savings for the treatment of a particular indication. For example, based on the treatment used in a pivotal trial for Neulasta—an Amgen biobetter version of their own Neupogen—a single treatment cycle costs $3400 for Neulasta and $6000 for Neupogen, despite the unit prices being $3400 and approximately $300 (body weight dependent), respectively (14). The biobetter in this case represents a 40% reduction in overall costs for the healthcare provider.

THE FUTURE BIOLOGICS MARKET

The global biologic market will grow—of that we can be certain—but what form will the market take? Globally, imitator

products will continue to generate significant sales volume, if not value, in the less regulated markets. Some manufacturers of these products may be able to develop sufficiently detailed quality, safety, and efficacy data packages to convert the imitator to biosimilar status in the regulated markets. With established production facilities and sales generated from the imitator product, the additional investment to bring the product up to biosimilar status may be justified by the returns from biosimilar sales. Direct entry to the regulated markets as a biosimilar is less likely to be economically viable.

Biobetters offer the opportunity to establish brand-to-brand competition within treatment indications. Where in the past the identification of a mode of action has prompted the generation of multiple analogues, or "me-too" products, such as Lipitor, Crestor, Zocor, and Lipostat in the statin market, the identification of a target receptor by the originator product will spur the creation of biobetters. With many possible routes of modification, several companies will be able to target the same indication with novel products and thus create competition within the market.

New targets will continue to be identified, and the first product to be marketed for an indication may be more sophisticated than a simple copy of the natural human protein. As platform technologies are established, partnerships between innovators and the owners of the platforms will grow.

SUMMARY

The follow-on biologics area appears crowded with product divisions dependent on what seem to be slightly different regulatory definitions. Much attention has gone to biosimilars as the class with the potential to reduce healthcare costs through increased competition. With the experience gained from the introduction of these products in Europe and the conservative approach to regulation in the US, it is unlikely that biosimilars will meet this hope. However, biobetters represent an opportunity to be innovative with reduced risk and increased sales for the manufacturer while improving the treatment of patients and reducing healthcare costs. Why be similar, when you can be better?

Product protection

The patent system exists worldwide as a mechanism for registering a novel idea or design to prevent others from using that intellectual property without paying the inventor. Typically, patents last 20 years and can cover the structure of a molecule, the design of a process used to produce that molecule, or a device for delivering the dose to the patient.

It is possible to challenge a patent in terms of obviousness (i.e. no leap of logic was required to arrive at the patented item), or because earlier patents that cover the same idea already exist. Therefore, a patent is not an absolute guarantee that the product or process cannot be copied, even within the nominal lifetime of that patent.

Also available to the originator of a pharmaceutical product is exclusivity—the interval between the approval to market the product and the time at which a competitor product may be approved by the same authority (i.e., branded exclusivity) or at which a competitor product may be submitted on the basis of safety data for the original product (i.e., data exclusivity). A product may have exclusivity without having a patent. For instance, additional periods of exclusivity are available to drug producers who conduct trials in children to ensure correct dosing (pediatric exclusivity) or who develop drugs to treat orphan diseases where the drug will not have high sales volumes.

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Development and Commercialization of Biosimilars in India

Anurag S. Rathore

India is expected to be a biosimilar powerhouse. The author discusses the reality of that promise.

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Biosimilars can be defined as biotech drugs that have been shown to have comparable quality, safety, and efficacy to the original product. Scientific and regulatory issues around approval of biosimilars have been a topic of great interest and debate lately in Europe and in the United States. A key concren is industry's limited understanding of how the different quality attributes (QA) of a biotech product affect its safety and efficacy. India is globally regarded to have great potential to become a significant player in the development and commercialization of biosimilars. This article, the 25th in the "Elements of Biopharmaceutical Production" series, aims to present the current status of India in this context, the challenges that need to be overcome, and some recommendations that may alleviate these challenges.

GLOBAL SALES PROJECTION FOR BIOSIMILARS

Patents of many top-selling biopharmaceuticals are set to expire (see Figure 1) (1). Expiries will begin in 2012 with the Enbrel (Amgen/Pfizer) patent and continue through 2017.

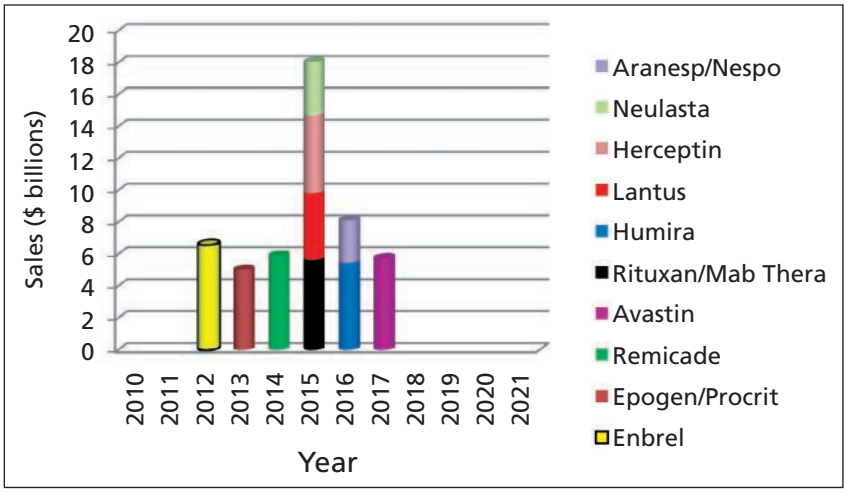
The peak will occur in 2015 when four products—Neulasta (Amgen), Herceptin (Roche), Lantus (Sanofi-Aventis), and Rituxan/MabThera (Roche)—face patent expiration. Sales of biosimilars will be primarily driven by sales in the developed countries (i.e., US and Europe) with the US constituting approximately 90% of the global market.

A regulatory pathway for approving bisimilars is already in place in Europe—14 such products have been approved to date (1). The pathway in the US, although approved under the Biologics Price Competition and Innovation Act of 2009, is still being implemented. In Japan, although a regulatory guidance for biosimilar approval was issued in 2009, the slow adoption of small-molecule generic drugs suggests that the approval of biosimilars is likely to begin later than in the US.

THE ECONOMICS OF BIOSIMILARS

Although the financial drivers for growth of biosimilars are widely recognized to be significant, there are some key aspects that distinguish biosimilars from the small-molecule generic drug market. Performance of biosimilars in Germany, the largest biosimilar market at present, shows that the substitution rate for biosimilar epoteins is about 35% of the total sales of epoetin. This percentage is quite different from the >90% rates that are commonly achieved for small-molecule generics (2). This difference is partly due to the observation that patients taking biotech therapeutics show an aversion towards switching to the corresponding biosimilars (3).

Figure 1: Upcoming patent expirations of 10 top-selling biopharmaceutical products (Data, Ref. 1).



Development and commercialization of a biosimilar requires anywhere from $10–40 million compared with the $1–2 million typicaly required for a conventional small-molecule generic drug. Furthermore, biosimilars are expected to be priced at only a 20–25% discount in comparison with their original products, a significantly smaller discount than what is common with small-molecule generic drugs.

Overall, the effect on overall drug pricing will be much more limited with biosimilars than with small-molecule generics. Patient behavior is also likely to result in slower adoption.

MANUFACTURERS AND CLINICAL RESEARCH ORGANIZATIONS

More than 30 biopharmaceutical companies and clinical research organizations are based in India (see sidebars on companies and contract organizations) (4, 5). The biopharmaceutical sector's estimated value was $1.9 billion in 2009–2010, and accounted for three-fifths of the approximately $3-billion in revenues of the biotech industry in India as a whole. More optimistic projections for sales revenues of biosimilars entering the US and Europe have been estimated as high as $21 billion for the next six to seven years. India is also emerging as a hub for conducting global clinical trials with its share going from 2% at present to 5% by 2012, based on low cost, large and diversified patient pools, easy recruitment, strong government support, availability of specialized doctors and trained investigators, and a gradual strengthening of the IP environment (5, 6).

The clinical research organization market has increased from $5 million in 2005 to $71 million in 2006, and reached $1 billion in 2010 with 50,000 clinical research professionals and 400 clinical trials involving 100,000 patients at 300 sites (7).

Indian companies have gradually advanced from making simpler biotech products, such as the granulocyte colony stimulating factor to manufacturing more complex biotech products, such as monoclonal antibodies (see Table I) (8). Dr. Reddy's has brought biosimilar version of Roche's Rituximab to market.

Overall, Indian companies have demonstrated that there is

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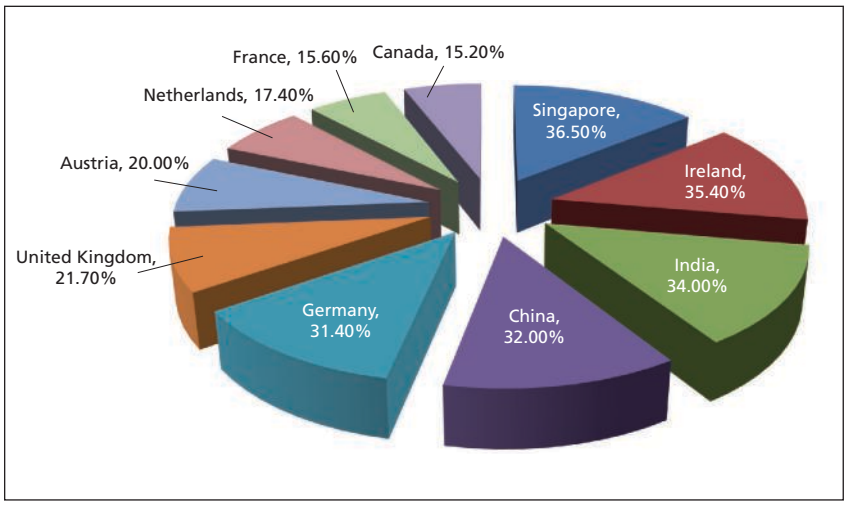
Figure 2: Countries that US firms are considering as potential outsourcing destinations. (Data, Ref. 9).



no dearth of technical competence as far as development and manufacturing of biotech products is concerned.

COMPETITION AND GLOBAL EXPECTATIONS

A recent survey focused on countries that US firms are considering for outsourcing (see Figure 2). Results showed that Singapore, Ireland and India are the top choices, followed closely by China and Germany. The competition for leadership in development and commercialization will be intense among the more established players (i.e., Germany, Singapore, and Ireland) as well as the emerging ones (i.e., India and China).

The developed countries (i.e., US, Europe, Japan) have a long history of manufacturing biotech products for regulated markets, but the developing countries have the key advantage of an availabile, qualified ,and relatively low-priced workforce. However, more is needed for the latter group to completely embrace the principles and expectations with respect to product quality and GMP compliance, and this will be a large obstacle on the path to these manufacturers emerging as major global players.

The complexity of the biosimilars market is further amplified by industry's inability to completely characterize biotech products through analytical means. As a result, there is limited understanding of how the biotech process affects the quality of the final product and how quality attributes affect clinical safety and efficacy of the product (10, 11).

In the current environment, any missteps by an Indian manufacturer may to result in a perception that the biosimilars manufactured in India (or any other developing country) are not of adequate quality. For example, the Swiss generic-drug maker, Acino, announced that it incurred a loss after European Union authorities required a recall of batches of its drug Clopidogrel, which had been formulated using an active ingredient from an Indian supplier (12). The European regulators ordered the recall after an inspection of the Indian firm led to the finding that it had compromised production records for Clopidogrel.

MOVING FORWARD

Much needs to be done for Indian manufacturers to graduate from being considered "manufacturers for the developing world" to the "manufacturers for the developed and the developing world."

Significant improvements have been made to the country's regulatory system. A systematic, science- and risk-based approach for review of regulatory filings and inspections has been put in place. However, there is a need to ensure greater transparency in the decision-making process so that the outcomes can be more consistent. The current system involves multiple organizations (e.g., the Department of Biotechnology, Central Drugs Standard Control Organization) and ministries of the government. A single point of decision-making and accountability will make the system more efficient, credible, and better coordinated, and will reduce confusion and delays that are otherwise suffered at times by the industry. In addition, basic training in areas such as GMP, documentation practices, scale up, technology transfer, and validation is needed by the industry and regulators.

Creation of modern technology platforms that can support the regulatory authorities in making appropriate decisions during the review and inspection process is needed. For example, approaches such as rapid analysis of a drug lot, fingerprinting, and use of chemometrics to quickly facilitate decision-making on product quality are already in practice by some of the major global regulatory agencies, and India could benefit from these technologies.

The Indian Pharmacopoeia Commission (IPC) is in the process of overhauling its monographs for biotech products. As

Table I: Major biosimilars being manufactured in India and their manufacturers (Data, Ref. 8).

Biosimilar products	Biosimilar manufacturers					
	Dr. Reddy's Lab	Intas Biopharmaceuticals	Shantha Biotech	Reliance Life Sciences	Wockardt Ltd.	Biocon Ltd.
Filgrastim	X	X		X		X
Rituximab	X					
Darbapoetin alfa	X					
PEGylated Filgrastim		X				
Human Interferon alpha-2b		X	X	X		
Erythropoietin		X	X	X	X	X
Streptokinase			X			X
Tissue plasminogen activator				X		
Insulin					X	X
Nimotuzumab						X

a creator of the minimum standards that a biotech drug must meet in order to be a commercial product, IPC can play a crucial role in contributing to the success of the Indian biotech industry by using a science-based and risk-based approach when creating these standards.

Professional organizations such as the Parenteral Drug Association (PDA) have been effective in the US in bringing the industry and regulators together in the form of various task forces to create best practices for industry and regulators. International trade organizations, such as the American Chemical Society (ACS) and International Society for Pharmaceutical Engineering (ISPE), also have been effective in the US by encouraging dialogue between academia and industry through conferences and other events.

India could benefit from similar academic–industry collaborations. The Small Business Innovation Research Initiative (SBIRI) from the Department of Biotechnology (DBT) is one step in this direction.

Quality by design (QbD) has gained significant momentum lately in the biotech industry with both regulators and the industry investing financial and staffing resources to implement the approach (13, 14). A QbD approach is likely to be expected of Indian manufacturers when they apply for FDA or EMA approval of biosimilars. The Indian biotech industry should see this as an opportunity to innovate and come up with more efficient ways of product development and commercialization without compromising product quality and consistency.

Major biopharmaceutical companies in India

Bharat Biotech
Bharat Serums and Vaccines
Biocon
Biological Evans
Cadila Healthcare
Dr. Reddy's Laboratories
Glaxo SmithKline
Haffkine Biopharmaceuticals
Indian Immunologicals
Intas Biopharmaceuticals
Panacea Biotech
Reliance Life Sciences
Serum Institute of India
Shantha Biotech
Syngene International
Wockhardt
Zydus Cadila Healthcare

Major clinical research organizations in India

Accutest
AceBimed
Asian Clinical Trials
Atemis
Clinigene International
Fortis Clinical Research Services
GVKBio
iGATE Clinical Research International
Lambada
Lotus
Manipal Accunova
Medicity
Neeman Medical International
Vimta
Wellquest

CONCLUSION

In summary, significant challenges lie ahead for the Indian biotech industry. The government, industry, academia, and regulators need to work together to surmount them. The business case for doing so exists as demonstrated herein. It remains to be seen whether India will capitalize on its strengths and emerge as a dominant global manufacturer of biosimilars.

> The complexity of biosimilars market is further amplified by industry's inability to completely characterize biotech products

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Integrity Testing of Flexible Containers

Vishwas Pethe, Mike Dove, and Alex Terentiev

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Defects as small as 10 µm can be detected without compromising product cleanliness using helium integrity testing.

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In the biopharmaceutical industry, flexible containers such as plastic bags or liners are often used for bulk intermediate storage, cell culture resuspension, viral inactivation, final formulation, final fill, or as bioreactors. In such applications, the bag is hermetically sealed and sterilized. Sterility of the bag must be maintained to avoid contamination of the product. Any breach of the sterile condition is considered a serious risk and often results in disposal of valuable product, sometimes after significant cost and effort has been expended in the course of making it.

At the moment, disparities exist between defect sizes that are readily detectable using current on-line technology, and the speculated value for the critical defect size (e.g., the defect size at which sterility of a package is lost). Lampi established, and Chen and Keller independantly substantiated, that the critical defect dimension for bacterial penetration for flexible bags is 11 µm or less, while Gilchrist determined that the dimension was nearly twice that: 22 µm (1–4). Blakistone later established that critical defect size was 7 µm (5). Discrepancies in the critical defect size could be attributed to differences in the bio-test methodology, concentrations of test microbes, test times, or positive/negative pressure

in the test bag. Therefore, one of the objectives of this paper is to determine the critical defect dimension at which sterility breach occurs in biopharmaceutical containers. Such information would provide a foundationfor avoiding inherently problematic conditions, as well as for empirically evaluating and tailoring leak detection equipment for their specific needs.

The package's integrity controls microbial ingress into the package and thereby preserves product sterility. The current technologies employed in the biopharmaceutical packaging industry (e.g., vacuum bubble test, dye-penetration test, pressure-decay test, constrained-plate pressure-decay test) are only capable of reliably detecting leak rates in the range of 10^{-2} or 10^{-4} cc/s, which is equivalent to detecting defect sizes in the scale of 90–500 µm. Thus, industry needs a method of testing the integrity of a flexible container that detects defects corresponding to the minimum size needed to block water-borne microbes. This article describes a new test methodology, called helium integrity testing (HIT), that can detect such defects.

Figure 1: Helium integrity test (HIT) apparatus.

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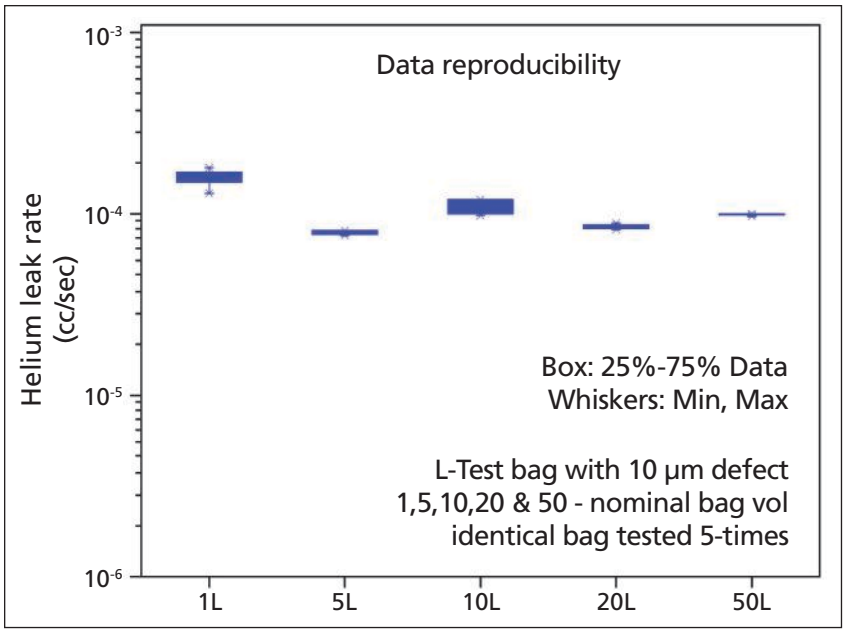
METHODS AND MATERIALS

The HIT apparatus includes the following main components (see Figure 1):

Figure 2: Data reproducibility of HIT system.



- Pressurized helium supply (not shown)
- Test chamber
- Spacer unit
- Vacuum pumps for bag evacuation and test chamber evacuation
- Process-control station
- Detector: Varian mass spectrometer.

Leak test method

The HIT apparatus is composed of a test chamber with a closable lid that when closed, forms a tight seal such that no helium can enter into the test chamber from outside. The test unit is a flexible two-dimensional bag with connecting ports that enable fluid communication with the helium source and vacuum pump. The test chamber is large enough to house the unit to be tested. The test chamber is connected to a vacuum pumping group equipped with the tracer-gas detector for chamber evacuation and gas detection. A second vacuum group is used to evacuate the unit under test before filling it with gas. A tracer-gas filling device (i.e., helium supply) completes the testing apparatus. The unit to be tested is put into the vacuum chamber and connected to service hoses, then the vacuum chamber and the unit are evacuated. During chamber evacuation, the test unit is pressurized with the tracer gas. After a stabilization time, the detector is linked to the vacuum line to detect the tracer gas flow through a leak and drawn in by the pumping group. This method can be made fully automatic, so it depends little on an operator. Its sensitivity can reach < 10^{-10} cc/s flow rates. To prevent long down times, the HIT system employs an in-line pressure-sensing test method as a preliminary leak test that detects gross leaks before the final automatic leak-test operation using a tracer gas is begun. This approach prevents large quantities of tracer gas from leaking into the atmosphere. A specialized pumping technique reduces the stress on the test unit by reducing the internal pressure of the test part along with the external (i.e., chamber) pressure.

To improve test time per test unit, the test chamber was modified to include spacers that allow the simultaneous testing of four units. The spacers are held upright by connecting rods, and each spacer has enough cavities in it such that when a test bag inflates against it, it does not block the path of helium molecules flowing through the defect. The spacers constrain the test bag in the test chamber, further increasing the helium pressure in the bag and resulting in increased sensitivity of leak detection. The spacers also ensure that test bags experience minimal stress during chamber evacuation by limiting bag expansion.

Test bags

The test bags were prepared by welding two sheets made from ATMI's proprietary TK8 film. To create defective bags, one of the two sheets was modified to incorporate a defective patch. A defective patch is a piece of TK8 film (4 in. x 4 in.), with a 10 µm ± 1 µm hole drilled by a laser. The laser-drilled holes were validated for defect size by measuring flow rate through the defect area. Hereafter, "defective bags" implies a test bag with a 10 µm defect, and "good bags" implies bags with no defect.

The size of the test bag depends on the size of the two sheets used in making one. The nominal volumes of test bags in this study were 1, 5, 10, 20, and 50 L. The helium leak rates through defective bags and good bags measured using HIT technology are discussed below. The test bags post-HIT tests were further characterized for product performance such as liquid particle count (*USP* <788>) to ensure

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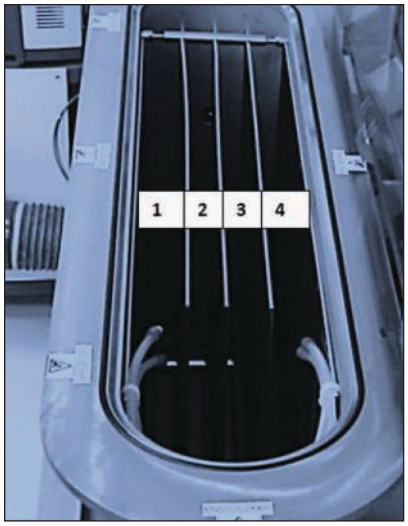
Figure 3: Test chamber with spacer, illustrating test bag positioning.



that the HIT process did not affect product performance.

Microbiological challenge test method

In this study, test bags for microbial challenge were prepared by thermally welding TK8 film sheets having defects of specific sizes (2, 5, 10, 15, 20, and 50 µm). The test bags were aseptically filled with 50 mL microbial growth medium (trypticase soy broth). The outside of the test bag was sprayed with a 0.9% saline solution containing approximately 10^6 CFU/mL of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus spizizenii* and approximately 10^5 CFU/mL of *Candida albicans* and *Aspergillus brasiliensis*. The test bags were then transferred to an incubator maintained at 30–35 °C and monitored for 15 d. The growth medium inside the test bag was periodically checked for microbial growth, which indicates microbial ingress.

Liquid particle count (LPC) test

A test bag was filled with ultrapure water (deionized water filtered using a 0.05 µm filter) and gently shaken to ensure that all the bag surfaces came into contact with the solution. A sample of the solution from the test bag was then passed through particle measuring equipment (PMS). The instrument reports the number of particles per mL of solution with particle sizes greater than 25 and 10 µm.

RESULTS AND DISCUSSION

Microbiological challenge test

While immersion biotesting has long been used to challenge packages, particularly cans, for pinholes and channel leaks, they are not the real conditions that a package generally encounters during its use. Hence, package integrity-evaluation methods that employ bioaerosols that simulate the conditions that the package will be expected to tolerate during storage and distribution are more relevant and are gaining prominence. Test bags with defects between 2 and 50 µm were exposed to a microbial environment and served as test sample. The test bags not exposed to bacteria served as negative control for the aseptic filling. For each organism, a bag containing no defect was injected with 0.1 mL of a 10^3 CFU/mL solution of the organism to serve as positive control. The positive controls exhibited growth after 1 d, thus validating the test conditions for detecting microbial ingress.

As expected, under the conditions of the test, microbial ingress into a package took longer as the defect size got smaller. Test bags with defect sizes of 50 and 20 µm took the same time (i.e., 5 d) to allow microbial growth, meaning that a clear channel for microbial ingress was already established at 20 µm. However, when the defect size was 15 µm, it took 14 d to show any microbial growth (see Table I). A significant slowdown in the microbial ingress at 15 µm and complete cessation of microbial ingress at 10 µm or smaller defect sizes are interesting, considering that microbial organisms are much smaller than 10 µm and should infiltrate through 10 µm defects just as easily as they did through 20 µm defects. The logical explanation for this observation lies in the threshold pressure inside the test bag (1). To initiate microbial ingress through a defect, the pressure inside the test bag (i.e., threshold pressure) must overcome the force of the liquid surface tension and initiate liquid flow through the defect, thus providing a channel for microbes to travel into the bag. The magnitude of threshold pressure required to initiate liquid flow depends on the location of the defect due to differences in the static head pressures. As defect size decreases, the threshold pressure for a given liquid increases. Thus, in test bags with 10 µm defects, the threshold pressure is lower than the force of the liquid surface tension, preventing the formation of a channel through which microbes can travel. One other reason offered in the literature for this behavior is the formation of a biofilm on the film surface, which prevents microbial ingress.

Although additional studies may be required to confirm the root cause for the lack of penetration of microbes through a 10 µm defect, these results are in agreement with researchers in the food packaging industry such as Lampi and Chen, who have shown the critical dimension for microbial ingress to be about 10 µm (2, 3). The differences in the critical defect size for microbial ingress between different studies could be due to

differences in the test methods employed and the contact materials that affect the surface tension of the liquid. Based on these results, it is clear that defects larger than 10 µm cause sterility breach. Therefore, integrity testing for on-line package testing must detect 10 µm defects to ensure product sterility.

Leak detection data

In an ideal world, the helium in the test background would be nonexistent, and a good bag would not leak helium at all. On the other hand, a defective bag would leak a definite amount helium, that would be detected, thus resulting in distinct separation of helium leak rates for defective bags versus those for good bags. This f ideal behavior would result in a high degree of resolution, allowing existing leak-detection methods to be used to detect bag defects.

However, the walls of flexible bags are often made of polymeric materials, which are intrinsically permeable to gases. Helium gas has a smaller molecular size and permeates faster through polymeric materials than air or nitrogen. Thus, even a good test bag can leak a significant amount of helium by diffusion through the bag walls. This diffused helium creates high helium background levels in the test chamber, thus making it difficult to quantify actual helium leaks through the defects in the test bag. The high helium background essentially masks the helium leaking from defects, limiting the lowest leak rate that can be reliably measured. HIT testing ensures that helium flowing through the defects is maximized, while the background helium concentration is minimized. The test time was kept as short as possible to prevent elevation in the helium background as the test progressed.

Figure 4: Effect of test bag position in the test chamber on the helium leak rate.

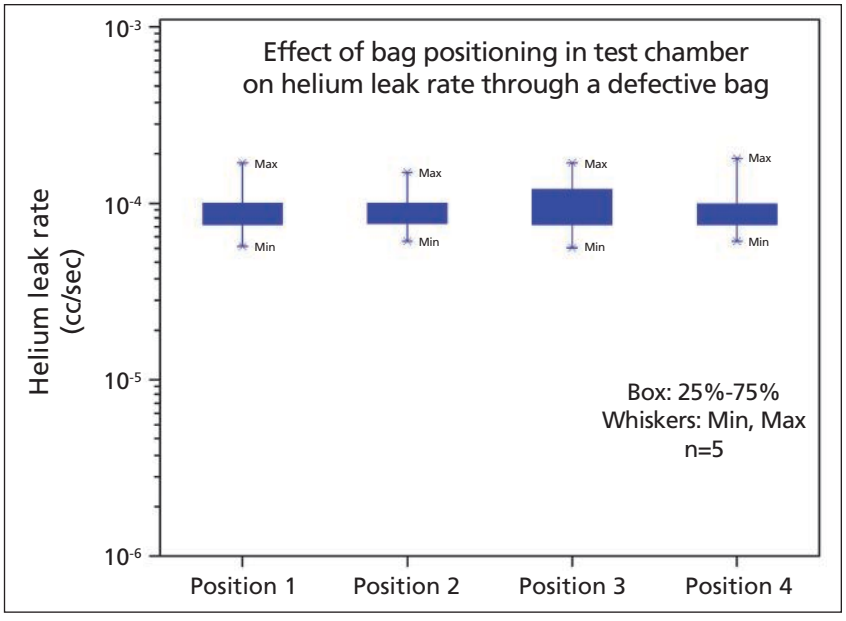
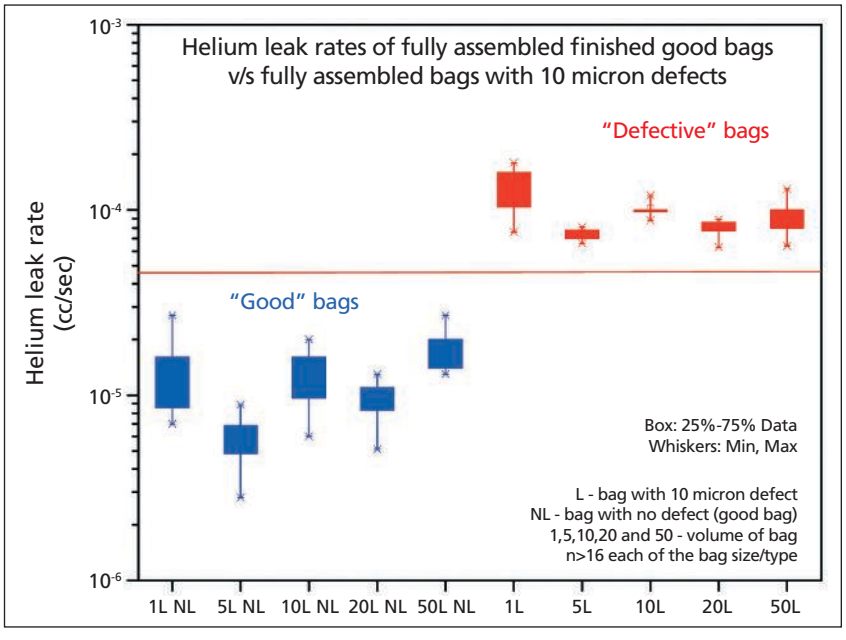


Figure 5: Box plot of helium leak rates (cc/sec) of good bags and defective bags.



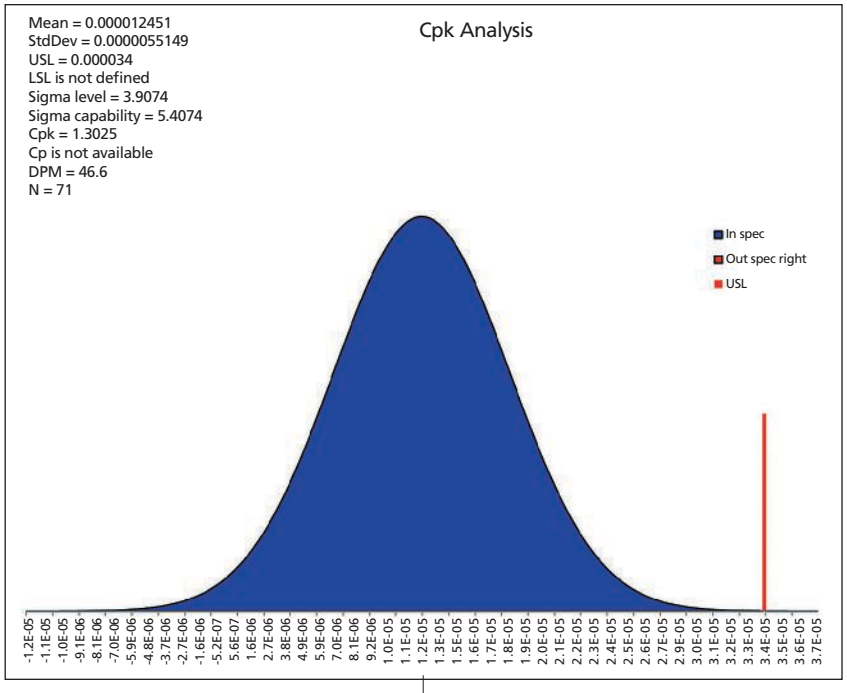
Data reproducibility

Often, even when a constant amount of helium flows through a defect, the ability of the pumping system to carry helium molecules to the detector can vary. In addition, the helium background varies as the equipment operates over a period of 8 h, which may result in variation of the measured leak rate for the same defect size. To minimize this variation, the volume of the test chamber external to the test bag was minimized and the pump-

Table I: Microbial challenge test data.

Defect size	Microbial growth	Negative control (not exposed)
50 µm	Growth on day 5	No growth
20 µm	Growth on day 5	No growth
15 µm	Growth on day 14	No growth
10 µm	No growth	No growth
5 µm	No growth	No growth
2 µm	No growth	No growth

Figure 6: Distribution curve for helium leak rate through good bags. The curve shows a good probabliity that good baks will have a leak rate lower than the baseline leak rate.



ing efficiency of the vacuum pumps was optimized. A single defective bag from each of the bag sizes (i.e., 1–50 L), was tested five times to verify the reproducibility of the leak rate through a defect (see Figure 2). The data show a standard deviation of less than 4% for leak rate in all bag sizes tested, except for 1-L bags, which had slightly greater variation at 10%. The higher variation in 1-L bags was due to the relatively high ratio of detector gas to test bag volume, resulting in higher sensitivity input parameters such as tracer gas fill volume and helium background. The box plot of the measured leak rates also indicates that the average leak rate for all defective bags, irrespective of the bag size is above a certain leak rate.

Effect of test bag position

The test chamber includes a spacer, which splits the test chamber space into four compartments that can each accommodate one test bag. Of the four compartments, the two compartments closer to the test-chamber wall are designated as positions 1 and 4, and the remaining two are designated as position 2 and 3 (see Figure 3).

During the leak-testing process, as the bag walls push against the container wall, some defects may be blocked by the container wall, thus reducing or eliminating helium flowing through the defects and, in turn, preventing its detection. To minimize this risk, spacer bars were placed between the walls of the test bag and container wall to provide a path for the tracer gas to escape. Experimental studies were performed to ensure that there was minimal variability in the amount of helium flowing through the defects due to the position of the bag in the chamber. Experiments were conducted with test bags placed in locations 1 through 4. For each location, five distinct sets of defective test bags per bag size were tested for helium leaks. The box plots showing the leak rates from bags at different positions indicates that there was minimal variation and that the measured leak rates were above a certain leak rate (see Figure 4). A *t*-test comparing the means of leak rates confirmed with greater than 86% confidence that no significant difference would be observed in the mean of the helium leak rates. Thus, irrespective of the bag position in the test chamber, helium leaks through a defective test bag reach the detector without appreciable loss in the process.

Validation of HIT technology for 10 µm defect detection

A series of experiments was performed to validate that the HIT technology could detect 10 µm defects. In the first set of experiments, good test bags of all sizes (i.e., 1–50 L) were run through the tester, the helium leak rates

Table II: Liquid particle count results (LPC/ml) in 1 L bags.

Sample #	LPC/ml, 10 µm		LPC/ml , 25 µm	
	Test sample	Control	Test sample	Control
1	0.88	0.33	1.11	0.11
2	0.88	0.55	1.11	0.55
3	1.33	0.33	0	0

were recorded, and a baseline leak rate was established. The defective test bags were then tested, and the leak rate through the defective bags was compared with the baseline leak rate. The sequence of testing defective bags versus good bags, smallest bag versus large bag was randomized to avoid trending issues. The helium leak rate through defective bags must be substantially higher than the maximum helium leak rate through good bags to detect 10 µm defects.

The good test bags averaged a helium leak rate of 1.17 x 10^{-5} cc/s, while the defective bags averaged 8.94 x 10^{-5} cc/s. The variation in the leak rates through good bags was largely due to variation in the helium background, while the variation in defective bag leak rates was largely due to variation in the laser-drilled defect hole sizes. The maximum leak rate, also referred to as baseline leak rate, is the sum of the average leak rate of good bags and four times the associated standard deviation. The baseline leak rate varied with bag size but for further evaluation, the baseline leak rate is the maximum leak rate possible for good test bags irrespective of bag size, 3.4 x 10^{-5} cc/s (see Figure 6). This meant that if the helium leak rate was higher than the baseline leak rate, there was a defect in the test bag, and the bag would be rejected. The helium leak rate value for all the defective bags tested was higher than the baseline leak rate value, allowing easy distinction between the good and the defective bags (see Figure 5).

A statistical analysis (*t*-test for means) of leak-rate distributions of defective bags versus good bags indicated clear separation ($p < 0.1$) of leak rates.

Liquid particle count

While methods using tracer gas have come close to detecting 10 µm defects, they have failed to maintain cleanliness of the test unit. Often such techniques involved connecting the test unit to the tracer-gas supply, evacuation pumps, which can be a source of particle generation. In addition, when the test bags are filled with tracer gas, they are pressurized to a great extent, resulting in film stretching and causing particle shedding. It was the intent of this test to show that no significant increase in the liquid particulate level occurred due to the use of this technology. The ratio of liquid particles generated to the nominal bag volume was highest for 1-L bag because of its higher surface-area-to-volume ratio, making 1-L bags more sensitive to a change in particle concentration as a result of the leak testing process. Therefore, only 1-L bags were tested for verifying particulate generation. It is expected that if 1-L bags do not show a significant rise in the particle count, large bags will not show a significant rise either. A sample of solution from each of the 1-L test bags was run through particle measuring systems equipment, and the data is reported in Table II. The data did not show significant increase in the liquid particle count level indicating that HIT technology does not cause particulate generation and is safe for-point-of-use applications.

CONCLUSIONS

Existing integrity-test methods for flexible containers can detect defects in the range of 500–90 µm, but are inadequate for ensuring product sterility. A microbial ingress study conducted on flexible sterile containers revealed that defects as small as 15 µm can compromise sterility, while defects equal to or smaller than 10 µm did not. A novel helium integrity test method developed by ATMI is capable of detecting 10 µm defects during in-line package testing without compromising the cleanliness of the product. The test bags with 10 µm defects had helium leak rate value higher than baseline leak rate value allowing clear distinction between good bags and defective bags. The cleanliness testing post integrity testing revealed that product performance is not affected by the test process.

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Protein Engineering

Trevor J. Hallam and Christopher J. Murray

Industrializing design, development, and manufacturing of therapeutic proteins.

From an industrial R&D perspective, the design and development of protein therapeutics today appears somewhat akin to the rational design of small-molecule discovery back in the 1970s when lead compounds were generated from known physiological substrates or ligands. Facing a need to find novel and diverse small-molecule leads, attention in the 1980s centered on high-throughput screening (HTS) technologies and compound libraries. Those libraries, albeit large, were hardly diverse, with most therapeutic agents coming from a few target protein classes. Complementation of libraries with natural products, the development of combinatorial chemistry, and application of focused-library sets followed. This evolution, together with automated methods for content-rich assay systems and fast make-test cycles, enhanced discovery of novel, potent, and diverse lead series.

Contrast this with the present analagous processes for protein therapeutics: the discovery and development of novel biologics is hardly diverse, efficient or rapid. State-of-the art protein discovery and development use multiple expression hosts (e.g., mouse, *E.coli*, Chinese hamster ovary (CHO), and NS0) and several reformatting steps between hosts are often necessary during testing, scale-up, and production. The process of developing cell-based protein expression systems that are efficient, consistent, and scalable often is difficult and sometimes impossible using currently available technology.

To date, more than 150 protein drugs have been approved for clinical use, nearly all of which are produced in cell-based expression systems, such as *E. coli,* CHO cells, and *Saccharomyces cerevisiae* (*S. cerevisea).* These cell-based systems have several limitations, and many biologics can't be developed in these systems. For example, these systems only allow the overexpression of proteins that don't affect the physiology of the host cells. For many expression systems, identifying cell lines that stably synthesize high protein titers of the desired product is a time-consuming and labor-intensive process. Ideally, the same production host for rapid variant discovery, production for animal testing, and manufacturing of a clinical candidate would be used.

Ideally, one would want to emulate the huge leap made in iterative drug design seen in small-molecule discovery, namely, rapid make-test cycles and generation of multiple parallel libraries of drug candidates with diverse structural elements to optimize activity while maintaining feasibility for manufacture. An ideal system would do the following:

- Make fast make-test cycles a prerequisite for re-iterative design on the order of three to five days, similar to those for focused small-molecule libraries
- Create efficient and rapid expression and purification that allows for libraries of hundreds to thousands of protein-sequence variants to be simultaneously tested per make-test cycle using standard off-the-shelf robotics equipment
- Incorporate preferred sequences defined from selection technologies, such as ribosome or phage display, into whole protein therapeutics for testing
- Enhance the diversity of chemical structures by expanding the library of available amino acids at specifically targeted points in the protein sequence from 20 natural to many hundreds of non-natural amino-acids
- Optimize several properties (e.g., agonist or antagonist, affinity, stabil-

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ity, and predictive manufacturability) simultaneously through rapid high-throughput make-test cycles
- Create processes that are not only rapid but amenable to rapid scale-up and cGMP manufacturing once the desired therapeutic construct has been identified.

As ambitious as such a system would seem, several exciting technologies are emerging that improve expression systems and enhance diversity to enable modification of intrinsic properties of proteins, such as enzyme catalytic efficiency or binding. Others combine different properties in single therapeutics by conjugation chemistries. Further emerging technologies can lead to more rapid and parallel expression of many protein drug candidates. Getting all of these desirable technologies into a single amenable platform that has the flexibility to be scaled and support cGMP manufacturing is in sight.

ADVANCES IN DEVELOPMENT

Early improvements in endogenous protein-based therapeutics produced new, commercially successful therapeutics with desirable properties by simply extending sequence incorporating fusion to proteins such as the constant fragment of antibodies (Fc) or by PEGylating to increase half-life. Beyond these early approaches, considerable effort to produce ever-more elegant constructs that combine two separate functions have been made. One promising approach, antibody drug conjugates (ADCs), involves using a targeting antibody to known tissue selective cell-surface antigens or receptors to target conjugated toxins or cytotoxic drugs and so enhance selectivity over normal tissue.

Successful design of effective ADCS is complex and requires linking cytotoxic drug payloads to tumor-targeting antibody constructs. The selection of an ideal antigen target for optimal internalization and specificity for tumor tissues is critical. The design of linkers that are stable in circulation, but cleave when internalized in tumor cells to release the cytotoxic drug, adds to the complexity, but the other major technical hurdle has been to define how the cytotoxic payload with linker are conjugated to the targeting antibody. The biopharmaceutical companies Seattle Genetics and Immunogen have developed robust platforms that depend on conjugation of linkers and cytotoxic warheads to available cysteine or lysine residues, respectively, in the tumor targeting antibody sequence.

Despite successes with ADCs, there are many examples where seemingly optimized functional components (i.e., antigen-binding motif, linker, and drug-conjugate) do not translate into a developable therapeutic candidate. ADCs produced using conjugation chemistries to endogenous cysteines and lysines inevitably lead to the production of multiple species of the ADC with the drug conjugated in varying payloads of between one and nine molecules per immunoglobulin G (IgG). Furthermore, all sites of conjugation are not equal. Some conjugations interfere with antigen-binding epitopes, thereby reducing binding affinity and/or drug half-life (1). All too often, poor efficacy is revealed in the clinic only after significant investment in cell-based expression systems and scale-up.

SITE-DIRECTED CONJUGATIONS

Several technologies aim to provide chemically amenable sites within a protein sequence for the posttranslational chemical conjugation of small-molecule drugs, peptides, or other constructs to improve or add functionality. For example, sequence-specific conjugations producing homogeneous ADCs with fixed payloads are aimed at improving tumor-cell killing and increasing therapeutic index. Engineered ThioMabs (Roche/Genentech technology) that use natural cysteine residues that must be carefully unmasked during production for subsequent site-specific conjugation have shown preclinical proof-of-concept (2). These approaches await further clinical validation.

Carlos Barbas' laboratory at The Scripps Research Institute exploited the use of exposed tyrosine residues within the complimentarity determining regions (CDRs) of IgG molecules as the basis for linking drug conjugates. CovX, acquired by Pfizer in 2008, was founded to develop this technology. These sorts of approaches are attractive in that posttranslational chemical coupling to a common IgG construct with resulting extended half-life represents a platform amenable to many different small-molecule or peptide agonists.

NON-NATURAL AMINO ACIDS

The introduction of non-natural amino acids (nnAAs), or those amino acids not part of the 20 naturally incorporated ones into proteins, plays an important role in basic peptide and protein research. They are increasingly used to develop biologics with enhanced pharmacological properties beyond providing sites for drug conjugation. NnAAs can be introduced through chemical synthesis in peptides or biosynthetically in proteins. Currently, only peptides and very small protein drugs with nnAAs are on the market because they can be made synthetically and avoid the limitations of cell-based expression systems. A prominent example is the semi-synthetic, broad-spectrum anti-

biotic, ampicillin, into which the nnAAs D-phenylglycine and D-4-hydroxyphenylglycine have been incorporated (3).

The opportunities to broaden protein diversity and properties with nnAAs are enormous, as is the ability to incorporate chemical modifications in proteins that can endow current biopharmaceuticals with improved or new properties. These chemical modifications can change the characteristics of proteins, including ligand-binding properties, stability, spectroscopic properties, folding behavior, catalytic efficiency, and substrate specificity. These modifications provide possibilities to develop biobetters and biosuperiors that have superior pharmacological properties, including improved safety profiles, longer half-life, and enhanced activity (4).

Much effort has been put into developing technologies that ensure a site-specific incorporation of the nnAAs with a high rate of yield. Various methods for site-specific introduction have been established, both semisynthetic and recombinant methods. Few methods, however, have made it from the bench at small-scale protein production to commercial scale.

A recently formed biotechnology company, Redwood Biosciences, is using an approach based on the work of Carolyn Bertozzi's laboratory at the University of California, Berkeley. Her work focuses on genetically encoded aldehyde tags and aims to exploit a specific sequence (originally found within the sequence of sulphatases) that is posttranslationally recognized and modified by a formyl glycine-generating enzyme to produce a so-called aldehyde chemical handle (5). The incorporation of the CxPxR sequence at specific positions in candidate protein therapeutics provides a means to produce a site-specific nnAA with a reactive aldehyde amenable to drug conjugation.

One of the oldest methods for nnAA incorporation into proteins uses auxotrophic strains from *E. coli* that cannot synthesize a specific natural amino acid and thus have to uptake it from the growth medium. A structurally similar nnAA can be supplied within the growth medium in place of the natural amino acid and will be alternatively incorporated into the protein. A major downside is that the specific nnAA will be incorporated at every site coding for the natural amino acid and can lead to misfolding and impaired function of the target protein, or the nnAA can be incorporated in the host-cell's proteins, which can have toxic effects (6). Allozyne has pioneered this type of cell-based expression system that incorporates nnAAs into protein sequences, but this approach requires extensive re-engineering of the target protein sequence used, due to the region-specific nature of the nnAA incorporation using this method.

Ambrx has developed cell-based nnAA incorporation systems where *E. coli* or CHO cells are engineered with orthogonal pairs of transfer (tRNA) and tRNA synthetases to charge and incorporate nnAAs at selected codons at specific points in the coding sequence of the expressed protein. This approach is a significant advance and provides answers to at least some of the questions raised about nonspecific sites of conjugations in ADCs. For truly expanding the number and variety of nnAA that can be incorporated to determine the effect on function, even at a single amino-acid position, the approach demands significant investment to engineer further orthogonal pairs of tRNA synthetases and tRNAs that can recognize a library of nnAAs. A further complication is that these pairs should be exquisitely selective over natural amino acids to avoid their incorporation over the desired nnAA, which can be challenging in a drug-manufacturing context with strict regulatory requirements. When this challenge is taken into account, along with the variability in efficiency with which nnAAs are absorbed into the cell, these systems will not likely be amenable to fast reiterative make-test cycles with libraries of nnAAs at multiple sites of incorporation.

All of these considerations suggest a clear need to move away from the conventional cell-based protein expression systems to address the critical requirement for a rapid make-test system that is amenable to many parallel reiterations of site-specific incorporations of defined natural amino-acid sequences or multiple nnAAs at multiple sites. The answer may not lie with cell-based systems at all, but with completely *in vitro* biochemical protein synthesis based on novel cell-free expression systems.

CELL-BASED EXPRESSION SYSTEMS: PARALLEL REITERATIVE DESIGN AND SCALABLE PLATFORMS

Adnexus Therapeutics (acquired by Bristol-Myers Squibb in 2007) has developed an *E. coli*-based platform for producing adnectins. Adnectins are derived from human fibronectin and many trillions of adnectin variants can be generated to represent a screenable library for desirable therapeutic properties. Scale-up from a selected lead is rapid, albeit with the requirement for PEGylation for clinical candidate manufacture (7).

Fabrus has recently addressed the rapid make-test cycle approach for biologics using arrays of predefined Fab antibody sequences produced in a high throughput expression system

based on production of proteins in *E coli*, cell-lysis, and subsequent protein purification (8). The large volumes of cell culture required for high-yield parallel production of Fabs requiring a significant investment in specialized robotics equipment. The method allows production of hundreds of variant Fab proteins over a one-week production cycle to begin variant testing in high-throughput biochemical-binding assays.

EXPRESSION SYSTEMS: CELL-FREE SYSTEMS

Although cell-free protein synthesis has been practiced for decades as a research tool, only recently have advances suggested its feasibility for commercial biologics drug development and production as an alternative to conventional cell-based expression systems (9). An ideal cell-free protein production platform would produce fully soluble and correctly folded proteins at high volumetric productivities at any scale. The platform would be rapidly and predictably optimized by systems-level process design and control without the demanding requirements for maintaining cell viability and be readily adapted to high-throughput methods, including *in vitro* evolution of proteins to allow incorporation of nnAA into polypeptides. The platform would be based on simple batch systems using standard bioreactors that are known to scale to thousands of liters for both cell fermentation and subsequent cell-free protein production (10, 11).

Early efforts at developing such a system focused on projected costs that were much too high, as well as on proteins with disulfide bonds that could not be folded effectively. By focusing on basic biochemical reactions and controlling cell-free metabolism, these limitations have been methodically addressed (12). Amino-acid supply has been stabilized, and metabolism activated to dramatically reduce substrate costs by requiring only the addition of nucleotide monophosphates to drive energy production. Commercially available *in vitro* transcription translation kits based on *E. coli*, wheat germ, rabbit reticulocytes, and insect-cell extracts do not offer this advantage and are suitable only for research exploration at small scale. Control of the sulfhydryl redox potential has been gained and a robust disulfide isomerase added to facilitate oxidative protein-folding (13). These advances not only suggest production feasibility for pharmaceutical proteins containing the 20 natural amino acids, but they also provide enabling technology for incorporation of nnAAs at commercial scale.

A recent publication demonstrates that this open cell-free system (OCFS) developed by Swartz and collaborators can be optimized for high-level production of proteins to allow for scale-up to commercial levels once the target protein is identified (14). The authors expressed a multidisulfide-bonded protein, biologically active granulocyte-macrophage colony-stimulating factor (rhGM-CSF), at titers of 700 mg/L in 10 h. Importantly, they could show that the product was linearly scalable from starting materials in 96-well plates up to 100-L culture volume (14). The open nature of the system allows mass spectrometry-based profiling of the cell-free metabolome and proteome. Rapid testing of the effects of addition and subtraction of various components for system optimization can be modeled without the requirements for tuning more complex cellular networks required for maintaining cell viability commonly encountered in mammalian cell-line development.

DIFFICULT-TO-EXPRESS PROTEINS

The lack of a membrane-barrier in the OCFS provides the opportunity to express and study proteins that are difficult to express in cell-based systems. Many proteins that can't be readily expressed by *in vivo* expression systems due to poor folding, inclusion body accumulation, or due to toxicity can be expressed in an *E. coli* cell-free lysate.

COMBINATORIAL SCREENING OF PROTEINS

The advancements in genomic research and increased numbers of sequenced genomes require expression systems that allow fast production of the proteins under investigation. Cell-free expression systems can provide a useful tool for rapid screening and analysis of protein function, which is important for protein-drug discovery and development. DNA molecules can be amplified, transcribed, and translated in microplate wells and the expressed protein can be assayed immediately (15). Recently, HTS in a cell-free wheat germ system led to the discovery of a novel malaria vaccine candidate (16). Finally, the linear scalability allows proteins identified in display-based selections and HTS to be immediately scaled for production of multiple gram quantities, thus avoiding the delays and challenges of conventional mammalian cell line development.

Currently, cell-based expression technologies exhibit several limitations with respect to protein production at all phases of the drug discovery and development pipeline. Rapid production of proteins with novel chemical modifications, such as ADCs, are particularly challenging. *E. coli*-

based cell-free protein synthesis systems, however, provide robust, rapid,and scalable protein production. The *E. coli*-based OCFS system, in particular, allows rapid and multiplexed production of various difficult to express proteins and opens the unprecedented ability to explore therapeutics beyond the 20 amino acids that define today's proteins (17). The OCFS, combined with rational protein design and the focused use of libraries of nnAAs, allows for rapid exploration and identification of protein therapeutics, moving from the exploratory stage to clinical scale-up on an unprecedented, rapid timescale.

CONCLUSION

Growing demand for new and better biopharmaceuticals has led to sophisticated advances in protein synthesis that now allow for:

- Rapid production of target proteins, including those that are difficult to express in cell-based expression systems
- Straightforward scalability of protein expression from HTS to commercial levels
- Combinatorial screening of many proteins to identify and optimize drug candidates
- Introduction of site-specific chemical modifications, including nnAAs into proteins to improve pharmacological properties

These new approaches to protein expression will revolutionize the development of biopharmaceuticals, and open up the possibility to create drugs that were previously inaccessible, and even unimaginable until now.

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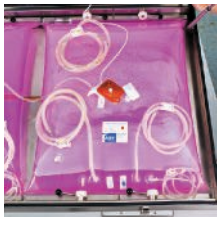

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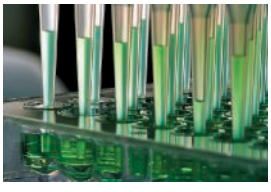

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Final Word – *Continued from p.58*

ECONOMIC CHALLENGES

The diagnostics industry is providing new tools that could improve product offerings to physicians and patients and create value for shareholders. Unfortunately, many diagnostics industry players believe the economics of innovation are undermined by low pricing and reimbursement of tests, and the diagnostics partner's low share of prescription–diagnostic (Rx–Dx) partnership values. In this context, it is important that the government and its agencies support required changes. According to research by PwC, diagnostics companies are seeking action in three main areas, as outlined below.

Pricing

Pricing should reflect the value of the test rather than its cost. The price should reflect a reasonable proportion of the test benefits or the cost savings. In the US, the concept of value-based pricing is making gradual progress. Europe, however, has yet to see value-based pricing applied to a personalized medicine test. The diagnostics industry fears, according to PwC research, that unless pricing is adapted to value creation, it will fail to achieve sufficient economic return to stimulate continued investment and innovation.

Reimbursement

The process to gain reimbursement for diagnostics should be accelerated and harmonized across countries. In many countries, reimbursement for a new test can take four to seven years following marketing clearance. Industry participants believe that health technology assessment (HTA) models need to be adapted to allow for faster reimbursement decisions. One practical solution to address reimbursement delays has been companion test subsidies from the bio/pharmaceutical partner. This is not ideal; but in cases where the test determines drug eligibility; the alternative would be that severe limitations would be placed on drug availability. This arrangement may not be acceptable to industry.

Diversity of health technology assessment procedures across countries is another issue in multicountry product launches. This problem has been recognized by the European Commission, which has sponsored the European network for Health Technology Assessment to work on greater cooperation. The US is also represented in this initiative through the Center for Medical Technology Policy.

Value

The share of value going to the diagnostic in Rx–Dx partnerships should be revisited. Diagnostics companies are concerned about not getting a fair share of the overall value of Rx–Dx combinations when negotiating deal terms with bio/pharmaceutical partners, and that they suffer from historically low recognition of the value of diagnostics. Traditionally, diagnostics represent less than 2% of healthcare spend despite influencing more than 60% of crucial healthcare decisions. Diagnostics partners are focused on trying to rebalance their share of the financial value in Rx–Dx combinations.

One avenue that diagnostics companies are pursuing is to obtain a royalty on sales of the companion product. Companies have resisted such a move because they believe the Dx partner has not shared the risk or investment associated with drug development. Diagnostics players insist that this move should happen, arguing that where Rx–Dx combinations are relevant, the companion drug would not be able to make it through clinical trials or be reimbursed and commercialized without the companion diagnostic. Thus, the value of the drug is critically dependent on the contribution of the companion diagnostic.

In the near-term, these challenges are not expected to undermine the pace of Rx–Dx deal activity. However, they may affect long-term diagnostics innovation if they are not addressed. At best, not addressing these economic issues could result in an undervaluing of diagnostic innovation by pharma. At worst, these issues could eventually discourage continued investment into diagnostics ventures and delay patient access to important new health technology. ◆

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The Economic Challenge of Prescription–Diagnostic Combinations

With the rise in therapeutics comes more complex partnerships.

by Gerry McDougall, a principal at Pricewaterhouse Coopers (PwC), and Loïc Kubitza, a director in PwC's Pharmaceutical & Life Sciences Advisory Services group, gerald.j.mcdougall@us.pwc.com. loic.x.kubitza@iu.pwc.com.

In-vitro diagnostic (IVD) tests are complementing targeted therapeutics to reduce side effects, improve efficacy, and help control healthcare costs. Analysts are projecting billion-dollar revenues for some new drugs linked to companion diagnostics.

As the pharmaceutical industry develops more targeted therapeutics, and their interdependence with companion diagnostics grows, companies are considering ways to access diagnostics technology to complement their evolving product portfolios. The main strategy has been to seek companion diagnostic solutions by forming partnerships with diagnostic companies. Yet, the allocation of the overall financial value of the drug-diagnostic combination has made deal-making a challenge.

SURGE IN DRUG-DIAGNOSTIC PARTNERSHIPS

Companion diagnostics partnerships in the industry more than tripled in 2010 compared with 2008, and the pace of deal activity has continued during the first half of 2011. The rising number of partnerships reflects the increasing seriousness with which bio/pharmaceutical companies view biomarker and diagnostic programs designed to accompany their drug-development efforts. They are making more systematic use of companion diagnostic programs to increase drug response rates and reduce side effects. Diagnostic companies, particularly those with strong molecular and tissue diagnostic capabilities, have been active in developing tools to respond to industry's specific needs.

Driving the momentum in companion diagnostics partnership activity is the increasing role of diagnostics in the regulatory approval, reimbursement, and performance optimization of new drugs. Increasingly, regulatory agencies insist on validated diagnostics prior to considering marketing clearance. In addition, the growing use of tests that identify patients who would *not* benefit from certain therapies has raised the bar for obtaining reimbursement for new drugs. Increasingly, payers see companion diagnostics as useful tools to allocate healthcare funds more effectively and to control costs. Many healthcare professionals insist on specialist testing before prescribing and reimbursing treatment regimens that are expensive and not efficacious in certain patient subpopulations.

In the US, some pharmacy benefit managers are adapting their business models by forming partnerships with, or acquiring, specialist clinical laboratories. Payers' preference for drugs that come with a companion test, particularly when these drugs are expensive and may lead to severe side effects, will likely increase over time with the rising pressure on healthcare budgets and greater availability of appropriate diagnostic tools.

THE PARTNERS: THE RX-DX COMBINATION

Bio/pharmaceutical companies have achieved some success in seeking improvements in drug-response profiles through better patient targeting. For example, drug-response rates of up to 80% have been reported for targeted subpopulations for cancers that generally have a 20% response rate.

The prospect of repeating such technological wins is encouraging industry to accept changes that appear increasingly inevitable, including the decline of the mass-market blockbuster drug model, the emergence of smaller targeted markets, and the need for high-performance diagnostic tools to dominate well-defined smaller market segments.

On the supply side, the technological feasibility of companion diagnostic programs is increasing. Companies continue to develop relevant expertise in molecular and tissue diagnostics, which will enable the development of better tools to guide treatment decisions.

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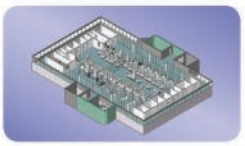

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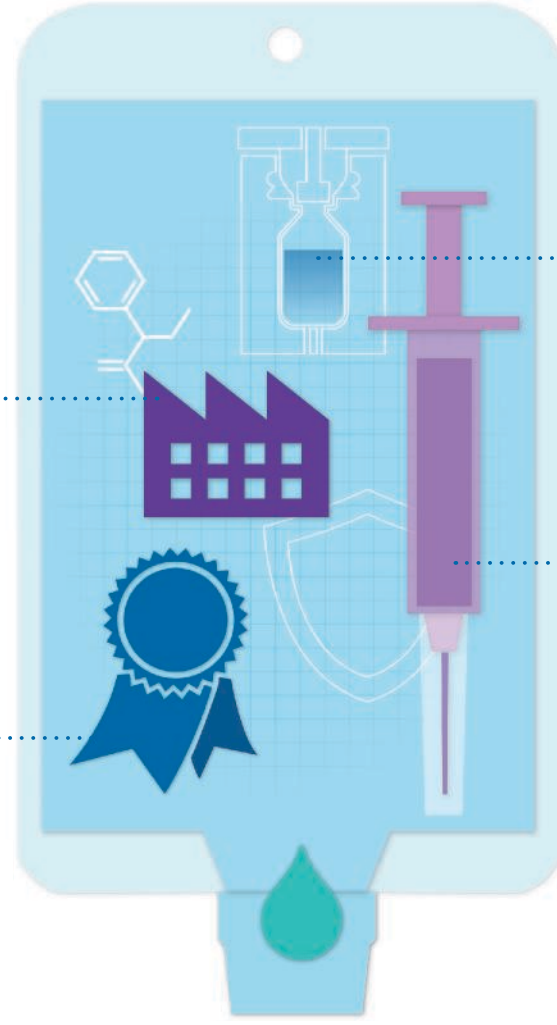

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