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SPECIAL REPORT

Cell Biology

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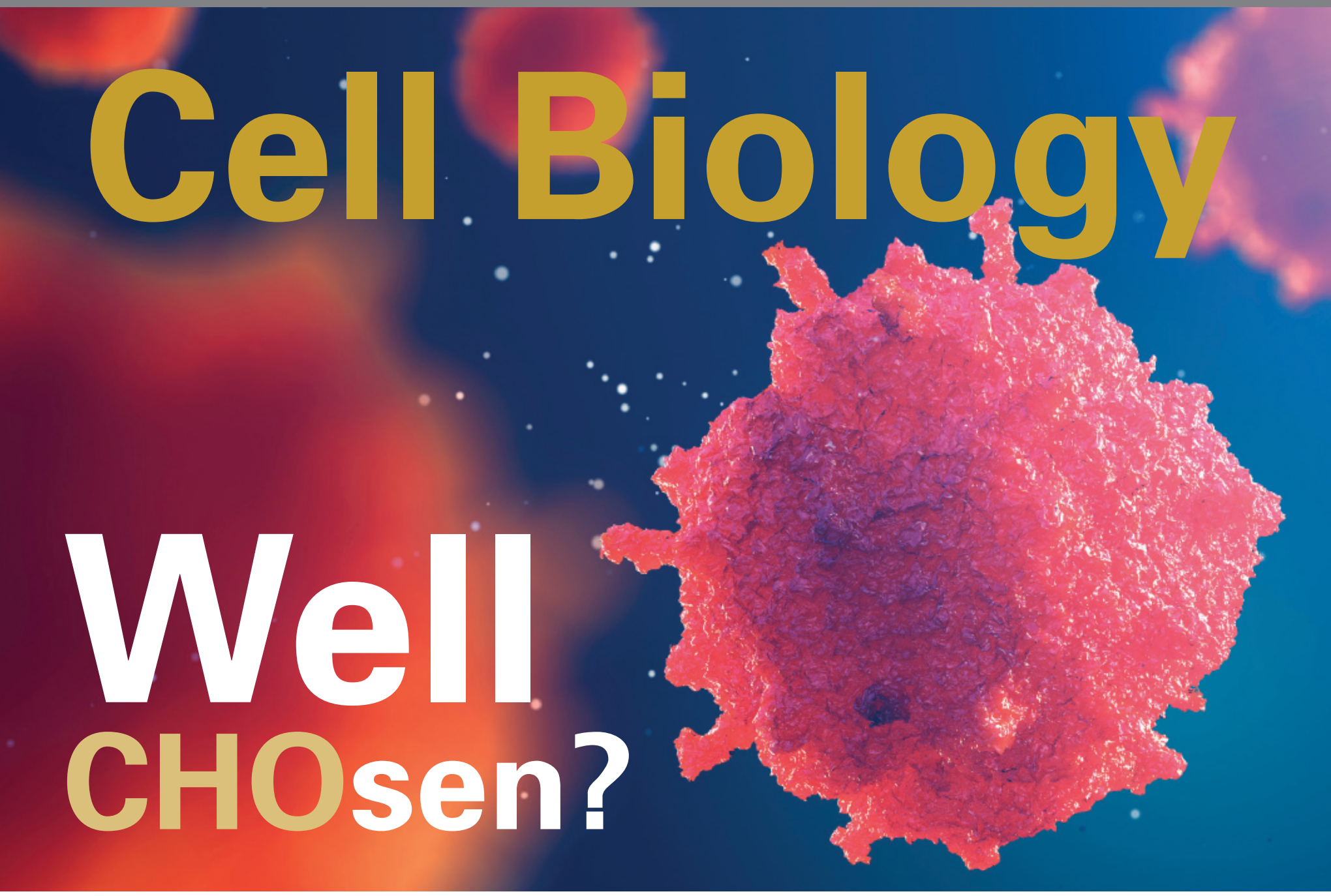

As important, potentially groundbreaking and valuable as much of recent biologics research is—with the creation of complex protein therapeutics prominent among them—the leaps forward are definitely pushing the limits of expression systems and cell lines.

Ever-more complicated biologics push technical boundaries

BY RANDALL C WILLIS

"IN A FUNNY WAY, our lack of success led to our breakthrough, because, since we could not get a cell line off the shelf doing what we wanted, we were forced to construct it. And the little experiment being done in the background, concerning hybridization between myeloma cells, developed into a method for the production of hybridomas."

There, in the middle of his Nobel lecture, César Milstein described the moment that his immunological journey pivoted toward the elucidation of the monoclonal antibody, leading him and Georges Köhler to share the 1984 Nobel Prize in Physiology and Medicine with Niels Jerne.

In the years since, monoclonal antibodies (mAbs) have become the lynchpin of some of the most advanced therapeutics, as well as transforming healthcare economics in the process.

And yet, although the basic structure of light and heavy chains remains the foundation of these protein complexes, new biologics constructs are making the simple mAb feel like the good old days, pushing the limits of expression systems and cell lines.

"Increasingly, we see scientists addressing complicated and intractable diseases by developing complex protein therapeutics such as bi- and tri-specific proteins, DARPins, triabodies, novel scaffolds decorated with peptides, enzymes and growth factors," says Igor Fisch, CEO of Selexis. "While this can be great news for patients, these complex molecules are often more challenging to manufacture, as most are non-natural proteins that need to be expressed at high enough levels to be commercially viable."

Where it can be challenging enough to produce a correctly folded, functional protein from a single gene, these more complicated therapeutics require the expressions of multiple genes, producing proteins at the right levels to work together, and then be secreted from the cells.

"A lot of those formats for bispecifics require three or four different genes to be expressed at the same time," explains Greg Bleck, head of Biologics R&D at Catalent Biologics. "So, instead of having one heavy chain gene and one light chain gene, we have two heavy chain genes and two light chain genes."

"Then the correct light chain needs to pair with the correct

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"Some of the issues we have seen make a protein difficult to express are improper folding, improper protein chaperone framework to usher the therapeutic through the secretory system, improper pairing of subunit proteins and metabolic stress to the cells from the high secretory load," notes Igor Fisch, CEO of Selexis.

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heavy chain, and the two heavy chains need to pair as heterodimers, not as homodimers," he presses. "So, we're creating pretty elaborate systems that need four genes expressed, and they probably need to be expressed at different ratios to get the best quality protein product produced as well as the best expression."

The routine needs for many of these proteins to be post-translationally modified to function in human tissues means that most products are produced in mammalian cell lines, typically Chinese hamster ovary (CHO) cells.

"CHO cells are not inherently 'designed' to express these novel proteins in the first place, [however], and certainly not at the extremely high levels necessary for making a product for patients," Fisch says. "For this to be achieved, companies engaged in cell line development will need to continually improve the tools and capabilities that will allow them to modify their CHO cells to address whatever transcriptional, translational, secretory or metabolic stresses these newer molecules place on the cells."

From plates to production

The first step in cell line development is being confident in and understanding your cell lines.

"CHO cells are very heterogenous, so there are lots of phenotypes within that population," explains Fay Saunders, head of Mammalian Cell Culture R&D at Fujifilm Diosynth Biotechnologies. "Some of those cells could have the desired attributes that we're looking for; others may not. So, we implemented the directed evolution strategies."

This was the birth of the company's Apollo platform.

In a white paper, Saunders' colleague Alison Porter, also in the mammalian cell culture group, expounded on the directed evolution approach.

"The selection of CHO cell variants with improved characteristics is typically an iterative process comprising several rounds of induced selective pressure," she wrote. "Examples include relatively simple approaches such as extended cultivation of cell lines in altered subculture regimes or limiting dilution cloning. Indeed, efforts to adapt cell lines to serum-free, chemically defined media will be a familiar 'directed evolution' approach to many."

"During the development of a new host cell line, a very large panel of potential new host cell lines can be obtained," she added. "It is therefore important to have a well-designed hierarchical screening strategy to gradually decrease these numbers and identify those cell lines with superior characteristics."

Saunders continues, suggesting that on the vector side of things, they went back to the beginning and looked at all components required for efficient protein expression, including promoters driving expression of heavy and light chains, promoters driving expression of the selection system and leader sequences.

As she explains, they looked not only at all those different components in isolation, but also in combination, to identify the optimal expression vector for their host cell lines.

Historically, screens during cell line development were quite far removed from the bioreactor environment, leading to repeated steps of optimization: during early selection and during process development for biomanufacturing.

"We wanted to incorporate state-of-the-art screens, getting various cell lines into suspension screens early on in that process to identify cells lines that will perform well in the bioreactor," Saunders offers. "Obviously, when you've got hundreds of cell lines, you can't put them all in the bioreactor, but it is trying to get those screens to mimic as closely as possible that environment early on."

Even with the Apollo launch in 2014, however, the company is well into development of its next-generation Apollo.

"We've done some further directed evolution on that host cell line, so altering the subculture regime to try to force a higher growth rate," Saunders explains. "We now have a cell line that is a quicker grower, which means timelines can be reduced, as well as improved expression capabilities. We're also looking at technology to improve the processing and reduce timelines."

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"We're creating pretty elaborate systems that need four genes expressed, and they probably need to be expressed at different ratios to get the best quality protein product produced as well as the best expression," says Greg Bleck, head of Biologics R&D at Catalent Biologics, talking about challenges in producing complex protein therapeutics such as bi- and tri-specific proteins.

According to Liza Rivera, Fujifilm Diosynth Biotechnologies senior director of global marketing, a significant rationale for developing the Apollo system and its microbial pAVEway expression sibling was to access clients the organization might never have otherwise seen. Whereas the company would traditionally transfer-in and

optimize cell lines from its clients, it now had the capability to offer services to clients who did not already have a preferred cell line.

"So, Apollo gave us access to clients at a much earlier stage, and we could be part of that journey from the very beginning," she enthuses.

According to Fisch, Selexis took a similar modular approach in the development of its SURE*technology* platform.

"At the core of the platform are our Selexis SGEs (Selexis Genetic Elements)," he explains. "These genetic elements protect integrated transgenes from chromatin silencing effects, allowing for robust recombinant gene transcription levels without the need for long-term gene amplification strategies to ensure sufficient transcription levels."

He adds that each component of the current system was developed to address specific needs.

Fisch offers the example of SURE*variant* Screening, which allows the company to screen production CHO cell lines rather than research cell lines, which are typically transiently transfected HEK, offering a significant time savings during drug candidate selection.

"The SURE*feed* Strategy was worked out to allow for cost-effective, animal-product-free protein therapeutic production that is readily transferable to CDMOs [contract development and manufacturing organizations]," he adds. "We built the SURE CHO-M*plus* Libraries to provide a comprehensive solution for overcoming secretion bottlenecks, and SURE*scan* can robustly establish clonality."

Seeing the endpoint

At a recent symposium on stem cell technologies in Toronto, Thermo Fisher Scientific's Tia Hexom noted that in developing the next generation of cell therapies, it was important to start with the end in mind.

There is some consensus that this is also a good approach in the protein biologics space.

As suggested above by Saunders, even during cell line development, her group is focused on identifying cell lines that will perform well in the bioreactor environment.

"We have knowledge and understanding of the large scale that we can mimic at the small scale," she suggests. "Yes, there are likely to be some changes, but very small tweaks, rather than really having to optimize and then spend months and months changing the process from what we identified in cell line development to get into manufacturing."

But the company also looks beyond the cells to consider the final product.

"We use pooled transfectants," Saunders explains. "You transfect your population and instead of cloning them out to get a monoclonal cell line, you just expand that population quickly and generate material."

"That material can then feed into your downstream processing, analytical development or you get an early readout of what its characteristics are," she adds. "Even before you've got your clonal cell line, you're already trying to get an understanding of the molecule and how it behaves—does it fit the downstream platform, can you purify it with no issues and

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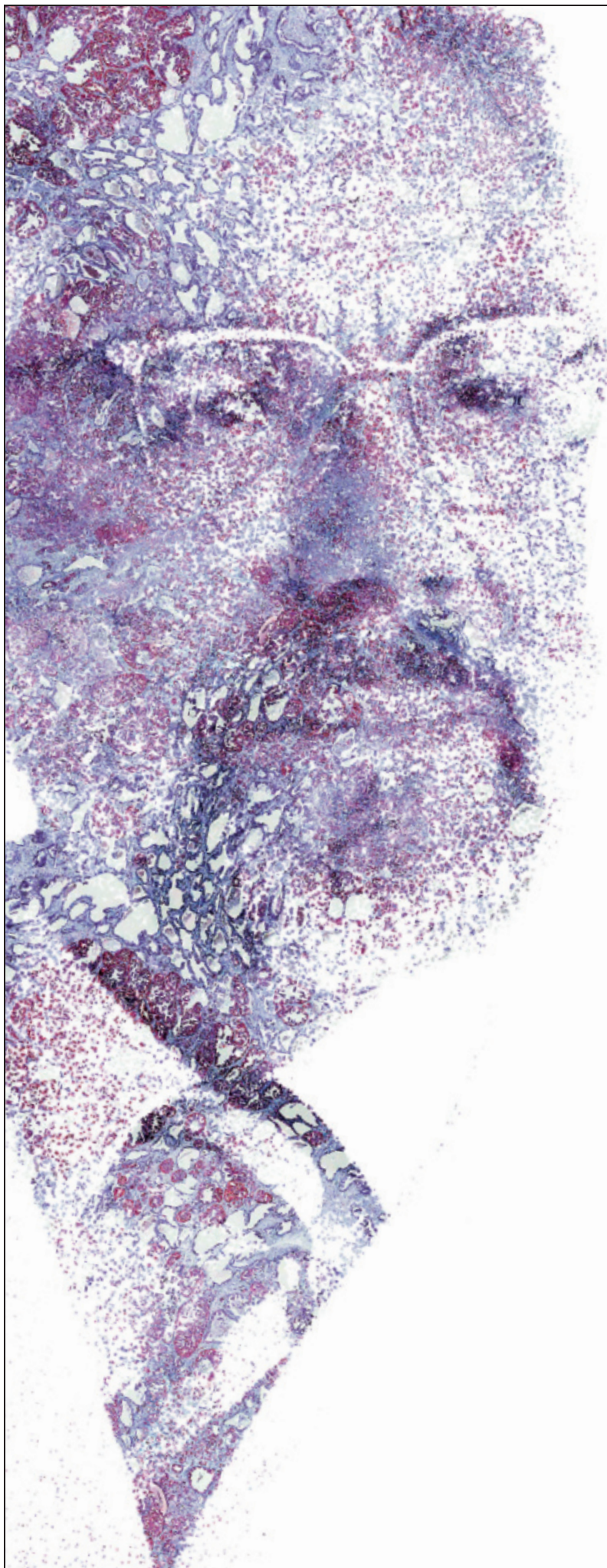
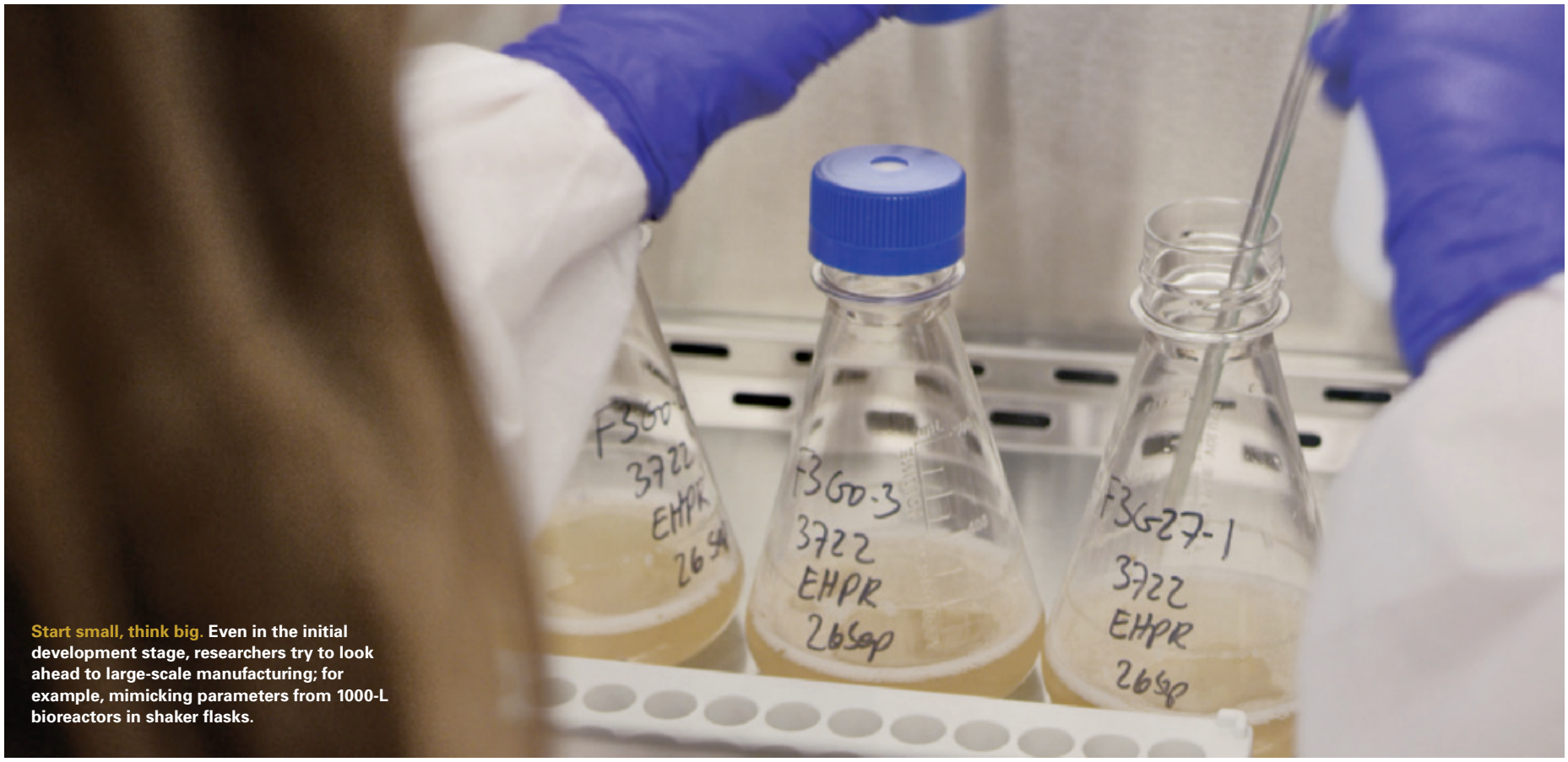
Fay Saunders, head of Mammalian Cell Culture R&D at Fujifilm Diosynth Biotechnologies





Start small, think big. **Even in the initial development stage, researchers try to look ahead to large-scale manufacturing; for example, mimicking parameters from 1000-L bioreactors in shaker flasks.**

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also what the formulation might be for your final drug substance and drug product."

Bleck suggests life may be a little simpler with basic protein manufacturing than it is for viral vectors or CAR-T therapies, but he thinks the train of thought is a good one.

"If you know you need certain things, whether it be an expression level to make sure your cost-of-goods (COGs) are in line or whether it is a profile of what the molecule needs to look like from a post-translational modifications point of view or if there are certain other desired characteristics that could be unique to the molecule," he says, "it's nice to know what those are heading into the program."

In general, he adds, because antibodies are pretty well established and process development is relatively mature, there is less concern at the front end.

"Other recombinant proteins or Fc fusions can have a few more issues being expressed; knowing how much material is going to be required for the clinical trial, and what might be a COGs that someone would be comfortable with if the product ended up being commercial, is good to know early in the process," he concludes.

Fisch echoes these sentiments.

"When novel biologics are being discovered and developed, it is important that the drug manufacturers characterize the quality and productivity levels of their biologic," he says. "If there is even a moderate amount of mismatching of subunits, protein degradation or disconnect between transcriptional and protein secretion levels, it will be important to address those issues early on."

Such concerted efforts are starting to gain traction as greater demands for consistent goods is increasingly highlighting the challenges of the traditional ad-hoc approach to cell line and bioproduction development.

Earlier this year, for example, Gino Stolfa and colleagues at Thermo Fisher Scientific examined the emergence of what they termed "CHO-omics" and a more coordinated multi-omic analysis of cell characteristics.

"The future of omics for bioproduction likely entails employing multiple omics technologies on individual CHO cell lines rather than an all-encompassing consensus model," the authors suggested. "Similar to the idea of personalized medicine, focusing omics strategies on individual CHO lines, beginning from the parental strain through to the final production clone, will maximize the gains in productivity and quality resulting from cell line engineering, and medium and process optimizations for each individual cell line."

The authors greatly appreciated the scale of the undertaking they have described, acknowledging that this will be a stumbling block. By the same token, the individual omic analyses have led others to wonder about what smaller changes might be made within cell lines to enhance productivity.

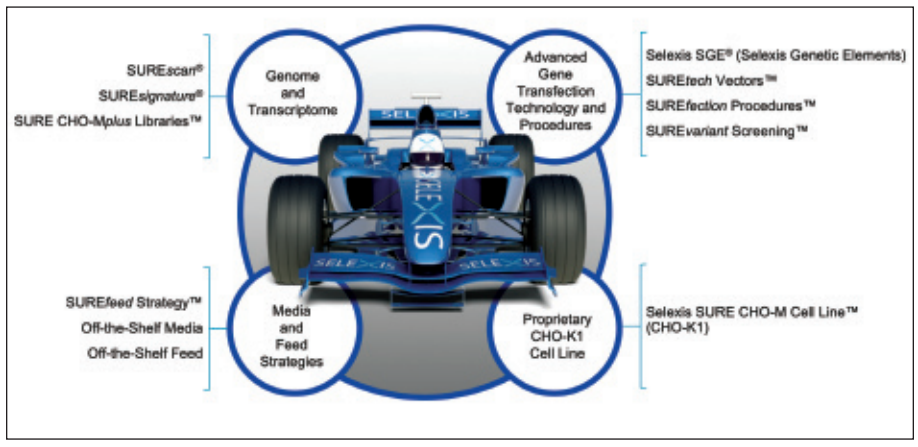
Fine-tuning vs. directed evolution

With an increased understanding of CHO-omics parameters, many labs have started to tinker directly with metabolic and secretory pathways in different cell lines, trying to manipulate how well cells express and/or tolerate proteins of interest or enhance cellular growth rates to potentially shorten production timelines. The recent flux of gene-editing tools like CRISPR/Cas9 has greatly facilitated such efforts.

Earlier this year, Tian-Yun Wang and colleagues at Xinxiang Medical University recounted their efforts to knock out the DNA methyltransferase gene Dnmt3a in CHO cells, thereby modulating epigenetic silencing and improving transgene expression and long-term cell stability in culture.

Despite its central metabolic role, Dnmt3a deletion did not significantly impact doubling time of the knock-out cells, nor did it have any effect on cell transfection. Compared to control cells, however, the Dnmt3a-deficient cells demonstrated higher and sustained transgene expression.

"After cultivation for 50 passages, the



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Selexis took a modular approach to its SUREtechnology platform, with company CEO Igor Fisch noting: "At the core of the platform are our Selexis SGEs (Selexis Genetic Elements). These genetic elements protect integrated transgenes from chromatin silencing effects, allowing for robust recombinant gene transcription levels without the need for long-term gene amplification strategies to ensure sufficient transcription levels."

3a-30 cells transfected with CMV still displayed high levels of eGFP, whereas the eGFP expression of the other transfected cells had obviously dropped, particularly in the cells transfected with EF1a," the authors wrote. "Overall, these results suggested that Dnmt3a [knock-out] can significantly delay the loss of CMV-driven recombinant gene expression."

A similar effort was described this year by Laura Abaandou and Joseph Shiloach of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) who followed up on an earlier effort to screen microRNAs for genes that impacted recombinant protein expression and identified ornithine decarbozylase antizyme (OAZ1).

Using CRISPR, the researchers deleted OAZ1 from luciferase-expressing HEK293 cells, examining the resulting knock-out line for growth and production efficiency in both stable and transient transfection systems.

"Compared with the parental cell line, both engineered cell lines demonstrated higher expression of luciferase, a stably transfected cytoplasmic protein, and alkaline phosphatase, a transiently transfected secreted protein," the NIDDK researchers wrote.

"Improved protein expression is often accompanied by undesirable side effects, such as growth and metabolic disadvantages, caused by increased metabolic load on the cells," they continued. "This was not observed in the OAZ1 deficient or knockout cell lines, where no significant differences in growth and nutrient utilization were observed."

Looking slightly more broadly than single gene knock-outs, Shiloach and colleagues Sarah Inwood and Michael Betenbaugh of Johns Hopkins University recently reviewed efforts to screen for microRNAs that might enhance growth and protein expression.

"MicroRNAs are currently the most frequently used non-coding RNA for improving CHO and HEK cell protein production capabilities," the trio wrote. "MicroRNAs can target multiple genes in the same pathway, making them good targets of a specific cell process, such as reducing apoptosis, leading to improved protein production."

As they explain, one advantage of microRNAs comes in their size, as a single molecule can target multiple genes simultaneously. That being said, the full scope of genes targeted by many individual microRNAs is yet unknown, increasing the likelihood of unintended consequences.

"Some small [non-coding RNA] such as

shRNA and siRNA are gene specific, narrowing the focus to one target gene and removing the uncertainty of undesired targets," the authors continued. "As more information becomes available concerning small non-coding RNA molecules, more applications become possible for improving protein production, such as the use of mitosRNA and SINEUP."

Such efforts aren't restricted to academic undertakings in research labs.

"We've looked at doing that," offers Catalent's Bleck. "We have added genes to certain programs where additional levels of that particular molecule might be important for that program moving forward."

He quickly acknowledges the complexity involved, however, in potentially modifying metabolic pathways and secretory pathways.

According to Fisch, Selexis also has used a variety of genetic tools to knock out or knock in genes in CHO cell lines to improve productivity, doing this on a case-by-case basis for clients who experience specific challenges with their proteins of interest.

For her part, Fujifilm Diosynth Biotechologies' Saunders understands the rationale behind these approaches, but she also wonders if they aren't potentially limiting.

"When you just want a better host with better growth and better expression capabilities, there are a lot of different mechanistic pathways involved," she offers. "When you go down a cell engineering route, you may not get your desired attribute at the end because you've only looked at one particular component, one particular pathway.

"Yes, that enzyme could be a good target for cell engineering, but it doesn't really follow through to an improved phenotype."

On top of that, she continues, there is the question of intellectual property when doing directed engineering, whether in the target or in the technology used to modify that target. She offers the example of CRISPR/Cas9 knock-outs.

"It's okay for research purposes," she cautions, "but if we were then wanting to use that to make a host cell line for a commercial purpose, then there is a lot of licensing and other things involved that would make it prohibitive for us to go down that route, which you obviously don't get if you go down the directed evolution approach."

As alluded to earlier, however, bioprocessing specialists aren't only adjusting the cells in which they express their target proteins.

Vexing vectors

In describing the origins of Catalent's GPeX platform, Bleck says that the initial goal largely focused on maximizing expression levels within cells, but as they developed the system further, other factors arose.

"We identified some unique characteristics about our approach that we felt gave us advantages in the areas of genetic stability, as well as the ability to titrate genes into the cell lines," he explains. "If you were expressing more than one gene, you could titrate genes into the cell lines to get the correct ratios of the proteins being produced."

As Bleck and his colleagues learned, even the order in which you transfect the transgenes can have a significant impact on cell viability. In one example, they found they needed to introduce light-chain constructs before they introduced the respective heavy-chain construct, as doing it the other way around made for less viable cell lines.

"A lot of heavy chain, when you express them by themselves, can be toxic to cells because they aren't secreted efficiently without a light chain present," he says, acknowledging that he has also seen this for some light chains.

"By being able to have different levels of expression in the clonal cell lines that you're screening for expression and functionality—that can be beneficial for those particular molecules," he adds.

Adding pressure to the host cell machinery is whatever selection system—*e.g.*, antibiotic resistance—you add to your expression vector. Knowing this, Catalent endeavored to limit this pressure.

"We also realized that traditional selection wasn't necessary because of the way that we were doing the process, and that we didn't have to use antibiotic selection and express another protein in addition to the protein of interest inside the cells," Bleck adds. "We could just express the protein of interest in the cell line."

In some cases, however, the addition of another gene is necessary to achieve the expression levels or modifications you need for your final product.

"While we are continually updating our SURE*tech* Vectors and SGEs based on our experience with evolving protein therapeutic products, the bigger change recently made with these vectors has been their utilization, not only for the therapeutic transgenes, but also for the introduction of auxiliary genes that help with CHO-M cell line productivity," Fisch recounts. "This is exemplified with the Selexis SURE CHO-M*plus* Libraries."

"These libraries are generated utilizing SURE*tech* Vectors and SGEs and contain sequences to a range of transcriptional, translational, secretory and metabolic proteins that can be rate-limiting for therapeutic protein production in CHO cells," he explains. "In other words, sometimes protein production is not limited by the amount of gene transcript, but rather, by secretion bottlenecks caused by limited levels of key proteins in the secretory process."

In May, Selexis and the International AIDS Vaccine Initiative (IAVI) published their efforts with colleagues to enhance production of HIV-1 Env trimers as a step to vaccine generation. Using a two-pronged approach, the group first generated a protein construct that assembled into a native-like conformation, and then applied the CHO-M*plus* Libraries platform, producing 30-fold more protein than possible with conventional methods.

A similar scenario is true for Catalent's SMARTag technology, which facilitates the conjugation of therapeutic or toxic payloads to antibodies to form ADCs. In this case, they first needed to develop a cell line expressing the formyl-glycine generating enzyme that would ultimately convert specific cysteine residues on the target protein into formyl-glycine and allow subsequent conjugation at the free aldehyde.

Even the location of the transgene can be challenging, as it can significantly perturb existing and potentially vital genes or the vector can integrate into a region of the genome that is transcriptionally quiet.

"People for years have been looking at ways to mitigate the effects of or trying to put elements in their vectors that alleviate the effect of where the gene inserts into cells," says Bleck. "Now, folks are starting to look at systems out there where they've identified landing pads in the genome that seem

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Despite mammalian cell lines like HEK293 and PerC6 having been commercially available for more than 15 years, nothing has displaced the primacy of CHO (Chinese hamster ovary) cells or is likely to do so, suggests Selexis CEO Igor Fisch.

Stepping off the hamster wheel

ALTHOUGH CHO CELLS are the workhorse of the biologics industry given their lengthy history and familiarity, they are not the only choice.

"I've been to numerous conferences where there've been talks around that if you were starting out again now, you probably wouldn't choose the CHO cell," recounts Fay Saunders, head of Mammalian Cell Culture R&D at Fujifilm Diosynth Biotechnologies. "It's not really designed for efficient expression of proteins, but they have been used for so long and characterized."

"For the transient expression work, HEK still do have an advantage over CHO cells," she says. "The transient expression systems have improved for CHO cells, and there are cells developed by individual companies that do give good performance, but HEK and the expression systems around that have a history on the transient side of things and they are a great workhorse for generating material at an early stage, more discovery phase."

Although Igor Fisch, CEO of Selexis, agrees that HEK cells yield protein therapeutics containing human glycosylation, he suggests that they are currently not as amenable to generating high-level, stable productivity cell lines as CHO cells.

"HEK cells, and PerC6 cell lines for that matter, have been available for cell line development for more than 15 years and have not displaced CHO," he offers. "Furthermore, over the last 20 years, so much progress has been made with CHO-based technologies in terms of productivity, stability and manipulability, I believe it will be a long time until another platform surpasses it."

Saunders' colleague Liza Rivera, senior director of global marketing, is quick to suggest that the company uses HEK cells quite often at its Texas site, which focuses on the viral and gene therapy space.

She adds that the company is also putting a lot of effort into Vero cells, improving processes for gene therapy applications and moving the cell lines from adherent to suspension culture.

Another reason to stick with CHO cells, however, is what Greg Bleck, head of Biologics R&D at Catalent Biologics, describes as the species barrier. Effectively, most human viruses don't infect hamster cells, and most hamster viruses don't infect human cells. Thus, when products are developed as therapeutics, there is less risk of passing along pathogens.

"If it was my product in development, I would need a very significant reason not to use CHO," he says. "Some of those exist out there, you just need to justify that to the regulatory authorities as to why you went that route."

He admits that it comes down to the individual project, and that certain molecules may be produced more effectively in one cell line over another.

"We've seen programs where you see a protease that might be more prevalent in one of the cell lines that cleaves your protein sequence, and so you can look to reduce that proteolytic cleavage by using one of the other cell lines," he offers as an example.

Extending that thinking even further, Saunders considers the opportunities that lie outside of mammalian cell lines altogether.

"If [your protein is] simple enough, then *E. coli* would be your route of choice," she says. "It's simpler and more straightforward. If you can use it, why wouldn't you?"

But as the protein gets more complex, bacterial and yeast expression platforms are less likely to cut it.

"All of the research around putting glycosylation machinery into *E. coli* and modifying yeast systems to get human-like glycosylation still seems to be at the stage where yes, it's happening in research," she explains, "but whether it's actually going to really change the way we do things in industry, I'm not sure."

Another consideration for moving out of mammalian systems, warns Saunders, is the hoops through which you have to jump to gain client confidence in the new cells or processes. This was something the company discovered when it introduced the Apollo system.

"It's CHO," she laughed. "Even though it's what's already on the market, clients look and ask what's new, what data do you have to show me that this works? You have to show a lot of data to get the confidence of the clients and the regulators that there are no issues with it."

That said, Rivera is excited about both Fujifilm Diosynth's microbial pAVEway expression systems and its work with insect cells and baculovirus.

"We have a commercial product that we are currently manufacturing, the antigen part, which is made in a baculovirus system," she enthuses. "That is something that we are very proud of because we were able to scale up a baculovirus culture to 2,000 L."

Fisch is less enthusiastic.

"There are some non-mammalian systems, such as *Pichia pastoris* or some insect systems, with post-translation machinery that can work for some biologics, but overall they are not as broadly applicable as CHO cells," he says. ■

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"Increasingly, we see scientists addressing complicated and intractable diseases by developing complex protein therapeutics such as bi- and tri-specific proteins, DARPins, triabodies, novel scaffolds decorated with peptides, enzymes and growth factors," says Igor Fisch, CEO of Selexis (a researcher and lab of which are pictured here). "While this can be great news for patients, these complex molecules are often more challenging to manufacture as most are non-natural proteins that need to be expressed at high enough levels to be commercially viable."

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to be really good for expression and secretion."

In some cases, however, the best-laid plans of cells and vectors are challenged by proteins given the epithet DTE: difficult-to-express.

Problematic proteins

As Fisch suggests, any number of intrinsic factors can lead a protein to be DTE.

"Some of the issues we have seen make a protein difficult-to express are improper folding, improper protein chaperone framework to usher the therapeutic through the secretory system, improper pairing of subunit proteins and metabolic stress to the cells from the high secretory load," he lists.

Bleck adds his experiences.

"There's obviously the toxicity part of things, where a molecule might not be secreted efficiently from the cells, so it causes the cells to become constipated and enlarge in size," he offers. "Or it could bind to something internal to the cell or external to the cell to cause issues."

The challenge of cell constipation was highlighted in a recent study by Sven Mathias and colleagues at University of Applied Sciences Biberach and Boehringer Ingelheim, who used fluorescence microscopy to visualize intracellular bottlenecks in CHO cells.

The researchers developed cell lines expressing a classical IgG1 antibody and a bispecific antibody (bsAb), and then monitored expression as well as localization.

Despite the bsAb-expressing cells growing better than the IgG1 cells, the former produced only about one-third as much product. Analysis by qRT-PCR and Western blotting suggested that the problem wasn't at the transcriptional or translational levels, respectively.

Using fluorescence microscopy, however, the researchers were able to watch the two products move through the secretory pathways.

"Notably, the bsAb-producing cell line showed no or only very weak colocalization of staining for the Cis-Golgi and the recombinant protein, whereas a very strong signal for the produced recombinant IgG was observed within the Cis-Golgi apparatus of the respective cell line," the authors commented. "This aberrant distribution of the bsAb additionally points towards a bottleneck within the ER of the respective cell line."

In keeping with Bleck's constipation metaphor, the researchers noted: "Additionally, the ER showed an affected morphology displaying distended or overloaded larger irregular-shaped structures which resemble previously described Russell bodies, where insoluble and slowly degraded aggregates containing immunoglobulins are hosted within the dilated cisternae of the ER or the cytoplasm."

As the researchers were more focused on methods development, they expressed high hopes for possible next steps.

"In combination with picture analysis tools, high-throughput microscopic analysis might enable automatic processing of the generated images leading to rapid identification of suitable clonal production cell lines," they suggested. "Furthermore, this method might enable investigations of diverse mutants or sequence variants of candidate molecules especially in combination with transient gene expression."

Unfortunately, as Saunders explains, CDMOs can be somewhat limited in what they can do when a client arrives with a DTE product.

Because they're making human proteins in CHO cells, codon bias will have some effect on protein expression. Thus, the company starts any project by having the requisite genes synthesized and codon-optimized for CHO expression.

They also use bioinformatic analysis early on to flag any potential downstream issues such as stability.

"For example, with protein folding and secretion, would a temperature shift be beneficial," Saunders offers. "That kind of slows everything down and that might help the proteins get through the cellular machinery."

Bleck shares the challenge. "I get asked all the time by potential clients," he relates, "well, can't you just look at the sequence and determine if this is going to be expressed well or not? I haven't seen anything that has been able to do that to date."

Obviously, he continues, there are some flags for problematic expression.

"If you have a free cysteine or a glycosylation site that's present in a variable region that's not normally there, those are the things you tend to avoid during antibody discovery because they can potentially cause problems with synthesis and secretion and expression," Bleck offers.

"I wish there was a program that we could utilize and avoid that extra work, but there isn't a good one that I'm aware of aside from those big red flags in sequences," he laments.

Ultimately, with so many moving parts, success in biologics development and bioproduction comes down to converting lengthy experimental experience into an individualized approach to each project. And just as the new molecules make mAbs look quaint, so too must the new technologies pale the old. ■

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