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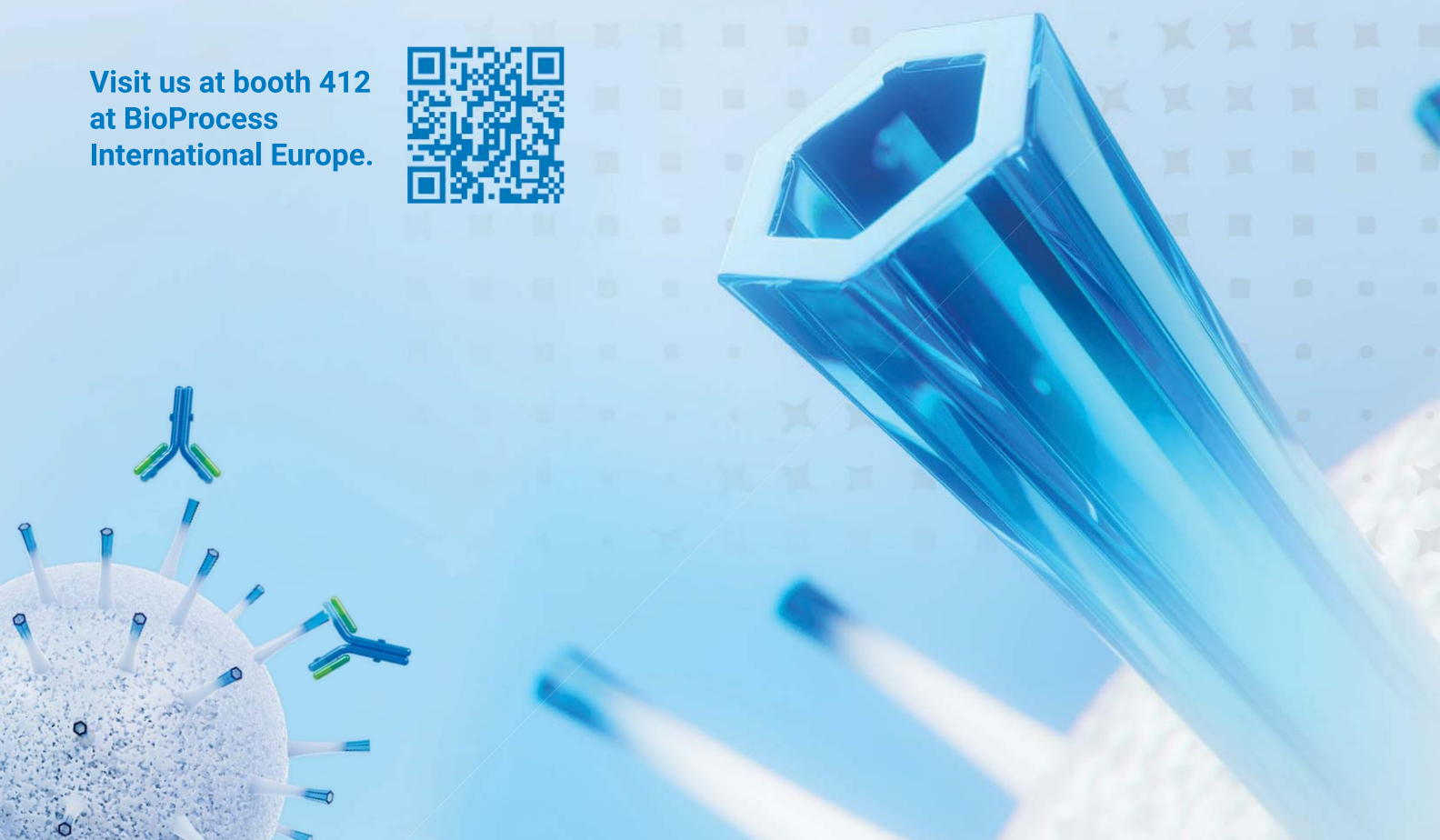

BioProcess International

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On the Cover A technician draws a sample from an eluate tank in this image from Sandoz in Kundl, Austria (HTTPS://SANDOZ.COM). *Find us online at https://www.bioprocessintl.com.*

FROM THE EDITOR

Cheryl Scott
Editor in Chief

Welcome to the first of our three special print editions for 2026! These issues are made available exclusively to attendees of our main events across the year: BPI West (San Diego, 9–11 March), BPI Europe (Vienna, 27–30 April), the Biotechnology Innovation Organization's (BIO's) annual convention (San Diego, 22–25 June), and the BPI Conference and Exhibition at Biotech Week Boston (22–25 September). We look forward to meeting you when you pick yours up.

When BPI decided to transition into "digital forward" for this year, we realized that we're not quite ready to go cold turkey on print. Each of these issues will showcase some of the best contributions we've had over the preceding months. For this edition, we're especially excited to share several articles from our featured-report series in the latter half of 2025. That series was our first foray into digital-only publishing, and we're proud of what we put together for it. You can always find a list of featured reports on our website at *https://www.bioprocessintl.com/publications/featured-reports*. Topics in 2025 included vaccines, cell-line development, formulation and fill–finish, biomanufacturing facilities, scale-up and technology transfer, and gene therapies. For 2026, we're returning to vaccines (online now) and planning for cell therapies, antibodies and their derivatives, cell lines and expression systems, facilities and process intensification, and formulation and fill–finish.

A few other articles herein come from our eBook series, of which we produce two each month. You can find them online at *https://www.bioprocessintl.com/publications/ebooks*. The eBook themes are a bit more specific than our more general featured reports and, as a result, tend to be shorter reads. Examples from 2025 including antibody–drug conjugate technology, bioreactor design, process- and product-related impurities, process intensification, chromatography, autologous and allogeneic cell therapies, and antibody engineering. For 2026, we'll revisit some of those and add new topics such as quality and analytics, outsourced manufacturing, continuous bioprocessing, technology transfer, and viral vectors.

You're probably familiar with the PDF format, but this year we're also offering some eBooks through a new interactive alternative. Don't miss our special benchmarking report for February, for example, which brings together perspectives on the current state of biopharmaceutical business, technology, regulation and more.

You can expect to see the same format for our annual "BPI Theater at BIO" collection this fall. There we summarize and connect you with recordings of the excellent presentations from our special stage in the BIO convention's exhibit hall. With the main program more devoted to business and other concerns, our popular venue is about the only place you can get into the technical details of biopharmaceutical development and manufacturing in San Diego that week.

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CGTs Tackle Alzheimer's Disease

by Millie Hoe

Dementia affects up to 55 million people worldwide; Alzheimer's disease accounts for 60–80% of all dementia cases. The disease burden of dementia is set to double every 20 years, with over 100 million patients expected to be diagnosed by 2050. But cell and gene therapies (CGTs) are catching up. During Cell and Gene Therapy International Europe 2025 (Berlin, Germany), two neurobiology showcases highlighted progress toward treating Alzheimer's disease.

The Alzheimer's "Master Gene": Tetraneuron, a Spanish biotechnology company, announced that its breakthrough gene therapy targeting Alzheimer's disease is preparing to enter phase 1 clinical trials. Tetraneuron's gene-therapy approach to Alzheimer's focuses on E2F4, a transcription factor that plays a central role in multiple pathological mechanisms. Research has linked E2F4 to >7000 genes involved in Alzheimer's disease pathology, including oxidative stress, neuronal cell death, and problems with DNA repair. Tetraneuron's website claims that E2F4 becomes dysfunctional during phosphorylation, and the company's patented molecule (E2F4DN) can restore cellular processes partially. "In animal models, we are seeing the release of factors that are helping to reduce amyloid plaques," noted chief executive officer (CEO) Ángel Lucio Pereira. "We also are able to maintain neurogenesis and prevent oxidative stress and neural death." Tetraneuron also has worked to recover synaptic long-term potentiation (LTP) in neurons. "We are able to recover LTP once it's completely lost," explained Pereira.

Growing Model Brains: Abraam Yakoub, lecturer at Harvard Medical School, spoke on his team's work with human-brain organoids. Yakoub's laboratory aims to reduce amyloid-plaque buildup that can lead to advanced progression of Alzheimer's disease. "We have developed 'constructs' that are undergoing patent processing in the United States," explained Yakoub.

The team's constructs successfully reduced β-amyloid plaque buildup in observed neurons. "The next step is testing in *human-brain organoids,* which are 3D structures reminiscent of the human brain that are grown from Alzheimer's patients' cells. These human-brain organoids do not just develop or express the amyloid-β peptide, but they [also] can develop the aggregate plaques, similar to what happens in human patients." Yakoub's team also tested their constructs in regards to gross effects on behavior and cognitive function in mouse models.

Yakoub's research is certainly promising, but because the nature and design of his team's constructs have not been disclosed publicly, patients may have to wait for a potential cure. However, the gene-therapy space is progressing within neurobiology with breakthroughs in Alzheimer's treatment not far behind.

Senate Blocks FDA Fast-Tracking of Rare-Disease Medicines

by Millie Hoe

A US Senate committee blocked a bipartisan bill to reintroduce the US Food and Drug Administration's (FDA's) Priority Review Voucher (PRV) scheme, a program designed to prioritize review and approvals of treatments for rare pediatric diseases. The bill was introduced to the House Energy and Commerce Committee's health subcommittee on 1 December 2025, in which it passed unanimously. But on 19 December, senators Bernie Sanders (VT-D) and Markwayne Mullin (OK-R) voted against reauthorization. The bill was blocked again after Sanders suggested an amendment to include the funding of community health centers, which Mullin rejected.

"It is disappointing and frustrating that the bill is stuck in the Senate, especially as it has broad support, and the blockage appears to be for reasons unrelated to the provisions of the PRV scheme itself," commented CEO of rare-disease biotechnology company SynaptixBio Dan Williams to *BioProcess Insider.* "The delay causes uncertainty for those smaller biotech [companies] that are developing rare-disease drugs."

"This is not a radical amendment," Sanders announced to the Senate, according to Fox News. "I'm not talking about passing Medicare for All here. I am talking about doing what the Republicans and Democrats agreed to a year ago." Sanders spoke in reference to a bipartisan agreement proposed in 2024 that included the PRV scheme and the "Give Kids a Chance Act."

In response, Mullin referred to Sanders as a "grinch," commenting that "the grinch is stealing kids' lives." He added, "I understand that [Sanders] intends to object to the Senate doing this, and I think it's wrong."

Several nonprofit organizations and patient-advocacy groups also have been critical of Sanders's decision. Nancy Goodman, founder and director of Kids V Cancer, reportedly was shocked by the vote. "Why doesn't our bill deserve support?" she asked in a public letter. "Why don't our kids with cancer and with other life-threatening illnesses deserve care?"

"Passing the bill is a major step forward, and we are grateful to any administration that supports it . . . but it's a question of when and how any further delay impacts the appetite of investors," added Williams.

Millie Hoe *is a reporter at* BioProcess Insider *and* BioXconomy*; millie.hoe@informa.com.*

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Combating Antimicrobial Resistance

Modeling the Untapped Potential of Monoclonal Antibodies

Irina Meln, Quentin Leclerc, Yvan Caspar, and Mateusz Hasso-Agopsowicz

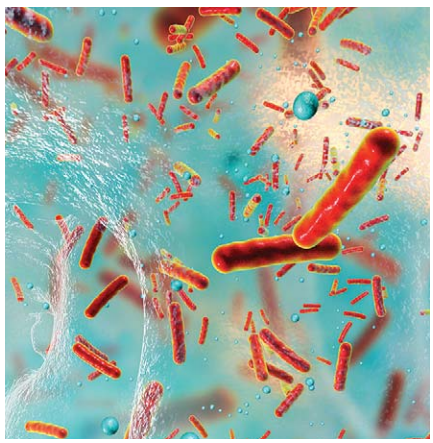
Antimicrobial resistance (AMR) remains one of the most urgent public health threats of our time. By rendering existing antibiotics less effective or even useless, AMR threatens to roll back decades of medical progress, increasing patient mortality and morbidity, prolonging hospital stays, and inflating healthcare costs. The development of new antibiotics is slow and economically challenging, but other biomedical tools such as vaccines and monoclonal antibodies (mAbs) are emerging as potential components of the AMR-response toolbox.

The PrIMAVeRa project — Predicting the Impact of Monoclonal Antibodies and Vaccines on Antimicrobial Resistance — is a five-year initiative funded under the European Union's Innovative Medicines Initiative 2 Joint Undertaking (IMI 2 JU). Bringing together 17 partners from academia, industry, and public health, the program is developing an open-access, web-based dashboard to model public health and economic impacts of such novel interventions on AMR to help policymakers, funders, and developers prioritize the most effective strategies.

Whereas vaccines are receiving increasing attention in public-health modeling, a review by PrIMAVeRa partners revealed that mAbs remain almost absent from AMR models (**1**). The gap is striking given the precision-targeting capability of mAbs and resulting potential to reduce antibiotic use if applied for outbreak control. Understanding why that gap exists — and how PrIMAVeRa's work can close it — is key to unlocking their full potential.

The Dual Role of Biologics

Vaccines reduce AMR mainly by preventing bacterial infections outright and/or by preventing viral infections that can lead to inappropriate antibiotic use and secondary bacterial infections. Vaccines work at the population level to lower pathogen transmission disease incidence/severity, thereby indirectly reducing the need for antibiotics. Overuse of such drugs directly contributes to increasing AMR prevalence.



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By contrast, mAbs work at the individual-patient level through multiple possible pathways:

- neutralizing bacterial toxins (e.g., *Clostridioides difficile* toxin B) or inhibiting their delivery into eukaryotic cells
- reducing pathogen adherence to host cells
- inhibiting virulence factors
- promoting immune-mediated clearance of bacteria either by increasing serum bactericidal activity through the complement system or by opsonophagocytosis mechanisms
- disrupting biofilms that protect bacteria from antibiotics and host defenses
- serving as adjunctive therapy to reduce antibiotic course length or need.

Precision targeting against specific strains and proteins gives mAbs high selectivity, making them less likely than antibiotics to generate AMR. Antibody specificity limits potential effects on nontarget bacterial species, limiting cross-resistance and reducing potential damage to commensal microbiota.

Due to the time needed for a specific immune response to be triggered, some delay follows the act of vaccination before a patient mounts full protection against infection. In the meantime, mAbs can offer immediate but temporary protection or targeted treatment, therefore making them ideal to complement vaccine programs.

Why the Modeling Gap?

In their systematic review of AMR transmission models, PrIMAVeRa partners found no published population-level models that quantify the impact of mAbs on AMR (**1**). That absence is not because mAbs are irrelevant to the problem; it reflects the barriers presented by a number of scientific and practical challenges to their progress.

Limited Clinical and Epidemiological Data: Although several mAbs are licensed for viral diseases, only a handful are effective against bacterial targets. None have been approved yet for the ESKAPE group of highly antibiotic-resistant pathogenic bacteria — *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species — that are responsible for most AMR-related health burdens. Thus, the availability of large-scale data is limited for tracking the population impact of mAbs in AMR-relevant settings. Most bacterial-related mAb data come from trials in specific patient populations, such as ventilated

intensive-care unit (ICU) patients. It is difficult to generalize such results to other populations (e.g., those undergoing hemodialysis) who also might benefit from the intervention but possess different epidemiological characteristics.

Highly Targeted Nature: Each mAb is specific to a certain pathogen or even a particular serotype or virulence factor. The effectiveness of mAbs depends on accurate and rapid diagnostics because the prevalence of targets will vary by setting. Accounting for all factors requires far more detailed and complex modeling for mAbs than for vaccines.

Narrow Deployment Scenarios: Rather than mass prevention, mAbs generally are used for postexposure prophylaxis, outbreak containment, severe infections, and adjunctive therapy. Modeling such highly targeted, complex intervention strategies requires equally complex mathematical algorithms.

Economic Uncertainty: Because of their high production costs, mAbs must be evaluated not just for their clinical potential, but also for relative cost-effectiveness in specific scenarios. The latter type of analysis is difficult because economic parameters vary widely among health systems and often are poorly documented.

Complex AMR Impact Pathway: In addition to treating infections by resistant bacteria directly, an important part of the influence of mAbs on AMR is indirect: By lowering disease incidence, they can inhibit antibiotic use, which in turn should reduce the selective pressure for resistant bacteria. However, the strength of the link between antibiotic exposure and resistance selection itself is difficult to quantify. That leaves substantial uncertainty surrounding the full extent of the importance of mAbs to AMR.

Bridging the Modeling Gap

The PrIMAVeRa modeling framework is designed to integrate both vaccines and mAbs into analyses of AMR intervention. The project's work involves data assembly, mechanistic modeling, economic evaluation, and scenario testing.

Partners are creating a high-quality repository of epidemiological data that includes incidence, prevalence, antibiotic usage, and pathogen-specific resistance patterns for priority bacterial pathogens. We use both deterministic and stochastic mechanistic modeling based on both compartmental and individual-based structures to simulate how interventions affect pathogen transmission, infection outcomes, and resistance dynamics. For the economic evaluation, we combine transmission models with cost-effectiveness analyses to assess the value of interventions under different use scenarios. And finally, different mAb deployment strategies can be tested, such as prophylaxis in outbreak settings, adjunctive therapy to reduce antibiotic courses, and preemptive administration in vulnerable populations. With mAb-specific modules built into a shared platform, policymakers can test different scenarios of hypothetical mAb use.

Target Pathogens for mAb Modeling in AMR: PrIMAVeRa modeling priorities align with the World Health Organization's priority list of antibiotic-resistant bacteria as well as European AMR burden data (**2, 3**). Existing mAb candidates are in development for several key AMR pathogens:

- *S. aureus*, including methicillin-resistant *S. aureus* (MRSA) — with toxin-neutralizing and opsonizing mAbs in clinical trials
- *C. difficile* — with bezlotoxumab approved to prevent recurrence
- *P. aeruginosa* — with mAbs targeting type III secretion-system components
- *K. pneumoniae* — with investigational candidates targeting capsule polysaccharides
- *Escherichia coli* — with some toxin-targeting approaches in development.

Of those, several pathogens currently are being examined within the PrIMAVeRa program. Each target presents distinct modeling challenges based on different transmission settings, patient-risk profiles, and resistance mechanisms.

Most Promising Applications: The most developed PrIMAVeRa case study focuses on MRSA, a leading cause of difficult-to-treat infections worldwide. The framework has identified a number of key mAb applications that could reduce the burden of AMR significantly.

A primary opportunity lies in preventing infections altogether in groups of patients known to be at risk of MRSA infection (e.g., ICU patients at high risk for bloodstream infections and those under mechanical ventilation for pneumonia). Outbreak control is another valuable mAb application that could enable targeted prophylaxis for rapid containment of healthcare-associated MRSA outbreaks.

In addition, mAbs can serve as adjunctive therapies, enhancing the effectiveness of antibiotics against MRSA, improving cure rates, and potentially shortening treatment durations. That would be particularly relevant in the context of biofilm-associated infections. Antibodies that disrupt MRSA biofilms on implanted medical devices could reduce the risk of chronic, antibiotic-resistant infections.

Such strategies position MRSA as an ideal pathogen for demonstrating mAbs' value as an innovative therapeutic tool while providing a model to tackle other priority AMR pathogens subsequently.

Integrating mAbs into AMR Strategy

Including mAbs in AMR strategy modeling is not just an academic exercise; it's essential for evidence-based decision-making. Without reliable models, policymakers are left guessing about potential population-level impacts and return on investment of such interventions.

It is equally important to acknowledge the scientific challenges that make the development of antibacterial mAbs more complex than those for viral pathogens. Viruses often rely on a limited number of receptors that can be blocked to prevent infection; bacteria display much higher diversity of surface structures even within single species. Different serotypes and virulence factors make a single mAb unlikely to provide broad-spectrum protection across all isolates. In many cases, mAb combinations or "cocktails" could be required to provide meaningful coverage of circulating strains. Moreover, targeting just one virulence factor or surface component might not be sufficient to block all bacterial adhesion or pathogenicity, which further complicates product development.

Continued on ***page 15***

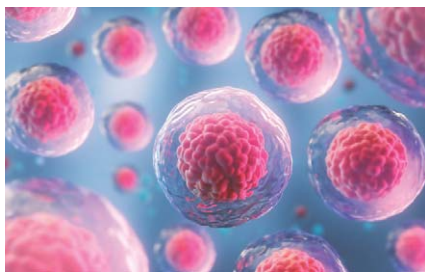
Regulatory Requirements for Human Cell-Line Development and Cell-Bank Manufacture

Steve Kornher

Many biologics used in the prevention and treatment of disease have been manufactured from cell cultures, both primary cell preparations and continuous cell cultures derived from established cell lines. The US Food and Drug Administration (FDA) has specific expectations regarding the selection of human cells for the manufacture of a master cell bank (MCB) to produce a therapeutic drug product. Cell-line selection and cell-bank manufacture typically take place during preclinical development, so this article will focus on selection of a cell line from human cells and preparation of a MCB to be used in the manufacture of a phase 1 clinical therapy.

Regulatory Challenges: Human Cell-Line Selection

The primary regulatory challenge for cell lines sourced from human donors is establishing their safety profiles, therefore confirming a cell line's suitability for use in production processes compliant with good manufacturing practices (GMPs). Potential cell donors must be evaluated to determine whether they are eligible to provide cells. To ascertain eligibility, potential cell donors must respond to questions regarding their relevant social behaviors and undergo medical assessments. Relevant medical histories must be recorded to identify risk factors for (and clinical evidence of) specific communicable disease agents and diseases, including human immunodeficiency virus (HIV); hepatitis B and C viruses (HBV, HCV); human transmissible spongiform encephalopathy (TSE); *Treponema pallidum* (the bacterium that causes syphilis); and human T-lymphotropic virus (HTLV). If reproductive cells need to be collected, then they must be tested for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhea*. Additionally, an assessment of communicable disease risks associated with xenotransplantation is required (**1**).



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Potential donors that meet screening criteria must be tested for specific adventitious agents (**2**). Adventitious agent testing requirements start with screening a donor specimen for evidence of infection by HIV, HBV, HCV, and *T. pallidum* (**3**). Donor specimens testing positive for any of those adventitious agents will result in donor rejection. Cells obtained from rejected individuals cannot be used to establish a cell bank for use in GMP manufacture of a drug product.

Donors of viable leukocytes also must be tested for HTLV and cytomegalovirus (CMV). Materials from donors who test positive for HTLV cannot be used to establish a cell bank. However, donors who test positive for CMV are not excluded automatically from donating cells. Their test results should be reported to physicians responsible for receiving the cells or drug product manufactured from them (**4**). In addition, a standard operating procedure (SOP) that addresses the release of cell banks and drug products manufactured from CMV-positive donor cells must be established by the investigational new drug (IND) sponsor (**5**).

The FDA considers several factors when determining the potential safety risk of a communicable disease or disease agent if infected cells are harvested from a donor for use in cell-bank production:

- likelihood of transmission
- clinical severity to an individual exposed to the disease agent or disease
- availability of screening procedures or tests for the disease agent or disease (**6, 7**).

Based on those criteria, the FDA has established that vaccinia virus (VV), West Nile virus, and agents that cause sepsis — including bacteria, viruses, and fungi — to be relevant and subject to screening measures. Sepsis is a broad category of clinical indications including, but not limited to, bacteremia, septicemia, sepsis syndrome, systemic infection, systemic inflammatory-response syndrome (SIRS), and septic shock (**6**).

Regulatory Challenges: MCB Production from Human Cells

Contamination of an MCB with adventitious agents is a major safety concern and, therefore, is a regulatory challenge that the IND sponsor must address. Contamination can arise from the following scenarios:

- introduction of contaminated raw materials into a manufacturing process; this can arise using "research use only" (RUO)–grade raw materials, which typically are not manufactured or tested to GMP standards
- introduction of animal-derived raw materials (e.g., porcine trypsin or

bovine serum) that have not been appropriately manufactured or tested

• improper execution of the manufacturing process by personnel

• poor segregation of cell-bank manufacturing processes at a manufacturing facility, resulting in bank cross-contamination during manufacture or storage

• improperly designed manufacturing processes (e.g., executing open process steps in areas of the manufacturing suite without proper environmental control).

Sponsors and cell-bank manufacturers must demonstrate a level of control over the MCB manufacturing process commensurate with GMP requirements. That starts with using the highest grade of raw materials available (compendial- or clinical-grade raw materials are preferred). If RUO-grade raw materials must be used, then they should be well characterized, including comprehensive testing for adventitious agents (**8**). An important aspect of qualifying RUO-grade raw materials for use in a GMP manufacturing process is executing small-scale pilot runs of the cell-bank manufacturing process using those raw materials to confirm that they will meet all release criteria, including those related to safety.

If **animal-derived raw materials** are used in MCB manufacture, then the following factors should be evaluated before adopting these materials: geographical origin, species of origin, potential microbiological hazards, donor-animal screening procedures, lot testing, and inactivation and removal steps in the process used to manufacture the raw material. For bovine-derived raw materials (e.g., sera), their manufacturers should test for the following adventitious agents: bovine viral diarrhea virus (BVDV), bovine polyoma virus, bovine circoviruses, rabies virus, bovine adenoviruses, bovine parvovirus (BPV), bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis virus (IBR), bovine parainfluenza virus type 3 (BPIV3), reovirus 3 (REO3), Cache Valley virus, bluetongue virus (BTV), and epizootic hemorrhagic disease virus. Testing raw materials for the presence of bacteria, fungi, and mycoplasma is also essential. Currently no test method adequately detects the presence of the prions that cause transmissible and/or bovine spongiform encephalopathies (TSEs, BSEs). Thus, manufacturers of bovine-derived raw materials must execute risk assessments based on the geographical source of the herds used, type of animal material used in manufacture of the raw material, procedures used to circumvent cross-contamination with high-risk materials, and the nature of the production process, with assurance that the manufacturer has established a quality system to ensure product consistency and traceability. Source animals must be deemed fit for human consumption based on pre- and postmortem inspections (**9**).

Currently, no test method adequately detects the presence of the **PRIONS** that cause transmissible and/or bovine spongiform encephalopathies.

Manufacturing processes for bovine-derived raw materials also should include viral inactivation/clearance steps based on irradiation or heating of those materials (**10**). US regulations stipulate that biologic products manufactured from a cell line must meet specific testing requirements for the detection of cytopathic and/or hemadsorbing agents and extraneous viruses (**11–13**). For sponsors using porcine-derived raw materials (e.g., trypsin) to manufacture cell banks, tests for the following porcine viruses should be conducted: adenovirus, parvovirus, transmissible gastroenteritis virus, and hemagglutinating encephalitis virus (**13**).

When selecting manufacturers of animal-derived raw materials, ensure that they are testing their product in compliance with specific regulatory standards. Confirm that the certificate of analysis (CoA) documents execution of such tests per compendial methods and that those test results confirm suitability of the raw material for use in a GMP manufacturing process.

Users also must confirm that raw materials are accompanied by certificates of origin indicating the geographical location of the source animals harvested for manufacture of raw materials. Some regions pose negligible BSE/TSE risks (class A) and are the preferred sources of biologic materials. Confirm that a BSE/TSE certificate is provided confirming that the raw material meets regulatory requirements to be considered TSE/BSE-free, and verify that the viral-inactivation process adopted by the manufacturer has been validated. Process steps that have not been validated cannot be considered to perform reproducibly from lot to lot of raw material and ensure the identity, purity, safety, and efficacy of the raw material.

A cell-bank manufacturer must have a raw-materials control program overseen by quality assurance (QA) to ensure that all raw materials are certified before they can be released for use in a GMP manufacturing process. Certification requires that each raw material entering a facility comes with appropriate documentation and meets all testing requirements as stipulated in a raw-materials specification sheet.

The risk of introducing adventitious agents during cell-bank manufacture must be addressed by thorough training of personnel on proper gowning methods, required behavior and equipment use in a GMP manufacturing suite, proper passage of raw materials and consumables, and execution of specific processes, including preparation of starting materials. Cell-bank manufacturers must reduce the likelihood of cross-contamination with cells from other sources by using disposable plasticware whenever possible, establishing proper line-clearance methods, and adhering to manufacturing schedules to prevent comanufacturing cell lines in the same suite. The latter strategy ensures multiple cell types are not brought into close proximity.

The FDA recommends that manufacturers test their cell banks for the following viruses: Epstein–Barr virus (EBV); CMV; HBV; HCV; human herpesviruses 6, 7, and 8 (HHV-6, HHV-7, and HHV-8); human polyomavirus 1 (BKV); human polyomavirus JC (JCV); human adenovirus (Ad); human parvovirus B19 (B19V); human papilloma virus (HPV); HIV; and HTLV. Testing for sterility and mycoplasma will be required, preferably using compendial test methods (**14, 15**). Alternate methods for sterility and mycoplasma testing may be considered if they can be justified (**16**). Sponsors should discuss cell-bank testing plans with the FDA before their implementation (e.g., at a pre-IND meeting), especially if the target patient group includes immunocompromised individuals.

In addition to tests for specific viruses, the following tests should be performed (**16**):

- Giemsa staining or spectral karyotyping of metaphase chromosome preparations
- amplification of short tandem repeats in the genome by polymerase chain reaction (PCR)
- inoculation of three cell lines (e.g., MRC5, Vero, and HeLa cells) to detect cytopathic and hemadsorbing/hemagglutinating viruses
- inoculation of embryonated chicken eggs and adult and suckling mice with cell-bank material; alternatively, testing of adventitious agents with a validated high-throughput sequencing method such as next-generation sequencing (NGS)
- transmission electron microscopy for detection of virus particles
- amplification assay for detection of reverse-transcriptase activity (such as the product-enhanced reverse transcriptase (PERT) assay); required if cell banks are grown on feeder layers of nonhuman cells
- species-specific virus testing, if nonhuman-derived materials are used to manufacture the cell bank — for example, if a monoclonal antibody (mAb) is used for an immunoselection step in the cell-bank manufacturing process, then testing for rodent viruses is mandatory, and use of porcine or bovine materials will trigger a battery of virus tests (**13, 17**)
- whole-genome sequencing and analysis, considering that expansion of cell numbers during cell-bank manufacture can introduce genomic mutations, some of them potentially deleterious (**16**)
- assessment of genomic stability and growth characteristics for continuous cell lines; tumorigenic potential of a product must be established using cells from the MCB.

Understanding the **REGULATORY EXPECTATIONS** for cell-bank manufacturers and cell-therapy sponsors is critical to ensuring production of cellular products in accordance with good manufacturing and tissue practices.

Technical details for those safety tests are discussed in the World Health Organization (WHO) guidance document on animal cell cultures for manufacturing biologics (**10**) and the FDA guidance document on qualification of biological materials for viral-vaccine production (**18**). For cell therapies manufactured directly from donor-derived cell banks (e.g., pluripotent stem cells, PSCs), the potential effects of donor-dependent variability on cell-bank quality attributes (e.g., cell doubling time, viable/total cell counts, yield in terms of vials of cell bank produced, identity, potency, purity, and stability) should be assessed. Factors that can contribute to variability include donor medical history, immune status, medication profile, age, sex, and ethnicity. Inconsistency in manufacturing also can contribute to variability in cell-bank quality attributes (e.g., among lots and raw materials) (**19**).

The FDA expects sponsors to evaluate potential effects of variable donor-cell quality on cell-bank quality attributes. A plan of action to address that issue should be discussed with the FDA before IND submission.

Adventitious-Agent Testing of Working and LIVCA Banks

Working cell banks (WCBs) should be tested for adventitious viruses through in vitro test methods or NGS. In vivo testing for specific viruses, retroviruses, or adventitious agents is not necessary if such tests already have been executed on MCBs and limit of in vitro cell age (LIVCA) banks derived from WCBs (**8**). Testing WCBs for sterility, mycoplasma, and identity still will be required (**16**).

LIVCA cells, also known as *end-of-production* (EoP) *cells*, must be subjected to retrovirus and adventitious-agent tests by the in vitro and in vivo tests described above. Such tests can enable detection of viruses that fell below the limits of detection (LoDs) for the MCB tests, viruses that have been activated from a latent state, slow-growing viruses, or viruses introduced into the manufacturing process subsequent to MCB production.

LIVCA should be established by expanding production cells beyond the passage number designated for cell harvest. A scaled-down version of the manufacturing process or a pilot scale production lot could be used for that purpose (**8**). Analysis of EoP cells must be carried out once the scaled-up manufacturing process for cell expansion is locked down, certainly no later than phase 3 trials. Sponsors should consider discussing the plans for such studies with the FDA.

Adventitious-Agent Testing of Unprocessed Bulk: One or more pooled harvests of cells and culture media are considered *unprocessed bulk*. If harvest from cell culture is continuous, then a pooled intermediate or unprocessed bulk sample may be collected from the process flow. The virus tests conducted should be based on consideration of the source cells, the results of the virus tests conducted for cell-line qualification, how the cells are cultured, and whether animal-derived raw materials are used in the manufacturing process (**8**).

Cell-Bank Stability Under Cryogenic Conditions: Many factors can affect the stability of cell banks at cryogenic temperatures, including donor-dependent cell properties, formulation-buffer composition, control of the freezing process when the cell bank is prepared for cryostorage, control of the cryogenic storage temperature, the number of passages the frozen cells underwent at the time of harvest, and so on. Therefore, it is important to establish a stability program for cell banks that assesses critical quality attributes (CQAs) such as sterility (**20**). Note that the FDA is encouraging sponsors to replace sterility testing with container–closure integrity testing for stability assessments (**21**).

Where To Go from Here

Much regulatory literature is available on cell-line selection and cell-bank manufacture. Understanding the regulatory expectations for cell-bank manufacturers and cell-therapy sponsors is critical to ensuring production of cellular products in accordance with GMPs and good tissue practices (GTPs).

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Opportunities and Obstacles for Personalized Cancer Vaccines

Ilona Baraniak-Lang and Anna-Lena Amend

Drawing on successful breakthroughs with preventive messenger RNA (mRNA) vaccines for infectious diseases such as COVID-19, many innovative vaccine developers are turning their attention to personalized cancer vaccines (**1**). Although the business models, product classes, and product designs are completely different, both preventive and therapeutic vaccines are manufactured using the same technology and share the same mode of action (MoA): eliciting an antigen-specific immune response. For cancer vaccines, an immunological response should be triggered that specifically targets an individual tumor's antigens (*neoantigens*), thereby helping a patient's body to recognize and destroy cancer cells while leaving healthy cells unaffected (**2**).

One such vaccine that is most advanced in development is mRNA-4157 (V940), a personalized neoantigen therapy (cancer vaccine) used in combination with the Keytruda (pembrolizumab) immune-checkpoint inhibitor (ICI) to treat patients with resected high-risk melanoma. Results from phase 2b clinical trials for the combination treatment showed a 49% reduced risk of recurrence or death compared with the ICI alone. Currently in phase 3 trials for both melanoma and non–small-cell lung cancer (NSCLC), the treatment is being codeveloped by Moderna and Merck (**3**).

BioNTech — which, like Moderna, has been at the forefront of mRNA COVID-19 vaccine development — also is conducting phase 2 clinical trials with an individualized mRNA vaccine. Autogene cevumeran was developed initially for treating patients with resected pancreatic ductal adenocarcinoma (PDAC), and other solid-tumor indications now are being evaluated in phase 2 studies. BioNTech is developing the drug jointly with Genentech (Roche) for administration along with standard-of-care (SoC) chemotherapy and a checkpoint inhibitor against programmed-death ligand 1 (anti-PD-L1). Results from a phase 1 clinical trial showed "substantial vaccine-induced T-cell responses that may correlate with delayed PDAC recurrence" (**4**).

RNA vaccines program cells to produce tumor antigen proteins that stimulate an immune response.
(HTTPS://STOCK.ADOBE.COM)

Meanwhile, French biotechnology company Transgene is evaluating an individualized neoantigen cancer vaccine (TG4050) for both ovarian and head-and-neck cancer patients who have undergone successful treatment but are at high risk of relapse. In such cases, vaccination offers a promising strategy for prevention of tumor recurrence and disease relapse, leading to meaningful improvement in clinical prognoses for those patient groups. The company has begun including patients in phase 2 studies after promising phase 1 results that showed specific cellular immune responses lasting up to seven months after vaccination (**5**). In a presentation to the 2023 American Society of Clinical Oncology (ASCO) annual meeting, the company stated that its preliminary data "demonstrate that TG4050 is safe, well tolerated, and capable of inducing T-cell responses in nonimmunogenic 'cold' tumors" (**6**).

Increasing Vaccine Immunogenicity

Personalized cancer vaccines rarely are used as standalone treatments. Usually, several additional measures are taken to ensure that adequate immune responses will be induced in vaccinated cancer patients. Examples include the use of adjuvants, special delivery systems such as lipid nanoparticles (LNPs), and the above-mentioned combinations with other treatments, such as ICIs (**7**).

Adjuvants are formulation components that, when administered with antigens, enhance the immunogenicity of the resulting vaccines (**8**). Such a strategy often is necessary because "naked" vaccines (antigens formulated on their own) often are poor inducers of adaptive immune responses, especially in the absence of inflammation or microbial stimulation.

The MoA for personalized cancer vaccines relies on a potent induction of a targeted immune response. With additional treatments or coapplied products, adjuvants can attract more immune cells to an injection site or trigger activation of antigen-presenting cells (APCs) (**8**). Adjuvants typically are classified as either *depot antigens* such as liposomes and emulsions or

immunostimulants such as toll-like receptor (TLR) agonists (**9**). Often, new adjuvants are codeveloped with vaccine products. Although some of them already have been approved — e.g., the Montanide water-in-oil emulsion system — many are still in development and not yet approved for cancer vaccines.

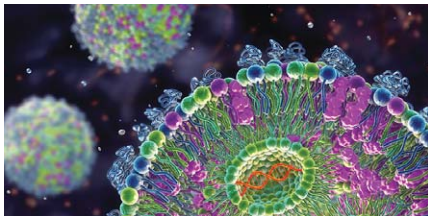
A number of specialized vaccine-delivery technologies are emerging (**10**). Delivery is especially important for DNA and RNA products, which cannot be administered "naked" either orally or intravenously (**11**). Circulating nucleases would rapidly degrade such products in a recipient's blood stream, just as they do to pathogens. Thus, RNA and DNA require delivery mechanisms that both protect and transport them to cells, thus preventing such enzymatic degradation. One option is encapsulation using specific and often proprietary nanoparticles (**12**). Those often serve not only as delivery systems but also as adjuvants to induce or heighten protective immunity (**13**).

Many types of nanoparticles are used in vaccine delivery: e.g., virus-like particles (VLPs), liposomes, and LNPs. Both Moderna and Pfizer–BioNTech use proprietary LNPs to deliver their preventive mRNA vaccines for COVID-19 (**13**), and those companies are building on such experience to produce personalized cancer vaccines.

Cancer vaccines also can serve as adjuvants themselves for ICIs in combination treatments. Based on current understanding of the human immune system and predicted MoA of ICI drugs, such synergistic therapies offer the potential to overcome ICI resistance in patients with cold tumors. An increasing body of evidence indicates that such combination treatments both improve the immunogenicity of cancer vaccines and restrain immunosuppression of ICIs. Thus, such combination therapies also could be effective for cold, unresponsive tumors — which could provide hope for patients who have exhausted all other treatment options (**14**).

Tackling the Challenges

Despite the clear promise of personalized cancer vaccines, companies developing them face a number of challenges from early research through manufacturing through testing and regulatory approval.



Lipid-nanoparticle cancer vaccine delivery system (HTTPS://STOCK.ADOBE.COM)

Barriers to Manufacturing: Chemistry, manufacturing, and controls (CMC) pose some difficulty for developers of personalized cancer vaccines. Personalized medicine follows a nonconventional production process that starts with identification, selection, and preparation of patient-specific input material using next-generation sequencing (NGS) techniques and computational vaccine design that could use artificial intelligence (AI) and machine learning (ML) in the near future (**15**). Applying such technologies brings regulatory scrutiny. NGS analysis must be conducted with validated protocols, on qualified equipment, and by trained staff. And although AI and ML could become integral to the design of personalized vaccines, currently no internationally approved regulatory framework exists for assessing the use of such innovative algorithms (**16**).

Ideally, the in silico protocol should remain unchanged throughout clinical trial phases; however, self-learning systems use data to train and optimize over time. So developers need to balance carefully their system modifications and training against a steady state that makes comparison possible among study participants and across different stages of clinical trials. We recommend that developers discuss with regulators when certain modifications can be introduced and to what extent.

Other key CMC challenges relate to potency assays, sterility testing, stability testing, and product release.

Establishing a potency assay is difficult because neither the treatment nor the disease will be comparable among trial subjects, and no surrogate models exist to investigate bioactivity of personalized cancer vaccines. Thus, standard potency assays are infeasible, so alternative solutions must be discussed with regulators early in product development.

Sterility testing can create a process bottleneck with tests that require long turnaround times and thus delay product release. Some alternative solutions have been proposed. Following the principles of real-time or parametric release, testing is not performed on each batch, but rather depends on demonstrating that predetermined, validated, sterilizing conditions have been achieved throughout a manufacturing process (**17**). Rapid sterility testing may be another alternative (**18**). And "sterility by design" concepts have been introduced, by which a manufacturing process is defined and documented to minimize risks of microbial contamination (**19**).

Stability testing presents challenges during development because a personalized cancer vaccine can be produced only after a patient enrolls in a trial (or enters treatment once a product has been approved). Potential approaches include testing a whole spectrum of possible product designs for stability. For example, peptide developers might test the most hydrophobic and the most hydrophilic variations. Another possibility might be providing a mix of stability data from engineering and clinical batches manufactured using the same process.

Nonclinical Challenges: One obvious challenge for personalized treatments is a lack of animal models available for preclinical testing. Each patient's tumor is unique, and each vaccine would be personalized. That obviates the application of classic nonclinical programs that rely on studying a product (typically bulk produced) in appropriate animal disease models — e.g., mice — to evaluate pharmacokinetics, pharmacodynamics, and toxicology.

Although use of specific in vivo models is extremely limited, cancer-vaccine developers can use surrogate models (e.g., mice with inoculated tumors). A mouse-specific surrogate product should be designed that targets the specific neoantigens of the inoculated tumor cell line. Such experiments can provide proof of concept and some information about safety and efficacy. Nevertheless, these studies ultimately are of limited relevance because they test surrogate products that target inoculated tumors rather than patient-specific products that target patient-specific tumors.

Toxicology studies are so complicated for personalized medicines that they may be **IMPOSSIBLE** to conduct because testing every individual product (and patient) would be infeasible.

Toxicology studies are so complicated for personalized medicines that they may be impossible to conduct because testing every individual product (and patient) would be infeasible. Such studies would not reveal toxicological effects based on immune reactions for personalized vaccines because both the drug product and the disease are different for each patient.

Note too that adjuvants often are used together with personalized vaccines (**7**). If an adjuvant is already in clinical use, or if data are available (e.g., published by another organization), then such information could be leveraged. However, if no data are available on a new adjuvant, then it is likely to require additional safety studies.

Clinical Challenges: Identifying the right patient population is a significant challenge with cancer vaccines. Patients often are terminally ill and heavily pretreated, having failed several lines of treatment already. Chemotherapy, a common first-line therapy, induces immune suppression. Yet for a personalized cancer vaccine to elicit an immunological response, that person's immune system must be functioning to some extent (**20**). If it is not, there will be no vaccine response, and the clinical trial endpoints will fail. A key step for developers is to set the right inclusion/exclusion criteria for each clinical trial.

The design, manufacture, and release of a personalized cancer-vaccine product begins only once a patient has been enrolled in a trial, which delays the start of the treatment. Patients often deteriorate further while waiting for a vaccine dose to be ready, even if the turnaround from biopsy to administration is rapid. Usually, developers target a turnaround period of under three months. During that waiting period, patients must be monitored closely.

Accompanying treatments present further difficulties. Late-stage cancer patients often require additional treatments to manage symptoms such as pain. It is important to define which additional treatments will be permitted and which will not, keeping in mind the natural history of a given disease and its associated side effects. Such specifications enable reliable evaluation of endpoints and appropriate trial-data analysis to identify the effects of an investigative treatment. For terminally ill patients, however, it would be unethical to withhold treatments that manage symptoms even if such treatments might compromise data analysis.

No single established treatment regimen will work for all personalized treatment approaches and/or all types of cancer. The many factors to consider when planning first-in-human studies include

- dose selection
- the number of neoantigens to include — which varies widely (4–20) in current trials (**21**)
- immunization schedules (consider the total amount of immunizations and the time between them)
- mode of administration — e.g., intradermal, subcutaneous, and intramuscular, as well as potential use of needle-free jet injectors or other devices
- uses of adjuvants or incorporation into combination treatments
- booster-shot protocols after initial treatment (e.g., timing of treatment-free periods and required numbers of boosters).

Regulatory Challenges: Some major differences in classification set Europe and the United States apart for developers of personalized cancer vaccines. The US Food and Drug Administration (FDA) classifies such products as *therapeutic cancer vaccines*; no comparable classification exists in the European Union, where products instead are classified according to their composition. Most are considered vaccines or chemical medicinal products, but some products (e.g., those composed of nonsynthetic nucleic acids) will be considered advanced-therapy medicinal products (ATMPs) and thus be subject to applicable regulations. Regulatory changes currently under discussion could lead to classification of all products containing synthetic nucleic acids as ATMPs. That would place the majority of cancer vaccines under the ATMP regulations in Europe (**22**).

Helping Patients Fight Cancer

Despite the many challenges to overcome, successes shown by a number of personalized cancer-vaccine products already in late-stage clinical trials and administered under compassionate use bring renewed hope to patients with hard-to-treat cancers. Many more products are yet in early development, offering great promise for advanced treatments in the future. As new ways to address the nonclinical, clinical, and CMC difficulties emerge — and as regulatory pathways become clearer — the industry can help provide more personalized cancer vaccines to the patients who need them.

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The PrIMAVeRa platform will offer the following for the first time:

- quantitative estimates of the potential for mAbs to reduce antibiotic use and resistance rates
- economic analyses to support reimbursement and procurement decisions
- scenarios to help optimize mAb deployment strategies alongside vaccines and other measures.

Addressing the current evidence and modeling gap will pave the way toward rational integration of mAbs into AMR control strategies.

An Important Role to Play

Antibodies are a scientifically potent but operationally underexplored weapon against AMR. Their precision targeting, low potential to drive resistance, and suitability for high-risk patient groups and nosocomial outbreak settings make mAbs an attractive complement to vaccines and antibiotics. Without robust transmission and economic models, however, their true value remains speculative.

The absence of mAb-focused AMR models reflects both data scarcity and use-case complexity, a shortcoming that the PrIMAVeRa program is designed to address. By combining high-quality data, advanced modeling, and economic evaluation, the project will deliver a flexible public platform to help global health decision-makers understand when, where, and how mAbs can make the greatest contribution toward curbing AMR. In doing so, we are not just modeling interventions, but also the future of biologics in AMR control. Backed by solid evidence and real-world applicability, the PrIMAVeRa project can help to ensure that promising technologies can be implemented where they will help most.

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From Crops to Cures

Intellectual-Property Considerations for the Emerging "Pharming" Industry

Bree Vculek and Paul Calvo

Using genetically modified plants, animals, or microbes as living bioreactors, "pharming" is poised to disrupt biopharmaceutical manufacturing. Although bioproduction methods in living systems have their difficulties (e.g., variability from environmental conditions), pharming offers faster scale-up, lower costs through reduced cold-chain logistics, and lighter environmental footprints than are associated with conventional protein expression systems, such as engineered Chinese hamster ovary (CHO) cells. Such systems can be designed to consume less water and energy while minimizing waste, reducing contamination risks and carbon emissions (**1**).

Analysts forecast robust growth across pharming sectors. Estimates predict that the global biotechnology industry could exceed US$3.5 trillion by 2033 (**2**), with plant, animal, and microbial pharming each showing a steady compound annual growth rate (CAGR) of 7–10% (**3–5**). Sitting at the crossroads of agriculture, pharmaceuticals, and biotechnology, pharming also introduces a uniquely complex intellectual property (IP) landscape that demands thoughtful and robust protection strategies.

Innovation entails not only developing new drugs, but also engineering novel organisms and cultivation/purification methods. Protecting such multifaceted inventions requires strategic IP management that might involve patents and other safeguards for trade secrets. For example, a "pharmed" crop might itself be a patented variety, raising "essentially derived" variety questions under plant breeders' rights (**6**). Freedom to operate must be evaluated across patents on seeds, transgenes, and even farming processes, while overlapping agricultural and pharmaceutical regulations add further complexity.

Plants such as *Nicotiana benthamiana* (pictured) that have been engineered for "pharming" are poised to disrupt biopharmaceutical manufacturing with their fast scale-up, low costs, and light environmental footprints.

Innovation for Plant-Based Pharmaceuticals

Genetically engineered plants could revolutionize drug production by serving as factories for therapeutic proteins, vaccines, and enzymes. Transgenic crops enable low-cost upstream production and significant potential for scale-up through open-field cultivation. Such benefits could reduce costs dramatically if appropriate downstream-processing methods are implemented. For example, seed-based expression systems can provide a favorable environment for storing biomass containing recombinant proteins for long durations at ambient temperatures, thus uncoupling upstream production from downstream processing and facilitating purification on demand (**7**).

A commercially successful example of plant-based recombinant protein production is Medicago's *Nicotiana benthamiana* platform, which enabled production of the Covifenz COVID-19 vaccine in 2022 (**8**). The rapid development and Health Canada approval of the vaccine platform underscores how quickly plant systems can be used as additional avenues for therapeutic production in response to pandemics (**8**). Likewise, plant-made influenza vaccine candidates have shown strong immunogenicity, validating transgenic crops' capacity to create viable human vaccines (**9**).

From an IP perspective, plant-based production enables innovators to patent not only final products, but also enabling technologies. Medicago, for instance, received patents for expression enhancers to boost transgene yields (**10**), protein-extraction technologies to improve purity (**11**), and glycoengineered *Nicotiana* lines to produce humanized proteins (**12**). However, patent applications become publicly available after 18 months. Therefore, a competitive edge can be gained by keeping certain aspects of production and purification systems secret.

Animal Pharming: Biofactories of the Future

Animal pharming uses transgenic livestock such as goats, cows, chickens, and rabbits to produce therapeutic

proteins in their milk, eggs, or blood. Transgenic livestock can be highly efficient and cost-effective "bioreactors" because they can produce large quantities of complex therapeutic proteins that are biologically active and correctly folded (**13**).

One leading success is Pharming Intellectual Property BV's Ruconest recombinant complement protein 1 (C1) esterase inhibitor, a drug produced in rabbit milk and approved by the US Food and Drug Administration (FDA) to treat hereditary angioedema (**14**). Similarly, goats and cows have been engineered to express antibodies in their blood and secrete proteins such as antithrombin in their milk, offering the potential for high-volume biologic production.

Related patents cover DNA constructs, promoters, modified animal lines, and purification methods. The developer of Ruconest protects both the recombinant protein and the transgene used to enable its expression in rabbit milk (**15, 16**). In addition to patents, trade secrets often safeguard breeding techniques and herd-management practices. Internationally, animal pharming raises additional hurdles, however. Some jurisdictions restrict patents on higher lifeforms, pushing companies to focus their claims on genetic sequences or methods. A layered IP strategy to balance patents and know-how is therefore essential.

Strategic IP Protection for Pharming Companies

Whether a large biopharmaceutical company or an emerging agritechnology start-up, all pharming organizations must be deliberate in protecting their innovations. The goal is twofold: to secure rights that attract investment and partnerships and to maintain a lasting competitive edge. Such a balance requires a thoughtful mix of patents and trade secrets.

Securing Exclusive Rights and Licensing Opportunities: Patents remain the backbone of pharming IP strategy. They provide exclusivity, create valuable licensing opportunities, and reduce investor risk by signaling market control. Because pharming platforms are designed to be versatile, innovators must look beyond narrow product claims to pursue broader protection, covering host organisms, expression cassettes, purification systems, and other enabling technologies.

> A vaccine grown in tomatoes or a therapeutic antibody expressed in a chicken's egg is more than a breakthrough product — it represents the **CONVERGENCE** of agriculture, biotechnology, and pharmaceuticals.

For example, Medicago's portfolio layered protection around its plant-based vaccine technology. That protection currently extends to Aramis Biotechnologies, which inherited the former company's IP in the wake of its 2023 closure. *Diamond v. Chakrabarty* (1980) confirmed the patentability of genetically engineered organisms in the United States; however, that is not the case in many other jurisdictions. Strong portfolios not only deter competitors, but they also underpin strategic partnerships and licensing deals that expand commercial reach.

Protecting Trade Secrets Behind Platforms: Not every innovation should be patented. Competitive advantages such as optimized growth conditions, purification recipes, cultivation techniques, processing steps, and stability enhancements may be better safeguarded as trade secrets. Unlike patents, trade secrets can last indefinitely if confidentiality is maintained.

For pharming, where yield optimization and process efficiency are critical, the most effective strategies combine patents for core inventions with trade secrets for refinements. Protecting such information requires restricted access, robust internal protocols, and confidentiality agreements with employees, collaborators, and suppliers. Those practices can ensure that know-how remains proprietary while still enabling collaboration.

Building Value Through Collaboration: Because pharming is a cross-discipline involving agriculture, pharmaceuticals, and biotechnology, partnerships are essential. Clear IP ownership and enforcement clauses are as critical as the patents themselves. iBio's FastPharming platform illustrates that principle: Collaborations have yielded new patents on therapeutic antibodies and glycosylation modifications while leveraging trade secrets to protect platform know-how (**17, 18**).

Cross-licensing and joint development can accelerate innovation, but agreements must ensure that resulting technology confers a competitive edge, either through exclusivity or favorable licensing terms. Given the overlap between agricultural patents on crops and pharmaceutical claims on biologics, rigorous freedom-to-operate analyses are especially important in the transgenics space.

Protecting Innovation Across Borders: A vaccine-producing crop might be engineered in one country, cultivated in another, and sold worldwide. Companies therefore should leverage the World Intellectual Property Organization (WIPO) to secure coverage in multiple jurisdictions, while tailoring filings to national laws that may restrict patents on living organisms. At the same time, trade secrets can provide a backstop, ensuring that even if protections differ across borders, proprietary know-how remains shielded from competitors.

The Future of Pharming with Smart IP Strategy

Pharming stands at the innovative intersection of farming and pharmaceutical production, offering a bold vision for the future of drug manufacturing. Its applications — e.g., to create vaccines in greenhouse-grown plants and therapeutic enzymes in goat's milk — could reshape global healthcare by making essential medicines more accessible and sustainable than before. As the field rapidly evolves from intriguing experiments to commercial reality, companies at the forefront recognize that technology alone is not enough; it must be matched with astute IP strategy.

Continued on ***page 46***

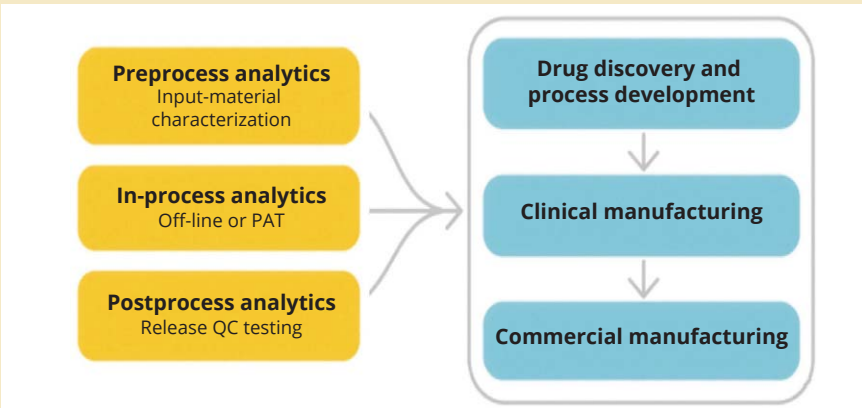
From Complexity to Control in Cell and Gene Therapy Analytics

Olympia Pachoumi

Cell and gene therapies (CGTs) have redefined the treatment landscape for many indications, but development and manufacturing remain uniquely challenging. They involve variable starting materials and complex, small-scale processes. As the CGT field pushes for faster development, more scalable manufacturing, and greater cost-efficiency — without compromising quality or safety — there is a clear shift toward automated, closed-system platforms. However, automation alone is not enough.

Extracting insights to support decision-making, enable process control, and facilitate rapid, cost-effective release testing will be crucial to accelerating delivery to patients. Those activities all will depend on effective analytics, which thus will be positioned at the heart of the CGT field's transformation.

Figure 1: Analytic tools used at different stages of cell and gene therapy development; PAT = process analytical technology, QC = quality control



The Importance of Analytics for CGT Development

The inherent complexity and variability of CGT processes necessitate a strategic and integrated approach to analytics. Variability in starting materials complicates process understanding, making it difficult to link inputs to product performance consistently. Simultaneously, the lack of deep biological insight into CGT mechanisms of action demands extensive in-process data to characterize and control manufacturing effectively.

The uniqueness of CGT products makes release quality control (QC) particularly complex, requiring rigorous analytical testing. Among other factors, such drugs have modality-specific critical quality attributes (CQAs), require specialized and therapy-specific potency assays, and cannot undergo filtration without compromising their function.

To help frame the spectrum of analytics used for CGT development, I categorize such technologies into three functional groups, with tools serving different purposes at each stage of a product life cycle (Figure 1).

Discovery and Process Development: Developers often lack clarity on which inputs and parameters are critical, necessitating high-fidelity measurement and monitoring of many variables. Capturing detailed data on input characteristics, process parameters, noise sources, and product outcomes helps analysts to build models that reveal key input–output relationships. Such efforts enable early control implementation to reduce variability and identify poor runs, shortening development timelines and lowering costs to achieve a robust, reproducible clinical process.

Clinical Manufacturing: Efficient production and variability control become critical due to high costs for scale-up and clinical trials. A comprehensive analytics dataset linking starting material, process parameters, and quality attributes helps analysts to uncover connections to clinical outcomes, improving CQA selection and prediction of product performance.

Commercial Manufacturing: At this stage, analytics refine the most relevant critical process parameters

PRODUCT FOCUS: Cell and gene therapies, viral vectors

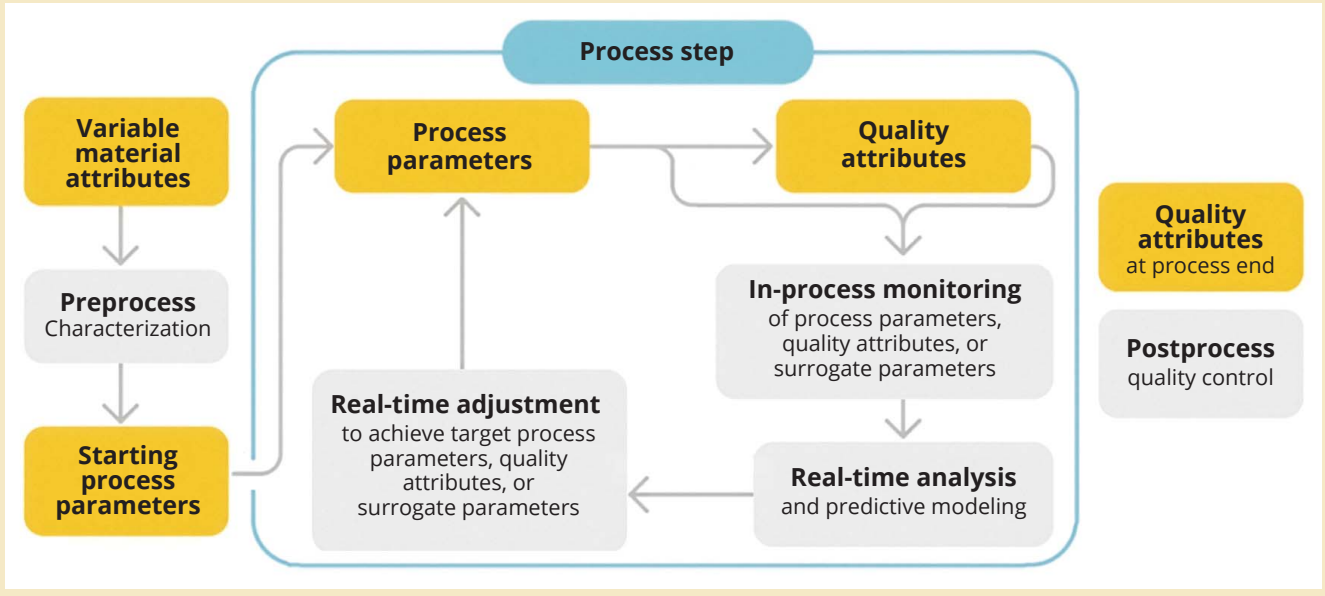
PROCESS FOCUS: Characterization

AUDIENCE: Process development, analytical, QA/QC, and manufacturing

KEYWORDS: Process and product characterization, process analytical technology (PAT), real-time analytics

LEVEL: Basic

Figure 2: Process development for cell and gene therapy production



(CPPs) and CQAs, with fit-for-purpose tools supporting real-time monitoring and predictive insights. Process analytical technology (PAT) enables manufacturing based on cellular activity instead of fixed schedules. That consideration is especially important for autologous cell therapies. Robust, scalable release QC ensures identity, potency, and safety for each batch (Figure 2).

Although significant progress is being made in leveraging analytical methods for CGT development, substantial technological advancements still are required to unlock their full potential. A major difficulty is the lack of analytical technologies that are fit for CGT processes. Many developers rely on legacy tools from traditional biologics analysis and academic research, which were not designed for the unique demands of CGT products. Traditional assays often require large sample volumes and long turnaround times, both of which are problematic for the small batch sizes and time-sensitivity associated with CGTs.

Additional obstacles remain: Most current tools are manual and difficult to integrate into CGT manufacturing workflows. Availability of real-time, in-line analytics for critical cell attributes remains limited, restricting process control. Release testing is complex and labor-intensive, often delaying product availability by 7–10 days, which might be incompatible with products that have short shelf lives. Many methods are complex, prone to variability, and dependent on specialist skills. And sampling needs can add strain to availability: With batch volumes as low as 50–150 mL, off-line testing can consume significant portions of a product, increasing costs and reducing yield.

Perhaps the most significant hurdle lies in converting measurements into actionable insights. Building predictive models requires large datasets, advanced analytics, and robust information technology (IT) infrastructure. Such resources are expensive and not widely accessible.

Unlocking the full promise of analytics in CGT will require development of fit-for-purpose tools that enable automation, work quickly with low sample volumes, facilitate use, and integrate easily into workflows.

Interviews with Industry Leaders

My team spoke with industry leaders to explore how the sector might realize the full potential of analytics in advancing CGT development and manufacturing. Our interviewees included Sharon Barkatullah, who is head of analytical development at the Cell and Gene Therapy Catapult (CGT Catapult), an independent not-for-profit organization in the United Kingdom dedicated to accelerating industrialization of advanced therapies. Among its many initiatives, the CGT Catapult leads collaborative innovation programs, including several with a strong focus on solving analytical challenges within the CGT industry.

We also spoke with Rachel Legmann, senior director of technology for gene therapy at Repligen. The company provides advanced technologies and analytics for production of plasmid DNA (pDNA), viral vectors, and messenger RNA (mRNA), enabling scalable, high-yield, and quality-controlled manufacturing.

Our final interviewee, Antoine Espinet, is chief executive officer of MFX, an early stage start-up developing automated bioreactor systems with integrated analytics. The company is leveraging its process-development–stage instrument to enable scalable cell-therapy manufacturing, ensuring reproducibility going into clinical and commercial activities.

Here, I highlight three areas of ongoing analytical innovation: characterization of starting material, process characterization and monitoring, and release QC. Improvements in these areas hold promise for enhancing efficiency, control, and insight across the CGT life cycle.

Input Material Characterization

Autologous Cell Therapy Donor Material Benchmarking: In autologous cell therapies, donor

material is foundational. Variability in starting material affects every aspect of the process, from manufacturing success to clinical efficacy (**1**). Factors such as donor age, disease burden, immune history, and leukapheresis-collection method (e.g., duration, access type, and patient tolerance) all influence the composition and quality of harvested cells. If such variability cannot be normalized, then a process might need patient-specific adjustments to ensure consistent product quality.

However, it remains unclear which attributes of input material matter most and how they influence manufacturing and clinical outcomes. That challenge is amplified by the high level of noise in small, patient-specific datasets, making statistical correlations difficult to determine.

Deep characterization tools such as flow cytometry, next-generation sequencing (NGS), mass cytometry, and multiomics analyses can assess T-cell subsets, surface markers, and phenotypes. Though powerful, those tools are expensive and require skilled operators, limiting routine use.

One solution, Barkatullah observed, is to create consortia that generate large, shared, intellectual property (IP)–neutral datasets linking input-material attributes with process and clinical outcomes. The CGT Catapult is undertaking such an initiative to define what constitutes "high-quality" donor material (**2**). That work and similar efforts eventually could enable targeted, one-off assessments to improve therapeutic-cell viability and to optimize individualized manufacturing strategies (**3**).

Plasmid QC: In gene therapy, pDNA is a foundational component for viral-vector production. Variability in plasmid quality can affect transfection efficiency, viral yield, and overall product consistency.

Although suppliers often provide a certificate of analysis (CoA) for such materials, contract development and manufacturing organizations (CDMOs) sometimes develop plasmids in house and perform internal QC to ensure consistency. Legmann noted, "Some developers just rely on the CoA and move forward, but if something goes wrong, it delays everything." She recommended that developers conduct independent QC despite having a CoA. "Evaluating [materials] upfront reduces the risk of failure," she said, and addressing issues proactively can save time and costs downstream.

QC of plasmids typically accounts for genetic identity, purity, percentage of supercoiled DNA, and residual impurities. Relevant tools include gel electrophoresis (GE), high-performance liquid chromatography (HPLC), ultraviolet-light (UV) spectrophotometry, and sequencing methods. Because pDNA QC happens before vector production begins, speed is less critical for plasmids than it is for therapeutic manufacturing, and current analytical tools are generally adequate.

Legmann recommended performing viral-clearance validation for all raw materials entering a process, including cell-culture media, transfection reagents, and cell activators. Although many reagents can be tested with existing technologies, some — such as transfection reagents — remain difficult to analyze because of complex compositions or proprietary formulations. Current tools might not assess such materials fully, which highlights a need for better analytical strategies in this area. As Legmann put it, "Raw material is extremely important, not only at the beginning, but all along the process. That's why you have to build a risk-mitigation strategy to monitor and control every material going into the process, including plasmids, media, and transfection reagents."

Process Characterization and PATs

Process development involves measuring key parameters such as pH, dissolved oxygen (DO), temperature, metabolites, and product attributes (e.g., cell count, viability, yield, and potency). Whether taken off, at, in, or on line, such measurements guide iterative protocol adjustments.

Instrumentation for Bioprocess Design and Development: Biomanufacturing involves multiple complex steps during which analytical data can be collected and assessed. However, establishing meaningful correlations between process parameters and outcomes remains complex and time consuming. Key limitations slowing process development include inflexible bioprocessing tools and the tendency of developers to optimize processes based on attributes that do not influence clinical outcomes significantly, so those attributes might not be "true" CQAs.

To address the limitations of traditional bioprocessing tools, Espinet said, MFX developed a scalable bioreactor platform that supports high-throughput parallelization and flexible control of process parameters. The platform integrates on- and in-line analytics, such as means for real-time cell imaging and metabolite measurement. It also is compatible with at-line measurement and small-volume sampling for external analysis. The system, designed to be what Espinet called a "DoE [design of experiments] machine," provides for systematic adjustment of inputs and measurement of outputs in small-volume bioreactors that are representative of manufacturing-scale vessels — thereby streamlining DoE studies and accelerating process development.

In a study of expansion steps for regulatory T cells conducted with AstraZeneca, MFX demonstrated how real-time analytics and control of agitation and feeding strategies enabled adaptive responses based on metabolic and phenotypic data, leading to efficient, data-driven optimization of the bioprocess under examination (**4**). "Once you know what you're looking for, you can start to zoom in on the analytics that are required to control that quality attribute — and use that to control your manufacturing," Espinet explained. "Control loops that drive manufacturing toward your product quality targets ultimately result in the best outcomes for patients."

Another hurdle in implementing PAT is that, during early process development, developers often do not know which parameters or CQAs are most predictive of clinical outcomes. Thus, CQAs can change over time. Barkatullah of the CGT Catapult highlighted a shift in the industry

toward building deeper process understanding and in-process control through more frequent — and ideally real-time — monitoring using at-, on-, and in-line analytics.

The CGT Catapult's analytical-development group is tackling that challenge by creating a deep, comprehensive database for in-process analytics. Using tools for transcriptomics, metabolomics, proteomics, and protein-expression profiling, the organization intends to identify biomarkers that predict batch quality and product efficacy. Once predictive attributes are identified, the goal is to develop fit-for-purpose analytics systems that directly or indirectly (through surrogate metrics) measure those markers, perhaps on/in line or rapidly at line. Then, the team will pursue targeted assay development to create rapid, robust, and scalable QC tools for batch release. Such work is meant to derisk adoption of advanced analytics by generating tools and insights for industry-wide use.

Barkatullah said, "This is an approach that we have been working on for several years — establishing the fundamental capabilities, working with various therapy and tool-development companies." She reported that her team is "moving into the next collaboration phase, and the sector is really embracing this approach due to the clear need. Once biomarkers are identified, [our analysts] could move from characterization tools into release criteria — helping eventually to predict whether a batch will perform well clinically."

Toward Continuous, Closed-System Processing: The monoclonal antibody (mAb) manufacturing industry, being more mature than that for CGTs, serves as a strong precedent for the benefits of continuous processing. Recent studies show that such a strategy can reduce production costs by up to 35% at lower annual scales than those associated with batch and fed-batch processes (**5**).

Integration of artificial intelligence and/or machine learning (AI/ML) and advanced analytics has enabled real-time monitoring and process control, enhancing consistency, efficiency, and product quality while improving access to mAb therapies. Continuous processing and analytics are becoming a scalable pathway to meet rising global demand while maintaining economic viability.

The benefits of continuous processing could be even more significant for CGTs considering such products' high variability and complexity. However, its application becomes especially challenging for autologous cell therapies, which by their nature require small-batch production. Realizing the potential of continuous methods for such therapies will require purpose-built, closed, sterile, and automated systems, incorporating modular single-use technologies. Integrated on-/in-/at-line analytics that are fast enough to control manufacturing based on feedback, seamless data acquisition, and good manufacturing practice (GMP)–ready control systems will be essential to supporting adaptive operation and driving the transition to continuous processing in CGT manufacturing.

Continuous Viral-Vector Production: Repligen's PAT solutions are designed to optimize performance across both upstream and downstream workflows. Perfusion can be applied for continuous upstream production of lentiviruses and other enveloped viruses. Integrating perfusion systems with capabilities for on-line metabolite analysis enables continuous clarification of feeds as they move into downstream process steps.

A compelling example of PAT-enabled continuous viral-vector production comes with integration of Repligen's Maven (**6**) and Maverick (**7**) analytics tools, which the company recently acquired from 908 Devices. The Maven system performs real-time glucose and lactate monitoring. As an in-line instrument for Raman-spectroscopy–based bioprocess analysis and control, the Maverick system provides real-time insights into critical process parameters (CPPs). Legmann explained, "If we want to reduce cost [for CGT products] without compromising safety, there is no doubt that the field will [need to] move faster into continuous processing than mAbs did."

Continuous mRNA Encapsulation: Another compelling example of PAT-enabled continuous processing comes from a Repligen collaboration with a CDMO manufacturing lipid nanoparticle (LNP)–encapsulated mRNA — a growing alternative to viral vectors due to mRNA's cost-efficiency. Repligen integrated its FlowVPX system for variable–optical-pathlength ultraviolet–visible light (UV-vis) spectrophotometry directly into the production line to enable real-time quantification of mRNA concentration and in-process impurities (**8**). On-line measurement supported continuous processing and reduced reliance on traditional off-line testing. The FlowVPX system also enabled assessment of encapsulation efficiency at the end of the process. By embedding its analytic capabilities on line, the CDMO proactively managed and reduced the risk of batch failure at release.

Realizing the potential of continuous methods for cell and gene therapies will require **PURPOSE-BUILT,** closed, sterile, and automated systems, incorporating modular single-use technologies.

Advancing PAT Integration: Progress toward continuous processing in CGT will hinge on seamless integration of nondestructive PATs. Many current sensors for pH, DO, and metabolite levels were adapted from traditional bioprocessing, and they often require large sample volumes, frequent calibration, and complex modeling, making them poorly suited for small-scale, aseptic CGT workflows. Modest redesigns and emerging innovations are addressing such limitations and facilitating integration.

Sterile connectors, presterilized single-use probes, and noninvasive sensors are enabling closed-system compatibility. For example, PreSens single-use, noninvasive optical sensors for oxygen and pH monitoring (from PreSens Precision Sensing) weld directly into cell culture bags (**9**). Single-use

A promising strategy for real-time monitoring of cell attributes involves **SURROGATE MARKERS,** PAT-measurable indicators that correlate with cell identity, function, and/or quality.

flow cells can be added to perfusion lines for real-time monitoring. And Repligen's Maven technology integrates single-use probes with bioreactors to provide critical data for cell-culture optimization and control.

Raman spectroscopy is gaining traction as a noninvasive in-line tool for tracking nutrients and metabolites (e.g., glucose, lactate, and ammonium) through chemometric models. However, adoption remains limited by calibration needs and data complexity. Solutions such as MilliporeSigma's ProCellics system simplify Raman monitoring through integrated software and technical support (**10**). Repligen's Maverick system applies Raman spectroscopy to measure multiple parameters in real time, enabling continuous monitoring and control of bioprocesses across different bioreactors and cell lines.

Addressing Gaps in Real-Time Analytics for Cell Characterization: Many CGT-specific attributes related to identity, potency, and function still lack suitable sensing solutions, particularly for on- or in-line cell characterization. Such technologies could yield major cost and efficiency gains. Currently, no tools can provide real-time, noninvasive analytics for key metrics such as total and viable cell counts, cell identity, CD4:CD8 ratios, vector titer, full:empty vector-capsid ratios, and impurity levels. Instead, cell characterization requires complex, typically off-line assays using such methods as flow cytometry, polymerase chain reaction (PCR) or Droplet Digital PCR (ddPCR, Bio-Rad Laboratories), and enzyme-linked immunosorbent assays (ELISAs). Those methods usually involve manual sampling, are slow and labor intensive, disrupting manufacturing. They are also difficult to miniaturize or automate for in-line and real-time use.

Reflecting on needs for cell characterization, Espinet said, "It's about streamlining the whole development life cycle and manufacturing workflow through data. Once you do that, you can shave days off production, reduce costs, and accelerate time to market. That's why analytics will be a game-changer."

"PATable" Surrogate Parameters: A promising strategy for real-time monitoring of cell attributes involves surrogate markers, PAT-measurable indicators that correlate with cell identity, function, and/or quality. A strategy proposed by the CGT Catapult is to combine high-throughput, high-content screening methods such as flow cytometry, gene-expression profiling, liquid chromatography–mass spectrometry (LC-MS), and sequencing methods with PAT-compatible technologies such as Raman spectroscopy to determine robust correlations between complex biomarkers that are measured off line and simpler analytes measured in/on/at line. The CGT Catapult is using such outputs to develop digital twins and enable advanced process-control strategies.

Some organizations are using cytokine expression as a surrogate for infectious titer, a critical metric in viral production that traditionally has been assessed through slow, manual assays. A recent study by Hsieh et al. showed that cytokine expression within hours of infection correlates strongly with infectious titer (**11**). Platforms such as Bio-Techne's Ella system for automated ELISAs enable fast, at-line cytokine measurement to support timely process control based on early infectious-titer insights (**12**).

Contactless, Label-Free Technologies: To overcome cell-characterization limitations, the CGT field is turning increasingly to technologies that are particularly suited for real-time monitoring. Rapid, label-free, and noncontact sensing modalities offer the most potential for on-/in-/at-line integration. Options include methods for optical and holographic imaging, electrical impedance, Raman spectroscopy, UV-vis spectroscopy, near-infrared (NIR) spectroscopy, and Laser Force Cytology (LFC, LumaCyte) methods. Integration with microfluidic devices, flow cells, and dynamic sampling platforms can enhance such methods' capabilities.

Emerging methods for AI-based image interpretation — e.g., systems combining two-dimensional (2D) imaging, microfluidics, and computer vision (**13**) — are enabling low-cost, real-time cell characterization. In such systems, microfluidic devices allow for precise, high-throughput manipulation of cells while 2D imaging captures cells' key morphological features. Computer vision then provides deep analysis, supporting rapid cell counting, identity classification, and morphology assessment. My organization, Team Consulting, has explored such a setup for in-process cell identification and sorting (**14**).

Holographic imaging extends such capabilities, providing 3D, label-free cell maps based on light-phase shifts in analyzed samples. Ovizio's iLine F Pro system is an on-line holographic microscope that provides continuous, noninvasive monitoring of CQAs in GMP settings. The instrument can be integrated with bioprocess bags, stirred tanks, and single-use bioreactors (**15**).

Optical spectroscopy techniques also are of growing interest in CGT analytics because they can take rapid and accurate measurements without need for sample labeling or contact. Such approaches are well suited for in- or on-line deployment. The most common method in this group, Raman spectroscopy, provides for real-time monitoring of nutrients and metabolites. Another promising approach is variable-pathlength UV-vis spectroscopy, which dynamically adjusts an instrument's optical pathlength to measure sample concentrations directly, eliminating the dilution requirements involved with traditional UV-vis methods. Repligen has demonstrated that its FlowVPX system, an in-line variable-pathlength spectrophotometer, can automate tangential-flow filtration (TFF) by using

concentration data in a feedback loop, replacing traditional monitoring based on mass balances (**16**). Thus, the variable-pathlength approach improves process precision while reducing human error. Legmann told me that Repligen also is exploring the FlowVPX platform's utility for in-line full:empty capsid analysis.

Proprietary sensing technologies such as LumaCyte's LFC method, used in the company's Radiance instrument, also are emerging as powerful tools for cell characterization (**17**). The LFC method infers intrinsic biochemical and biophysical properties by measuring how individual cells respond to precisely controlled laser light and microfluidic flow. Optical and fluidic forces are measured to capture parameters such as cell velocity, size, shape, optical force index, and cellular deformability — all of which depend on cell morphology, refractive index, internal density, complexity, and membrane properties. The Radiance instrument can detect subtle phenotypic changes, rapidly quantifying early indicators of cellular responses to viral infection, activation, transfection, transduction, and differentiation — without need for labels and other reagents. LumaCyte's technology has been used in descriptive and predictive analytics for both CGTs and vaccines.

Measurement of electrical impedance can provide rapid, label-free characterization of single cells by assessing their electrical properties as they pass through a microfluidic chip. When an electric field is applied across embedded electrodes, each cell disturbs the field, enabling measurement of resistance and capacitance without the need for stains or labels. Sophisticated analysis of impedance signals can reveal subtle phenotypic or functional changes. For example, Cellix's Inish impedance-based flow cytometer can collect data for cell counts, cell viability, and transfection efficiency (**18**). Cytomos is developing a dielectric-spectroscopy–based platform for rapid at-line analytics (**19**). And Agilent's xCELLigence RTCA eSight platform combines impedance sensing with live-cell imaging to monitor cell behavior in real time (**20**).

Product Release

Accelerating release QC will be essential to reducing delays and ensuring CGT product consistency. Traditional QC methods such as quantitative PCR (qPCR), flow cytometry, ELISAs, sequencing, and culture-based assays are often manual and complex with testing timelines that can extend to weeks.

Much promise lies in shifting toward rapid, automated analytical systems that can be validated against established compendial methods. For example, sterility and mycoplasma tests are moving from days-long culture assays to automated molecular qPCR, rapid nanopore sequencing (**22**), and sensitive optical microbial detection (**23**), cutting time to results significantly. Such methods can be adopted in regulated environments when validated as comparable to established compendial methods (**24**).

Furthermore, identification of CQA biomarkers that correlate strongly with batch quality — and, eventually, with clinical outcomes — will spur on development and validation of rapid, targeted assays. The CGT Catapult's analytical-development group, led by Barkatullah, is combining insights from CQA-biomarker studies with diagnostic-industry experience to identify and adapt proven diagnostic technologies for in-process monitoring and release testing of CGT products. The diagnostics industry, she explained, offers CGT developers valuable lessons in developing rapid analytics, particularly considering the industry's move toward point-of-care testing for infectious diseases. Technologies such as the LumiraDx multimodal diagnostic system — which Team Consulting supported during development and which has since been acquired by Roche — demonstrate how automated, closed, plug-and-play instruments can deliver laboratory-comparable accuracy with rapid turnaround times, high throughput, and ease of use for nonspecialists (Photo 1) (**25**). Adapting such engineering approaches for CGT could streamline release testing and reduce delays significantly.

Condensed or Streamlined QC Analytics: Barkatullah offered a starting point for accelerating QC release testing: "We just need to look at QC as a whole for each product and ask, 'Can we start to streamline this [workflow] and make it cheaper and less complex to meet the demands of scale-up?'" Alternatively, scientists can use *condensed-analytics approaches*, which leverage single technologies to capture multidimensional data, enabling multiple release tests within one platform. She explained, "Condensing methodologies into formats such as digital PCR or NGS could reduce sample volume, cut costs, and speed up release times." Doing so also could enhance data richness and traceability. For instance, analysts are exploring NGS not only for identity testing, but also for detection of adventitious agents, vector integration sites, residual host DNA, and replication-competent virus. Thus, the technology could consolidate several assays into one workflow.

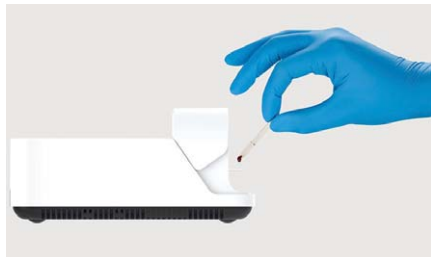


Photo 1: Technologies such as Roche's LumiraDx multimodal diagnostic system demonstrate how automated, closed, plug-and-play instruments can deliver laboratory-comparable accuracy with rapid turnaround times, high throughput, and ease of use for nonspecialists (**25**).

Multiplex flow cytometry also shows promise for cell-therapy release testing through parallel measurement of multiple CQAs, including those related to identity, viability, transduction efficiency, activation, and potency (**26**). Advancing this multiplex approach will involve automating on-board sample preparation and staining to cut labor, developing standardized panels, and optimizing workflows and data analysis to improve usability and consistency. Further validation of surrogate functional assays for high-throughput release testing will help to realize the technology's full potential.

Reflections

CGTs involve distinctive manufacturing challenges because of their biological

complexity and variability. Advanced analytics are essential across the CGT life cycle, from input characterization to process development and release testing.

Rapid, low-volume, automated analytical tools that integrate into closed systems will be vital to accelerating and derisking CGT manufacturing. Such technologies enable real-time insights and predictive control, helping drug developers to manage variability and ensure consistent product quality.

Progress in PAT is deepening process understanding and enabling steps toward real-time batch release. However, gaps remain, particularly for standardized, noninvasive cell characterization.

Emerging surrogate markers, enabling technologies, and AI-driven analytics offer promising solutions. Rapid, multiplexed QC testing and diagnostic-inspired tools can reduce delays and improve patient access.

Ultimately, the convergence of innovation, data sharing, and strategic analytics will unlock CGT's full potential by enhancing process efficiency and delivering transformative therapies to patients faster and more reliably than is currently possible.

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Synchronizing CMC Activities with Clinical Development

for Robust and Compliant Biomanufacturing from Bench to BLA

NaveenGanesh Muralidharan, Austin Turner, Harald Michor, and Mark Davis

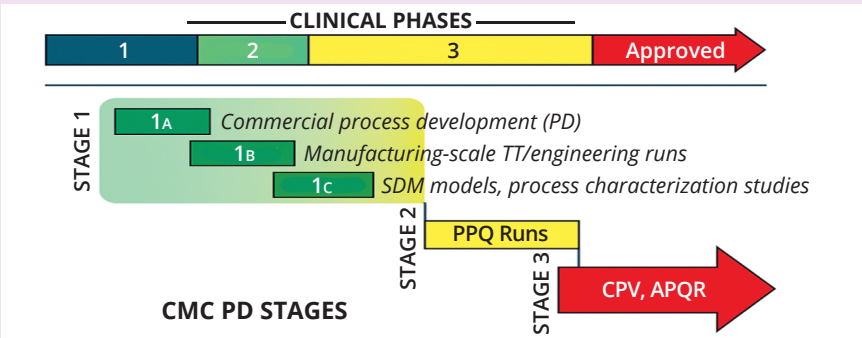
For a biopharmaceutical program to progress successfully from early stage clinical trials to commercial launch, a coordinated evolution of chemistry, manufacturing, and controls (CMC) activities must parallel that path. CMC encompasses two interdependent but distinct domains: manufacturing process understanding — regarding the design, characterization, scale-up, and control of a manufacturing process — and analytical method development to inform it. The latter ensures that product quality attributes (PQAs) will be measured with accuracy, precision, and regulatory compliance throughout a biotherapeutic's life cycle.

As investigational products advance through clinical phases, both domains must mature together. Manufacturing process understanding evolves through the life cycle from initial feasibility studies at bench scale, through risk-based process characterization, to commercial-scale process performance qualification (PPQ) and control-strategy refinement. Analytical method development progresses from early fit-for-purpose assays to fully validated methods that are good manufacturing practice (GMP) compliant and integrated into a product's control strategy and continued process verification (CPV) framework.

Aligning those two streams with clinical milestones is essential for derisking drug development through early identification of process and analytical risks. Doing that enables data-driven decision-making by generating reliable, representative process and product data. And that ultimately ensures supply continuity by scaling process capability and analytical readiness in step with demand.

Here we present a life-cycle–based, dual-stream CMC framework that integrates manufacturing-process and analytical activities with clinical development phases. We detail how each stream progresses from phase 1 through phase 3, highlight key integration points, and show how risk-based strategies (e.g., process characterization, analytical life-cycle management, and control-strategy development) converge to meet regulatory expectations and support robust commercial manufacturing. Figures 1 and 2 illustrate how the streams advance in parallel from bench-scale experimentation to full-scale GMP manufacturing, thus ensuring technical readiness, regulatory compliance, and reliable product supply across a product's life cycle.

Figure 1: Integrated alignment of activities for clinical development and manufacturing process understanding



APQR = annual product-quality review; **CMC** = chemistry, manufacturing, and controls; **CPV** = continued process verification; **PPQ** = process performance qualification; **SDM** = scale-down modeling; **TT** = technology transfer

PRODUCT FOCUS: All biologics

PROCESS FOCUS: Chemistry, manufacturing, and controls (CMC)

AUDIENCE: Process development, analytical, QA/QC, and manufacturing

KEYWORDS: Life-cycle framework, analytical development, process characterization, process performance qualification (PPQ), control strategy

LEVEL: Basic

Alignment of Activities

Successful development of biopharmaceutical products relies on the coordinated progression of CMC activities alongside clinical development. As investigational products move from early safety and pharmacokinetic studies into late-phase efficacy trials and eventual market authorization, the associated CMC functions must evolve in complexity, rigor, and operational scale to meet

Figure 2: Life-cycle framework for biopharmaceutical process validation

increasing regulatory expectations and ensure supply continuity (**1**).

Our life-cycle–based framework strategically aligns CMC milestones with clinical phases to ensure that process understanding, control strategies, and manufacturing readiness mature in step with product development. During early clinical phases, CMC efforts focus on small-scale laboratory operations, including initial process optimization, preliminary formulation development, and establishment of early analytical methods to support the production of clinical trial material. As a product enters phase 2, the CMC focus shifts to transitioning that process to manufacturing scale, where bench/pilot-scale performance is demonstrated to be reproducible at manufacturing scale under either engineering or GMP conditions. This phase also marks the initiation of risk-based process-characterization studies, which are critical to identifying key process parameters, refining a control strategy, and supporting commercial process-control readiness.

By phase 3, a process must be fully defined, robust, and validated at commercial scale. CMC activities at this stage include PPQ, control-strategy finalization, and completion of all validation studies required for regulatory submission. That work culminates in preparation of comprehensive CMC documentation for filings such as biologics license applications (BLAs) in the United States and marketing authorisation applications (MAAs) in Europe (**1**).

To support that evolution, CMC development is structured into a tiered life-cycle framework of three major stages (Figure 1). Stage 1 focuses on process and product understanding and is further divided into 1A (small-scale commercial process development), 1B (technology transfer to larger-scale engineering or GMP batches), and 1C (formal process characterization). Stage 2 encompasses full-scale process qualification and validation, and Stage 3 ensures CPV during routine commercial manufacturing (**1, 2**).

Figure 2 illustrates the integrated relationship between clinical development phases and CMC operational scales, showing how activities transition from bench-scale experimentation to full-scale GMP manufacturing. That roadmap highlights the critical role of synchronized CMC planning throughout process development, technology transfer, characterization, and validation for reducing technical risk, ensuring regulatory compliance, and supporting reliable material supply throughout a product's clinical and commercial life cycles.

Integrating Clinical Development and Analytical Method Activities: Analytical methods mature alongside the process and product knowledge base. Their development is tightly interwoven with process-development activities, ensuring that decisions made are based on reliable, fit-for-purpose data. By the time of commercial launch, the methods must meet full validation standards. The "Analytical Development" box details how analytical methods evolve alongside clinical testing.

Stage 1: Gaining Process Understanding

A robust and compliant manufacturing process begins with systematic process understanding. ICH Q8(R2) outlines the principles of pharmaceutical development based on quality by design (QbD) (**2**). Stage 1 encompasses bench-scale development, pilot-scale transfer, risk assessment, and experimental qualification. The goal here is to generate scientific knowledge needed to identify, characterize, and control sources of variability that could affect product quality, safety, and efficacy.

Stage 1A — Bench-Scale Development, Process Optimization, and Cell-Line Stability Assessment: Process development begins with small-scale bioreactors and purification systems for establishing process feasibility and optimizing key performance indicators (KPIs) such as yield, purity, and critical quality attributes (CQAs). Early upstream activities focus on evaluating basal and feed-media composition, seeding density, culture duration, pH, dissolved oxygen (DO), and temperature-control strategies to maximize productivity and ensure cell-culture process consistency. Preliminary analytical methods and formulation prototypes developed in parallel will support clinical-material production and stability studies.

Essential at this stage, cell-line stability assessments evaluate genetic and phenotypic stability of a production clone over extended population doublings. Developers monitor productivity, growth kinetics, and PQAs (e.g., glycosylation patterns, charge variants, and aggregation) across multiple passages. Such studies ensure that a selected cell line maintains consistent expression of therapeutic protein under manufacturing-relevant conditions to meet regulatory expectations for long-term performance.

Concurrently, impurity-clearance assessments begin, with spiking studies that simulate worst-case impurity loads to start defining downstream-process robustness. Known concentrations of host-cell DNA (hcDNA) and proteins (HCPs) — and elemental impurities, where applicable — are introduced into process intermediates to assess their removal through chromatography and filtration steps. These studies help process engineers quantify log reduction values (LRVs), select resins, and sequence downstream steps.

Viral-clearance studies are a critical regulatory expectation introduced during this stage. Low-pH and/or detergent-based virus inactivation steps are followed by virus filtration, both of which are evaluated using spiked model viruses to establish clearance capabilities and generate target LRVs. Process parameters such as hold time,

pH, protein concentration, and filter loading are optimized to ensure robust and reproducible virus removal. These studies are conducted at both bench and manufacturing scales, serving as a critical component of the overall viral-safety strategy to align with regulatory guidance, including ICH Q5A(R2), by providing evidence of effective removal and/or inactivation of potential viral contaminants.

In stage 1A, an initial virus-spiking study is conducted using a two-virus panel to assess the feasibility and robustness of key purification steps. Such early studies typically use two model viruses: xenotropic murine leukemia virus (XMuLV, an enveloped retrovirus relevant to Chinese hamster ovary cells) and minute virus of mice (MMV, a nonenveloped parvovirus known for its high resistance to inactivation). Using small-scale chromatography columns and virus-filtration units, scientists identify which unit operations exhibit inherent viral-clearance capabilities. Although data from these small-scale studies are not intended to support licensure submissions, they provide valuable insights during early development for investigational new drug (IND) applications. These bench-scale efforts inform process-development decisions by highlighting which steps warrant further optimization or scale-up validation. Typical LRV expectations at this stage range ≥4–6 $\log_{10}$ for enveloped viruses and ≥2–4 $\log_{10}$ for nonenveloped viruses, depending on the unit operation.

Also at this stage, purification parameters for chromatography, filtration, viral filtration, and tangential-flow filtration (TFF) operations are optimized to reduce cost of goods (CoG) and improve impurity-removal efficiency while maintaining product recovery and process robustness. Together, the integrated activities of Stage 1A (Figure 3) form the foundation of a scalable, compliant, and robust manufacturing process. These efforts ensure that both upstream and downstream operations are capable of consistently producing material that meets quality, safety, and regulatory expectations throughout clinical development and into commercialization.

Stage 1B — Scale-Up and Pilot-Scale Technology Transfer: Following successful bench-scale development, a process is transferred to larger-scale systems that bridge laboratory findings with production realities. Scaled-up operations provide an essential intermediate step for evaluating process scalability, assessing equipment compatibility, and generating material for subsequent development activities. During this stage, platform knowledge and historical data are leveraged to ensure process comparability and identify scale-dependent risks proactively **(1, 2)**.

For process validation, a series of targeted risk assessments (Figure 4) is conducted in parallel with scale-up and technology-transfer readiness activities. Such assessments help developers understand potential risks to product quality during manufacturing at scale and guide the implementation of appropriate mitigation strategies. Process modifications, material adjustments, and/or addition of specific in-process or release testing of formulated drug substance (DS) can help to ensure continued product integrity and regulatory compliance.

Analytical Development Aligns with Clinical Development

Phase 1 is rapid development of fit-for-purpose methods to characterize an investigational product and assess key critical quality attributes (CQAs) for early batches. Key features include

- analytical target profile (ATP) drafting, which defines the quality attributes to be monitored, performance criteria, and each method's intended use
- selection of analytical platform technologies based on target attributes and available timelines
- partial method qualification following ICH Q2(R2) for specificity, precision, and range relevant to the clinical phase
- stability-indicating assessment (preliminary) using forced degradation under key stressors to determine stability-indicating capabilities
- integration with process development to provide CQA data that support bench-scale process optimization, enabling early comparability studies across process conditions to detect early process-induced product variants (e.g., glycoforms, charge variants) that could influence later control strategies.

Phase 2 increases method robustness, expands qualification parameters, and ensures sensitivity for scale-dependent process changes. Key features include

- ATP refinement to adjust performance criteria based on deepening knowledge of product variability
- qualification expansion to add linearity, intermediate precision, and accuracy for key assays
- method optimization to adjust parameters for increased robustness
- transfer of method from development to manufacturing quality control (QC) laboratories, including site-specific qualification
- integration with process development and characterization methods to support engineering runs to confirm scale-up feasibility. This work provides high-resolution analytical data for risk-based process-characterization studies (e.g., linking pH shift to aggregate formation) and enables assessment of process robustness by detecting subtle quality shifts under deliberately varied critical process parameters (CPPs).

Phase 3 is full validation, making good manufacturing practice (GMP) methods ready for process-performance qualification (PPQ) execution and lifecycle management. Key features include

- complete ICH Q2(R2) validation, including robustness, detection limit, quantitation limit, and robustness
- life-cycle documentation linking analytical validation reports to the control strategy set forth in the chemistry, manufacturing, and controls (CMC) submission
- GMP control readiness efforts, with analytical methods used in QC laboratories at manufacturing scale and all criteria (e.g., for system suitability and acceptance) and documentation in place
- integration with process characterization and PPQ. Methods are used to generate release and stability data from PPQ batches, providing critical evidence of process reproducibility. That supports comparability protocols for changes made to process/site/scale before product launch. Results feed into continued process verification (CPV) planning by establishing baseline analytical performance trends.

Figure 3: Stage 1A scale and technology transfer process-validation activities

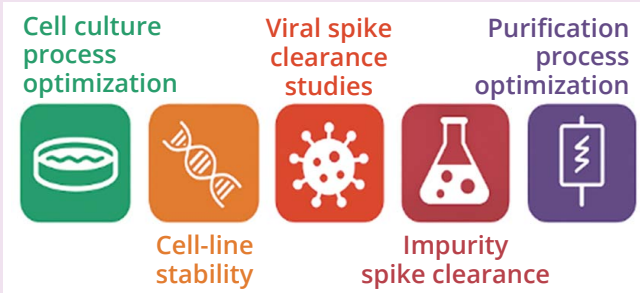
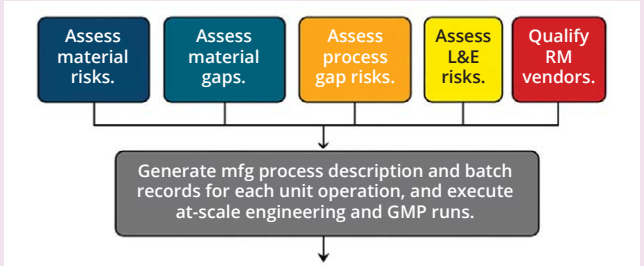


Figure 4: Stage 1B scale and technology transfer process-validation activities; L&E = leachables and extractables, mfg = manufacturing, RM = raw materials



Material Risk Assessment: Crucial to biomanufacturing, aligning material risk assessments enables proactive identification and control of raw materials that could compromise product quality, safety, or regulatory compliance. Through a structured evaluation framework, developers assess contamination risks (biological, chemical, and particulate), product and process impacts, regulatory status, and variability potential. High-risk materials include nutrient-rich cell-culture media, animal-derived components, and excipients without compendial support, which are prioritized for enhanced control (e.g., sterility assurance and detailed testing protocols). Tools such as weighted risk-scoring systems integrate multiple attributes into a unified classification, allowing manufacturers to assign raw materials to risk tiers (low, moderate, and high) for applying tailored control strategies.

Such a risk-based approach supports regulatory alignment with standards such as ICH Q3D, USP ‹1043›, and current GMP (CGMP) expectations — while also fostering operational efficiency and supply-chain resilience. By linking material risks to process performance and CQAs, manufacturers can mitigate variability, reduce failure rates, and ensure lot consistency. Material risk assessments also help to streamline supplier qualification efforts, facilitate defensible regulatory filings, and support life-cycle management of raw materials through continuous monitoring and control (**3, 4**).

Elemental Impurity Risk Assessment: In alignment with ICH Q3D, manufacturers must identify and control elemental impurities from raw materials, equipment, and the environment. A control–identification–risk-based stratification (CIRS) framework categorizes materials by compendial status, point of use, and incorporation into final products. Elements such as Pb, As, Cd, and Hg are evaluated based on permitted daily exposure (PDE) values. Wherever supplier data are lacking, conservative models can estimate impurity levels based on use and batch data. Clearance factors from chromatography and ultrafiltration/diafiltration (UF/DF) help to refine model predictions. Identified acceptable materials can be exempted from routine testing with proper justification. Risk rationales and mitigation plans must be documented in regulatory filings (**4, 5**).

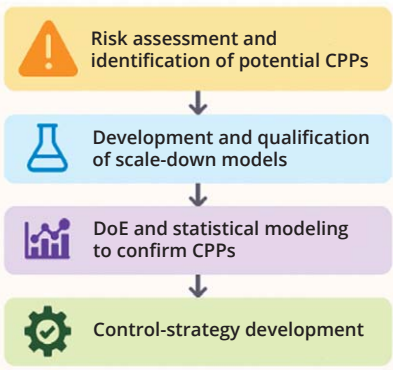
Extractables and Leachables (E&L) Risk Management: E&L risks stem from single-use systems (SUS) such as tubing, bags, and filters. Extractables are identified under exaggerated conditions; leachables are measured under actual use. E&L risks are scored based on where in the production stream a system is used, product-exposure temperature and duration, process-fluid interactions, and ratios of surface area to solution. SUS component risks are ranked low to high using cumulative risk scores. Mitigation options include material changes, assessment of vendor data, flushing, and additional testing. Safety thresholds guide acceptability. When vendor data do not cover process conditions, in-house studies are needed. E&L controls must be integrated into overall change-control and qualification systems (**6**).

Raw-Material Vendor Qualification: Process robustness and supply-chain assurance necessitate qualification of raw-material suppliers. Vendor qualification extends beyond basic sourcing and pricing considerations; it is a formal, risk-based evaluation of a supplier's ability to consistently deliver materials that meet predefined quality, regulatory, and performance requirements. This process typically includes a comprehensive review of the company's quality management system, manufacturing practices, analytical capabilities, and change-control processes. Site audits, questionnaire assessments, and historical performance evaluations are common tools used to assess vendor reliability and CGMP compliance (**7**).

Such efforts generally begin during Stage 1B of the process-validation life cycle (as part of process design), as process understanding is being established and key materials are selected. Early qualification supports process-development activities by ensuring material consistency throughout development and scale-up. These efforts continue to mature into Stage 2 (process qualification), when a finalized vendor list, quality agreements, and change-notification expectations must be in place for executing PPQ batches. Ongoing performance monitoring is part of Stage 3 (CPV) to ensure sustained compliance and reliability throughout commercial manufacturing (**8**).

A number of real-world examples underscore the importance of stringent vendor oversight. Variability in raw-material compositions (e.g., inconsistent amino-acid levels in cell-culture media) has caused batch failures and reduced product yield in some monoclonal antibody (mAb) manufacturing campaigns. Microbial contamination traced to some suppliers' insufficient environmental controls has led to costly deviations and discarded lots. Such incidents highlight the need for proactive

Figure 5: Stage 1c process-characterization activities; CPP = critical process parameter, DoE = design of experiments



qualification to identify potential risks before they affect manufacturing (**8**).

To mitigate those risks, biomanufacturers often establish tiered qualification strategies based on material criticality and leverage supplier performance data, quality agreements, and dual-sourcing plans. A structured approach ensures that raw-material inputs remain controlled, traceable, and fit for use across a product's life cycle.

Stage 1c — Process Characterization: This stage marks a pivotal transition from process development to a well-defined, science-based manufacturing framework. The primary objective is to deepen process understanding by systematically identifying, evaluating, and confirming the process parameters that influence CQAs. By integrating risk assessment, qualified scale-down models, and statistically designed experiments, manufacturers generate robust data to establish process-control strategies and define a QbD design space (Figure 3). Such foundational knowledge enables proactive risk management, supports regulatory expectations, and lays the groundwork for a consistent and reproducible commercial process. The activities in Stage 1c bridge development efforts with process validation and commercialization (**2**).

Critical Process Parameters (CPPs): Comprehensive risk assessments are conducted to identify potential CPPs that could influence product quality. Tools such as failure modes and effects analysis (FMEA), Ishikawa (fishbone) diagrams, and risk-ranking matrices help developers assess each unit operation. Parameters are ranked based on their likelihood of variation, effect on CQAs, and detectability.

For detailed characterization studies, scale-down models (SDMs) replicate full-scale unit operations at laboratory scale. The models must predict reliably the performance and quality attributes observed in production-scale equipment. SDMs are especially valuable for high-cost biologics and when sample-material supplies are limited. SDMs are qualified by showing comparable performance in yield, impurity profiles, and product-quality metrics.

SDMs enable design of experiments (DoE) studies to evaluate relationships among process inputs (e.g., agitation speed, temperature, pH) and outputs (e.g., titer, aggregate levels, and impurity clearance). Statistical tools such as response-surface methodology (RSM), multiple regression analysis, and principal component analysis (PCA) help developers identify key drivers of process performance. Parameters with confirmed influence on CQAs are designated as CPPs; those with negligible effects are categorized as non-CPPs. These studies provide the knowledge needed to establish a design space defining acceptable operating ranges and parameter interactions (**2**).

Control-Strategy Development: The knowledge gained during Stage 1 feeds into the development of a science- and risk-based control strategy. This strategy outlines how CPPs will be monitored and controlled to maintain product consistency. Proven acceptable ranges (PARs) are defined and integrated into batch records, automation systems, and quality controls. In-process controls (IPCs), feedback loops, manual interventions, and process alarms are implemented to ensure proactive response to deviations and maintain a state of control (**2**).

Viral Safety SDM: Following small-scale verification, a comprehensive virus-spiking study is performed under manufacturing-representative conditions using a qualified SDM. This study includes a four-virus panel selected to represent a range of physicochemical properties and resistance levels: XMuLV; MMV; reovirus type 3 (Reo-3), a nonenveloped, double-stranded RNA virus; and herpes simplex virus type 1 (HSV-1), a large, enveloped DNA virus. Those are chosen to challenge key downstream unit operations under worst-case conditions and provide a broad assessment of viral clearance capability.

Evaluated steps can include low-pH inactivation, protein A affinity chromatography, ion-exchange chromatography, and virus filtration. Each unit operation is assessed independently for its virus-reduction potential. For example, low-pH hold inactivates enveloped viruses (e.g., XMuLV and HSV-1), typically providing ≥4 $\log_{10}$ reduction. Virus filters — e.g., the Planova family from Asahi Kasei and Viresolve membranes from MilliporeSigma — target small, nonenveloped viruses such as MMV with typical LRVs of ≥4–6 $\log_{10}$. Chromatography can provide additional clearance of 2–4 $\log_{10}$, depending on virus-binding behavior. The overall process is expected to achieve ≥6 $\log_{10}$ cumulative reduction for enveloped viruses and ≥4 $\log_{10}$ for nonenveloped viruses in line with global regulatory expectations for biologics produced by mammalian expression systems.

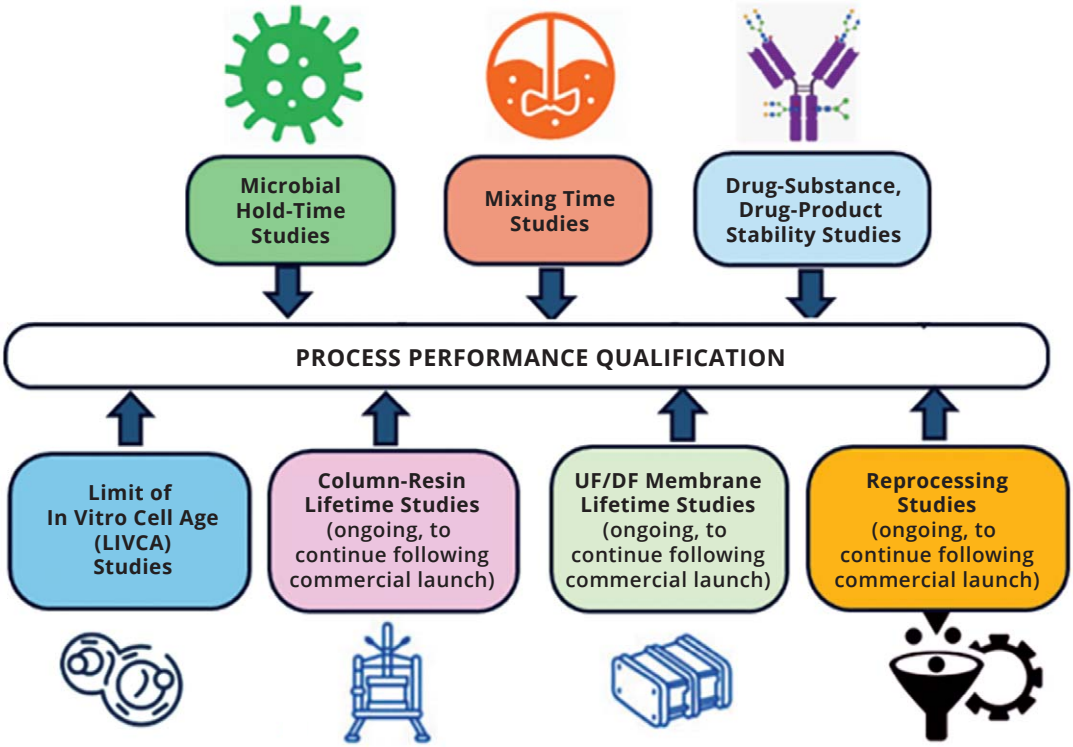
Supported by qualified SDMs, data from the manufacturing-scale virus-spiking study are included in BLA/MAA regulatory filings to demonstrate viral safety of a drug substance (DS) and confirm that a downstream process can clear adventitious viruses robustly and consistently.

Stage 2: Qualification and Validation

Stage 2 validates a manufacturing process under real-world conditions in a GMP-compliant environment. PPQ and analytical-method validation verify overall system readiness for commercial production. Such validation efforts are essential, but here we focus primarily on process validation based on integrated guidance in ICH Q8(R2) and published practical insights (**1, 2, 9**). Rathore, Baseman, and Rudge's 2023 book offers an applied, industry-based perspective that complements the standard harmonized framework to form a conceptual foundation for our validation strategies (Figure 6) (**1**).

Process Performance Qualification: PPQ serves as the final confirmatory

Figure 6: Stage 2 process performance qualification and associated at-scale validation studies; UF/DF = ultrafiltration/diafiltration



stage of process validation, demonstrating that a manufacturing process performs as intended under routine commercial conditions. Typically, three or more consecutive, successful full-scale batches are manufactured under defined, controlled parameters that represent commercial production. Executed batches must be produced by qualified personnel using calibrated and qualified equipment, qualified raw materials, and approved procedures in a GMP environment.

Each PPQ batch is evaluated rigorously against predefined acceptance criteria: CPPs, IPCs, release specifications, and CQAs. Additionally, intermediate process outputs — including step-recovery yields, intermediate yields, and processing trends — are monitored carefully and well documented. Developers must confirm process robustness, control consistency, and the ability to reproducibly manufacture product that meets quality standards (**1**).

Peripheral Validation Studies: In addition to the core PPQ batches, several supporting validation studies are used to confirm the robustness and reliability of processes that influence product quality and manufacturing consistency (Figure 5).

Limit of In Vitro Cell Age (LIVCA) Studies: End-of-production (EoP) and LIVCA studies are critical components of cell-bank qualification and process characterization. These studies are meant to verify the genetic stability and consistent performance of a production cell line over the full extent of population doublings used for routine manufacturing. A common misunderstanding is that EoP or LIVCA samples must be collected precisely at the point of bioreactor harvest. In practice, it is widely accepted to collect them a day or two before peak viable cell density (VCD) has been reached, as long as all cells have been exposed at least once to the full regimen of supplements (e.g., nutrient feeds, base titrants, and antifoam agents). Provided that an EoP sample is passaged subsequently for additional generations to reach the estimated total generation number at harvest, the resulting data will be scientifically representative and compliant with regulatory expectations.

Such sampling flexibility is especially important for processes in which culture viability declines significantly at harvest and when excessive accumulation of toxic metabolites limits the ability to recover viable cells. Once a culture has reached peak cell density, its population doubling rate slows or plateaus, so further in-bioreactor culturing has minimal effect on cell age. For such scenarios, a well-established and validated strategy is to collect EoP samples just before they attain peak cell density and passage them in shake flasks or other scale-down systems to match the final generation count. It ensures robust and meaningful genetic-stability assessment while reducing operational risks associated with sampling from late-stage or compromised cultures (**1**).

Mixing Validation: To ensure uniformity and consistency in biopharmaceutical solutions, mixing validation is especially useful for multicomponent systems where inadequate mixing can diminish product efficacy, stability, and safety. These studies focus on attributes such as turbidity, pH, conductivity, and osmolarity as proxies for homogeneity. Dynamic mixing evaluations across different volumes, agitation rates, and viscosities simulate operational extremes. Bracketing and matrix strategies can streamline testing across vessels, impellers, and fill volumes (**10**).

Chromatography-Resin Reuse Validation: As a key downstream step, chromatographic purification must be validated to confirm resin performance,

Figure 7: Stage 3 continuous process verification (CPV); SOP = standard operating procedure

reproducibility, and robustness. Process engineers evaluate binding capacity, pressure profiles, flow uniformity, and cleaning efficiency over multiple cycles. Carryover is assessed through product-blank sequencing, and cleaning validation targets removal of HCPs, DNA, and impurities. Supported by statistical trending, resin-lifetime studies often exceed reuse cycles with documentation of column packing, sanitization, and flow distribution (**1**).

Membrane-Reuse Validation: UF/DF membranes are reused to reduce costs while maintaining safety and performance. Prospective scale-down cycling studies and concurrent production monitoring assess metrics such as normalized water permeability (NWP), transmembrane pressure (TMP), product yield, and impurity clearance. Cleaning validation relies on total organic carbon (TOC) testing, enzyme-linked immunosorbent assays (ELISAs), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and blank runs. Validation includes cleaning protocols, storage, sanitization, and reuse tracking (**1**).

Microbial Hold-Time Validation (MHTV) is critical for ensuring that buffers, media, and process intermediates maintain acceptable microbial quality throughout designated storage periods. During biomanufacturing, some in-process solutions (e.g., clarified harvests, chromatography eluates, pooled fractions, and formulated bulk DS) can be held for extended durations before they are processed further. MHTV studies are designed to verify that bioburden and endotoxin levels remain within predefined alert and action limits in such materials during extended storage. The studies must be performed at scale and often are executed alongside PPQ runs (**11, 12**).

Bioburden and endotoxin specifications are tiered based on process stage and associated risk. For sterile upstream operations, limits typically are measured in colony-forming units (CFU) and endotoxin units (EU), set at ≤1 CFU/10 mL for bioburden and ≤10 EU/mL for endotoxin. During intermediate stages — such as clarification, which often involves nonsterile but sanitary operations — bioburden allowances may increase to ≤200 CFU/10 mL, before tightening to ≤100 CFU/10 mL for purification steps such as chromatography. Endotoxin thresholds also reduce progressively, from 5 EU/mL in early purification to 1 EU/mL as a process nears the DS stage. In final downstream operations, particularly just before sterile filtration, bioburden limits must not exceed <10 CFU/100 mL. To ensure patient safety, endotoxin levels at that stage typically are constrained to 0.25–1.00 EU/mL. For nonsterile DS or formulated intermediates awaiting filtration, a bioburden limit of <10 CFU/100 mL generally is acceptable when supported by validated microbial clearance data.

MHTV provides scientific justification and regulatory assurance that microbial control is maintained during hold periods, thereby safeguarding product integrity, patient safety, and CGMP compliance.

Reprocessing Validation: Reprocessing of operations such as refiltration and buffer exchange is permitted under strict controls when deviations occur (e.g., equipment failure or microbial excursion). Potency, purity, aggregation, and stability assays must validate product comparability for such cases. Protocols should define acceptable scenarios, maximum reprocess limits, and documentation requirements. Regulatory filings should predefine reprocessing options with data to demonstrate safety and efficacy. Reprocessed batches must meet all original release and stability specifications.

Note that *reprocessing* is distinct from *rework* and *remanufacturing* (**1**). Reprocessing validations are justified through root-cause investigations, clear procedural controls, and robust comparability testing. Regulatory filings must include reprocessing strategies if repeated actions are likely. Internal change-control systems track reprocessing frequency, yield impact, and patient risk.

Stability Testing: Stability studies ensure that biopharmaceutical products maintain quality, safety, and efficacy throughout their shelf lives. These studies assess the effects of temperature, humidity, and light over time, focusing on degradation mechanisms such as aggregation, oxidation, and chemical modification. Stability testing is staged across clinical development, with small-scale batches under accelerated conditions to identify degradation pathways during phase 1; intermediate and long-term studies to evaluate technology-transfer batches for container compatibility and stress conditions during phase 2; and long-term testing of PPQ batches (up to 2–3 years) to confirm shelf life and support regulatory filings during phase 3.

Batch selection for such testing aligns with ICH Q1A, typically requiring three representative batches. One-batch studies may be allowed for low-risk scenarios. Regulatory guidance recommends new stability studies for site or process changes. Testing frequency follows regulatory expectations: every three months in year 1, every six months in year 2, and annually thereafter. Bracketing and matrixing to reduce testing burden must be justified scientifically, although matrixing is rarely used for biologics due to their complexity (**1, 13**).

Stage 3: CPV and Life-Cycle Management

CPV ensures that validated parameters remain in control throughout a product's life cycle. Continuous/periodic review of process data confirm consistent performance and identify emerging trends, shifts, and variabilities that could compromise product quality (**13**).

The US Food and Drug Administration's (FDA's) 2011 process validation guidance formally introduced CPV as an essential part of life-cycle process monitoring. Shewhart control

charts serve as a primary statistical tool for tracking CPPs, key process parameters (KPPs), IPCs, batch yields, step-recovery yields, and product release specifications (**13, 14**).

Control charts often are interpreted using Nelson rules to detect out-of-control (OoC) points (data beyond control limits) and out-of-trend (OoT) behavior (nonrandom shifts or patterns). However, false alarms (signals that are statistically outliers but operationally insignificant) and nuisance alarms (alerts with low practical impact) can overwhelm the signal-to-noise ratio. To mitigate that problem, control-chart design is optimized to balance statistical sensitivity with practical significance — e.g., by reducing the number of rules applied or refining data-distribution assumptions to account for skewness and kurtosis (**14, 15**).

A robust CPV program is built on four key functional components to ensure systematic oversight and control of a manufacturing process over time (Figure 7): A standard operating procedure (SOP) should outline the criteria for selecting parameters to be monitored, the frequency of data review, team responsibilities, applicable statistical methods, documentation practices, and reporting timelines. Based on that document, a CPV plan is developed for each product or intermediate stage, defining specific parameters to be trended, statistical tools to be used, and review cadence tailored to a product's risk profile and manufacturing frequency. Data-review meetings are held periodically with crossfunctional representation to evaluate statistical signals, assess associated risks, and determine appropriate actions. Meeting discussions and decisions are documented in formal meeting minutes. Finally, CPV reports are compiled quarterly/annually, summarizing trend analyses, capability indices, significant statistical observations, and corresponding responses. The reports also serve to refine CPV plans over time by recommending updates to the list of monitored parameters or adjusting their monitoring frequency in response to observed process performance and life-cycle maturity (**15**).

Parallel Paths to Success

Consistent delivery of high-quality biopharmaceuticals depends on parallel advancement of manufacturing process understanding and analytical-method development. By aligning those streams with clinical milestones along a product's life cycle — linking process characterization with analytical refinement and embedding both into a unified control strategy — manufacturers can derisk development, meet regulatory expectations, and ensure commercial readiness. An integrated, risk-based approach safeguards product quality, patient safety, and regulatory compliance from early development through sustained commercial supply.

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Risk-Assessing Manual Cleaning Processes To Reduce Error Rates in Biomanufacturing

Tim Sandle

Equipment cleaning is of fundamental importance to biopharmaceutical manufacturing. Such activities prevent cross-contamination between product batches and, where applicable, remove and control microbial bioburden. In this context, *cleaning* refers to controlled and consistent application of chemical and physical methods to remove residues down to acceptable levels. The presence of residues can be detected directly on biomanufacturing equipment or indirectly in water from a final equipment-rinse step. Whether remaining residues have been reduced to satisfactorily low levels depends on predetermined acceptance criteria, which usually specify the absence of visible residues.

Facilities staff use either of two approaches for achieving a desired level of equipment cleanliness: automated or manual cleaning, with the former option being preferred by both biomanufacturers and regulatory agencies. Automated cleaning can be achieved in situ using clean-in-place (CIP) systems followed, if needed, by steam-in-place (SIP) systems. Manual cleaning generally involves immersing instruments and equipment parts in water with detergent, then having personnel scrub, brush, flush, suction, and spray items to remove residues. For verification of manual cleaning, the pharmaceutical industry can draw upon lessons from the healthcare sector (**1**).

An operator applies a pressure washer to rinse manually cleaned biopharmaceutical equipment.
(HTTPS://STOCK.ADOBE.COM)

It might seem quaint in a world of automation and robotics to write about manual cleaning, yet the practice remains common in many parts of the biopharmaceutical industry. Manual cleaning also can go wrong; thus, it requires regular training and supervision. The topic of manual cleaning appears with regularity in regulatory findings.

Manual cleaning persists because some types of equipment cannot be connected to automated CIP technologies due to legacy issues or bespoke designs. In other cases, certain parts might require manual cleaning even when the main piece of equipment can be processed through an automated system. Although manual cleaning is necessary and fairly ubiquitous, it presents many difficulties for biomanufacturers, including the need to ensure cleaning consistency and efficacy. Depending on the intended use, a piece of equipment can exhibit an elevated risk of cross-contamination. For instance, product-contacting equipment poses greater cross-contamination risks than does noncontacting equipment. Moreover, certain product types pose greater risks than do others. Thus, manual cleaning is a perennial concern for auditors, and a system of assurance is required to demonstrate its effectiveness. Here, I explore risks associated with manual processes, then provide a basis for a control strategy and a framework for periodic evaluation.

PRODUCT FOCUS: Recombinant proteins and antibodies

PROCESS FOCUS: Manufacturing

AUDIENCE: Manufacturing, facilities, operations, QA/QC

KEYWORDS: Cleaning validation, equipment cleanability, residue detection, visual inspection, risk assessment, Sinner's circle

LEVEL: Basic

Relative Risks of Manual Cleaning

According to Pluta and Sharnez, "Manual cleaning processes are high-risk operations that must be adequately controlled through a comprehensive program that includes robust procedures, clear documentation, and thorough inspections and training" (**2**). Regulatory agencies often express concerns about cleaning consistency and reproducibility during facility inspections. For example, US Food and Drug Administration (FDA) concerns with manual cleaning date back to the agency's initial guidance from 1993 (**3**).

The biggest weakness of manual cleaning is inconsistency of practice. Thus, controls must be in place to minimize variability. They can be constructed by identifying performance parameters, documenting cleaning performance against selected parameters, and maintaining operator competency through education and training.

Manual cleaning presents several types of risk. In addition to equipment composition and age, relevant factors include the type of soil to be removed, the stage of manufacturing at which a piece of equipment is used, the amount and complexity of disassembly required, the design's cleanability, the ease of drying, and the level of equipment damage (e.g., damage to surfaces from use of incorrect cleaning tools). Personnel must ensure that dirty hold times are not exceeded and that cleaning equipment is not exposed to dirty-equipment storage areas. Because manual cleaning is a repetitive, labor-intensive task, biomanufacturers also must rotate personnel regularly (**4**). The more monotonous a task is, the more likely personnel are to lose concentration and make errors.

Key Control Factors

Overall control is achieved through establishing a validated process. *Cleaning validation* is a methodology applied to give assurance that a cleaning process has removed residues and contaminants from a piece of equipment or machinery. Practitioners often ask about the extent to which manual cleaning can be "validated."

Control is strengthened by being able to identify and maintain critical quality attributes (CQAs) and critical process parameters (CPPs). For cleaning validation, CQAs include residue levels from product solutions, microbial contamination, and cleaning agents, as well as equipment-design consistency (e.g., drainability) and cleaning-agent concentration and conductivity. Relevant CPPs are clean and dirty hold times, process run times, pressure, flow rate, mixing, activity (e.g., chemical exposure and number of water rinses), chemical concentration and contact time, and cleaning temperature.

Some CPPs cannot be controlled easily under manual-cleaning regimes. Time can be measured, but the duration and strength of agitation applied during scrubbing will vary across operators. Pressure and water flow rates can be preestablished, but operators still need to apply them consistently. And although equipment-related factors might seem self-explanatory and standardized, not all systems that are said to be "like-for-like" are sufficiently similar. Disparities can lead to variations in cleaning methods. For instance, the ability to free-drain a piece of equipment will influence residue retention.

Cleaning Procedures

Exacting Detail: Some inconsistencies in manual cleaning can be addressed through clear and effective procedures that describe — and illustrate — disassembly, cleaning techniques, and common pitfalls. A strong standard operating procedure (SOP) gives clear, step-by-step directions. It tells employees who does what and outlines the tools, cleaning agents, and quality checks to use.

Sufficient guidance needs to be provided in several areas. For instance, a protocol should identify specific pieces of equipment to be cleaned and establish the method, frequency, and sequence of each cleaning step. Instructions should include details about required cleaning between batches of the same or assorted biological products. Operators will need information about equipment disassembly and reassembly, with details about parts to be removed and, if applicable, assembly aids.

Cleaning detergents and application instructions should be listed. An effective SOP will describe detergent volumes, concentrations, temperatures, dwelling times, and application methods. Note that *cleaning* and *disinfection* are not synonymous terms in cleaning-process SOPs. *Detergents* are cleaning agents that remove soils — e.g., dirt, blood, and protein residues — from surfaces, whereas *disinfectants* eliminate microorganisms.

Likewise, an effective SOP provides a complete account of the cleaning process. Instructions should specify what type of water to use (e.g., deionized, distilled, or potable) and at what temperatures, pressures, and flow rates. Cleaning SOPs should codify immersion protocols (e.g., the need to immerse items fully during cleaning to minimize splashing), soaking periods, scrubbing tools (e.g., sponges, brushes, scrapers, sprayers, wipes, and cleaning aids), agitation time and strength, siphoning techniques, and numbers of rinses and equipment rotations. Operators also need guidelines for drying and storage as well as instructions for postcleaning visual inspection.

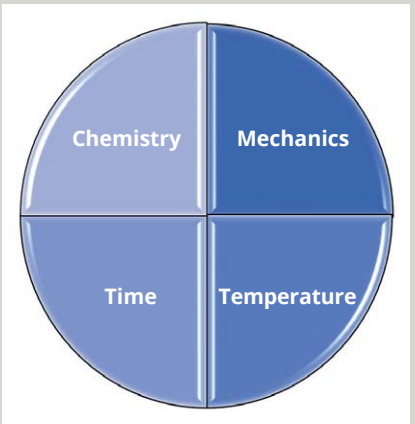
The more monotonous a task is, the more likely personnel are to lose concentration and **MAKE ERRORS.**

Points of Emphasis: Selecting an appropriate detergent and ensuring water-rinse consistency are arguably the most important measures for equipment cleaning. A detergent works by penetrating soil and reducing the surface tension that adheres it to an equipment surface. That property allows for soil removal. Thus, a detergent increases the "wettability" of water.

Many detergents are synthetic *surfactants* (a portmanteau of *surface-active agents*). Surfactants contain molecules with hydrophilic and hydrophobic regions. Detergents remove particles from surfaces by either capillary action or electrostatic forces. Differently charged ions cause microorganisms and soil particles to repel each other, disassociating dirt from equipment surfaces and leading to its suspension in the cleaning solution. Enzymatic detergents are effective cleaning agents because they are supplemented with enzymes to advance cleaning action and maximize residue removal. However, nonenzymatic detergents can be used across a wider range of materials and pH levels (**5**).

During rinsing, water is necessary to penetrate soil deposits. It can break soil into fine particles and hold them in suspension until they can be rinsed

Figure 1: Sinner's circle in balance for evaluation of cleaning procedures (**6**)



away. Regular water flow is needed to prevent residues from redepositing onto cleaned surfaces.

Several technologies can improve manual processes for cleaning disassembled equipment. For instance, ultrasonic cleaners emit sound waves at high frequencies. Bubbles produced by the sound waves generate microscopic shockwaves (tiny, high-pressure implosions within the liquid cleaning solution). Those shockwaves remove dirt and contaminants from complex machines without causing harm. Ultrasonicators also enable adjustment of cleaning frequency and temperature.

Pressure washers represent an alternative. They use high-pressure water jets to remove dirt, contaminants, and grime. Such systems are effective for nonsensitive equipment — e.g., work surfaces.

Both technologies enable control of factors in Sinner's circle. During the late 1950s, German chemist Herbert Sinner developed a model for effective equipment cleaning through washing (**6**). His model represents the cleaning process as a circle with sectors for chemistry, mechanics, temperature, and time (Figure 1). Sinner's circle effectively describes how reducing the size (effectiveness) of one factor requires increases in other factors to compensate. That is, if temperature decreases, then cleaning time, detergent efficacy, and application of mechanical action (e.g., scrubbing) must increase to achieve effective cleaning (**7**). Controlling such variables provides some consistency, although they remain reliant on personnel activity and practice.

Examining human factors can strengthen cleaning procedures. Designing processes to suit how people naturally approach their tasks can enhance the execution of those activities. That is true for manual cleaning, as well (**8**).

Evaluation of Cleaning

Whereas spot checks suffice for an automated system that has been subjected to rigorous validation, with manual cleaning, experts recommend that each item and cleaning session undergo an assessment (such as a supervisory check) with periodic verification by quality assurance (QA). Evaluation can be achieved through visual inspection and periodic sampling.

The most effective way to detect residues on equipment is through visual inspection. Although precise determination requires microscopy or swabbing, assessing whether a surface is free from streak marks, powders/particles, rouging, pitting, inclusions, and so on provides quick and straightforward assessment of surface cleanliness under appropriate lighting conditions (**9**). The person performing a visual inspection must understand what distinct types of residues look like. Visual inspection must be supported with appropriate documentation so that inspection outcomes can be recorded. Gamblin indicates that the limit of visual detection is thought to be in a concentration range of 1 µg/cm^2 to >10 µg/cm^2 depending on the products investigated and inspection conditions (**10**).

Visual inspections should be conducted when equipment is dry and under lighting conditions that are typical for a manufacturing environment. Inspection using magnification and additional illumination might identify residues more readily than the unaided eye. However, if such tools are used for training and verification, then they must continue to be used in the manufacturing area. For consistency, the three visual-inspection parameters requiring standardization are viewing distance, angle of viewing, and light level. Inspecting difficult-to-access surfaces also requires a mirror.

When visual inspection is supported by sampling, inspection should be performed first. Doing so helps to demonstrate the inspection method's suitability and degree of limitation. Furthermore, as Walsh points out, visual inspection should be performed before equipment reassembly (**11**).

Formal revalidation might be unnecessary (arguably, manual cleaning cannot be validated formally). However, regular sampling of manually cleaned equipment is important to verify that accepted cleaning levels are being achieved consistently. What is sampled, where, and how often are based on an assessment of the equipment complexity and on results of trend analysis (to help identify the hardest-to-clean items). At minimum, chosen sampling sites must be identified as difficult to clean through an appropriate risk framework. Where the same item of equipment is used with different product types, the sampling approach should ensure that the worst-case residue is tested (**12**). Sampling frequency will depend upon the risk of patient harm from low levels of an active pharmaceutical ingredient (API). Sole reliance on visual inspection during changeover between products containing potent APIs is appropriate only with clear evidence that residues can be seen readily and consistently at an established acceptance level.

When evaluating sampling results, recorded residues must be compared with the original cleaning validation and validation risk assessment. At this stage, it is important to understand whether a recorded residue level exceeds what is tolerable and hence would present a risk of product adulteration if the affected equipment was used during manufacturing.

Residue detection through either sampling or visual inspection carries potentially serious consequences. Cleaning failures should be recorded as events, which could lead to deviations, as defined in the company's pharmaceutical quality system. Results should be made available as inputs to review the cleaning validation.

Training and Reassessment

Training frequency for manual cleaning depends on a few factors (**13**). For

instance, QA teams should consider equipment size and complexity. Personnel considerations include the number of times that an employee will perform a cleaning activity and the range of equipment formats and sizes that will be cleaned. Teams also should examine outcomes from supervisory or QA checks on manually cleaned items — e.g., how often items were deemed insufficiently clean and sent back for additional processing. Such data can relate to staff members or specific types of equipment. The latter information can provide an index of difficult-to-clean items, which would be sensible to include in a retraining program. Manual cleaning should be evaluated at least annually using a practical exercise based on technique evaluation, visual inspection, and sampling. Such an exercise will highlight variability in practices across operators and potential for inconsistency.

Personnel who perform sampling, especially those performing surface swabbing, should have their techniques revaluated regularly. Periodic recertification is recommended to reinforce the manual dexterity and skills required for swabbing.

Training also must extend to supervisors and QA personnel who are tasked with visual inspections. Training must be completed with several residue types on different surfaces under representative lighting conditions. That could involve viewing soiled coupons of representative material. Spiking studies should be conducted to determine what concentrations of different residues are detectable. Such evaluations are also useful for operator training to help people who perform manual cleaning learn to differentiate between clean and unclean surfaces. Spiking evaluations should be based on an understanding of the maximum levels at which surface residues are safe. There is little point in relying upon visual inspection above that level because doing so could lead to surfaces being labeled *clean* when they contain unacceptably high levels of product residues (**5**).

To simplify acceptance criteria, straightforward classification as *clean* or *dirty* should suffice. Phrases such as *slightly clean* and *slightly dirty* are meaningless in this context. An inspector must detect all dirty surfaces to receive certification, and the accrediting organization will need to define an acceptable number for clean surfaces that were wrongly identified as dirty. Additional tests can be used to support verification of dirt, such as the same visual acuity tests used for personnel tasked with inspecting finished products for particulates.

Operator Safety

Manual cleaning presents risks to employees performing it and to people in their immediate vicinity. Operators can be exposed to splashes and airborne droplets. Although personnel should be protected adequately through personal protective equipment (PPE, such as shoe covers, gowns, gloves, face masks, eye protection, and head coverings), droplet dispersal remains a risk. For example, one healthcare facility undertaking endoscope reprocessing tested face shields affixed to procedure-room walls for traces of gastrointestinal fluids, with shields hung 6 ft (about 2 m) from uncleaned equipment. After exposure, 21% of shields tested positive for trace fluids (**14**).

Safety factors also can lower manual-cleaning efficacy. Certain cleaning chemicals pose too great a hazard to be managed by personnel, even with appropriate protective measures in place. Hence, in some circumstances, manual cleaning simply cannot be as effective as an automated process because of what chemicals can be used.

Segregation of Clean Equipment

As indicated above, manual cleaning activity is likely to generate droplets and aerosols. It is important not only to protect operators, but also to ensure that cleaned equipment is kept away from material that has yet to be cleaned or that is undergoing cleaning. Ideally, all cleaned equipment and parts will be held in a separate room. If that is not possible, then the distance between cleaned and dirty equipment must be at least 3 m. Consider that one healthcare study found that infective droplets can travel at least a meter from sinks during manual cleaning activities (**15**). Where airborne cross-contamination is a concern, Oftead and colleagues recommend affixing moisture-detection paper to environmental surfaces and PPE to assess droplet dispersal during manual cleaning tasks (**16**).

> Formal revalidation might be unnecessary. However, regular sampling of manually cleaned equipment is important to verify that accepted cleaning levels are being achieved **CONSISTENTLY.**

Mitigating Risks from Manual Cleaning

Manual cleaning is an inherently high-risk activity compared with automated cleaning. Variations will occur across operators because of differences in technique, cleaning time, cleaning-chemical volume, and amount of scrubbing. It follows that manual cleaning procedures must be monitored regularly and that training should be reassessed routinely. Thus, for manual cleaning operations, each biomanufacturing facility should set clear requirements in its procedures to help ensure consistent application of selected cleaning methods.

Overall, a biopharmaceutical manufacturer must be able to convey to an auditor that the relative risks of manual cleaning are understood and that process controls are in place to minimize them. My advice in this article can go some way in addressing such concerns.

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Antibody–Drug Conjugate Quality

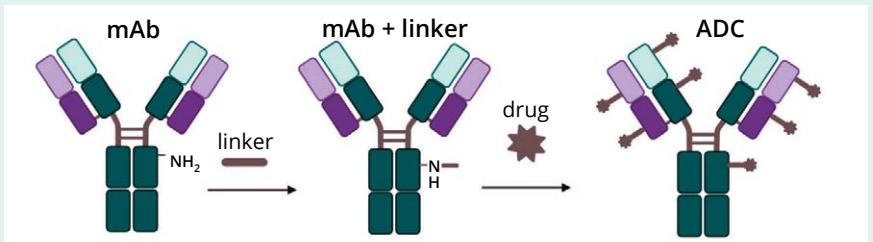
Key Attributes to Controlling the Molecule and Its Mechanism of Action

Jessica Bridges Weaver, Bryan Thacker, Likun Duan, and Jeff Patrick

Antibody–drug conjugates (ADCs) have become an important modality in biotherapeutics. By early 2025, there were 19 such products approved on the global market, with dozens more under development at more than 25 different companies (**1**). Largely considered to be oncology treatments, ADCs also are finding utility in other areas, such as autoimmune diseases and metabolic disorders due to their widely ranging mechanisms of action (MoAs). The appeal of this modality is antibodies' ability to deliver drug payloads to well-selected targets.

However, ADC development and manufacturing processes are complicated by the chemistry involved, as depicted in Figure 1 (**2**). Manufacturers need to monitor the biologic (e.g., a monoclonal antibody, mAb) and its attributes as well as all chemically derived intermediates and the combined end-product. Further complexity comes from the nature of the payload (the chemical entity that gets conjugated to the antibody), and the conjugation chemistry including linkers and other related factors. The developer's challenge is to ensure that all safety, identity, strength, purity, and quality (SISPQ) attributes are monitored appropriately and controlled as critical quality attributes (CQAs).

Figure 1: Representation of a typical antibody–drug conjugate (ADC)



ADC Structure and Complexity

- Amino-acid sequence: chemical modification, degradation, ragged ends
- Glycosylation: multiple glycan species
- Conjugated drug: abundance, distribution, spurious chemistry

Those CQAs are the main focus in creating a control strategy using multiple analytical methods based on a broad range of technologies to analyze and control at least three different entities: the starting biologic, the ADC drug substance (DS), and the ADC drug product (DP) (**3**). Such a control strategy centers on the basic MoA (Figure 2), in which the ADC binds to a target and delivers its payload to act on the target (usually by triggering cell death), thus providing patients with relief from a disease.

Table 1 depicts such a control strategy adapted from Bechtold-Peters et al., listing the typical combination of assays used to demonstrate that an ADC is both safe and efficacious (**4**). Many of the indicated assays are compendial assays commonly used for monitoring the following attributes in nearly all injectable biologics: appearance, osmolality, pH, content, sterility, endotoxins, and particulates (**5**). Other ADC assays test attributes that are specific to the modality and its MoA, manufacturing process, and chemical nature. Such attributes include DS size and charge variants, host-cell residuals, the presence of free/unconjugated drug (payload) and antibody molecules, and processing residuals.

Two of the most important CQAs for an ADC are the drug:antibody ratio (DAR) and cytotoxicity, the latter being based on potency assays. These closely

PRODUCT FOCUS: Conjugates

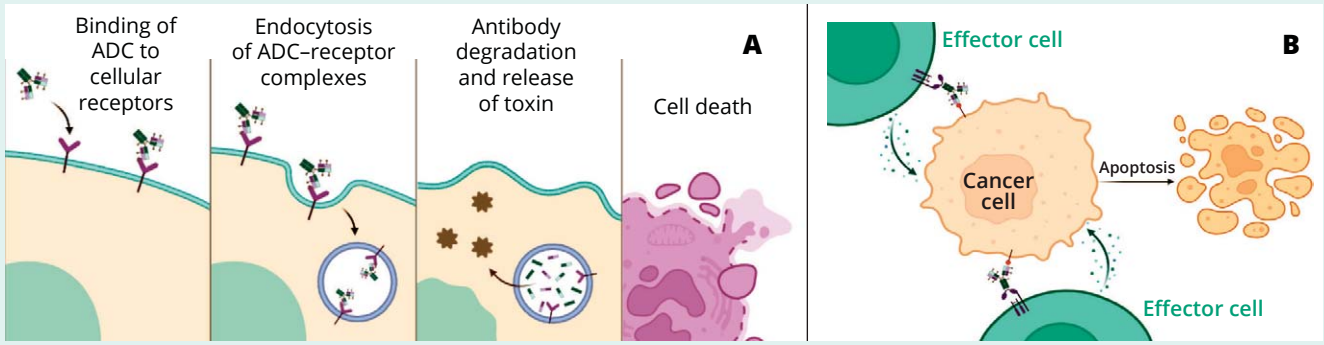
PROCESS FOCUS: Manufacturing

AUDIENCE: Process development, manufacturing, and quality control

KEYWORDS: Drug–antibody ratio, critical quality attributes, mechanism of action, ADCC assays, cell-based assays, HPLC-HPMS

LEVEL: Basic

Figure 2: Mechanism of action (MoA) for a typical antibod–drug conjugate in (A) a cytotoxicity-type activity and (B) an antibody-dependent cellular cytotoxicity (ADCC) activity



correlated attributes are critically important to ensuring the efficaciousness of an ADC drug and its dosing.

The "ADC Structure" box lists general material attributes to be monitored and key qualities to be assessed that affect the fundamental attributes of ADCs, including potency and efficacy. Variances in those attributes will also manifest in other assays, including those for potency or activity.

Below are discussions and results from our application of representative assays to model ADC compounds. These examples demonstrate the typical results and data to be expected when developing an ADC control strategy.

Table 1: Representative control strategy to support a typical antibody–drug conjugate (ADC), as adapted from Bechtold-Peters et al. (**4**); this assumes a four-step process in which a monoclonal antibody (mAb) or similar molecule is modified with a linker (mAb + linker), a drug is coupled to the linker to create an ADC drug substance (DS), and the ADC is then formulated to a finished drug product (DP).

Quality Attribute	mAb	mAb + Linker	DS	DP
Appearance	●	●	●	●
Osmolality				●
pH	●		●	●
Content	●		●	●
Sterility				
Endotoxins				●
Particulates				●
Size variants	●		●	●
Charge variants	●		●	●
Host-cell proteins	●			
Host-cell DNA	●			
Residual protein A	●			
Target binding	●		●	●
ADCC/effector function	●		●	
Cytotoxicity bioassay			●	●
Drug–antibody ratio (DAR)			●	
Drug–antibody profile			●	
Unconjugated mAb			●	
Glycosylation	●			
Variants	●			
Chiral impurities		●		
Oxidation				●
Conjugated impurities		●	●	
Free drug		●	●	
Metals		●	●	
Water content		●		●

ADCC = antibody-dependent cellular cytotoxicity

Cytotoxicity

The cell-based cytotoxicity assay (Figure 2) reflects the capacity of an ADC to target cells that present a specific receptor, delivering a payload drug that subsequently kills or otherwise disables those cells (Figure 2A). In antibody-dependent cellular cytotoxicity (ADCC) assays (Figure 2B), for example, an antibody (whether mAb or bispecific) binds to and elicits an effector cell to facilitate the final activity, which typically is cell killing. This effect often is described as the "magic bullet" approach of an ADC, selectively affecting only cells that can be targeted by the antibody used. In some instances, the MoA can be made even more complex for increased selectivity, such as by using a bispecific antibody that targets two different antigens.

We analyzed a representative ADC product using a cellular cytotoxicity assay. Target-expressing cells were plated and dosed with the ADC through serial dilution. Then the cells and drug were incubated for a prescribed period, after which we determined the viable-cell population by adding a detection reagent and reading luminescence on a plate reader.

Figure 3 shows that higher drug concentrations induce greater relative cell death. We averaged triplicate determinations to create a dose–response curve, with testing repeated across an assay range of 50–150% of the nominal dose concentration (NDC) (Figure 3). Such analyses typically are performed with a relative error of 10–15%. The cell type chosen can be critical for cellular toxicity assays because it must express the correct surface marker to engage the antibody

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Figure 3: Representative data from a cellular cytotoxicity assay; (A) <100% nominal dose concentration (NDC), (B) ≈100% NDC and (C) >100% NDC; DP = drug product

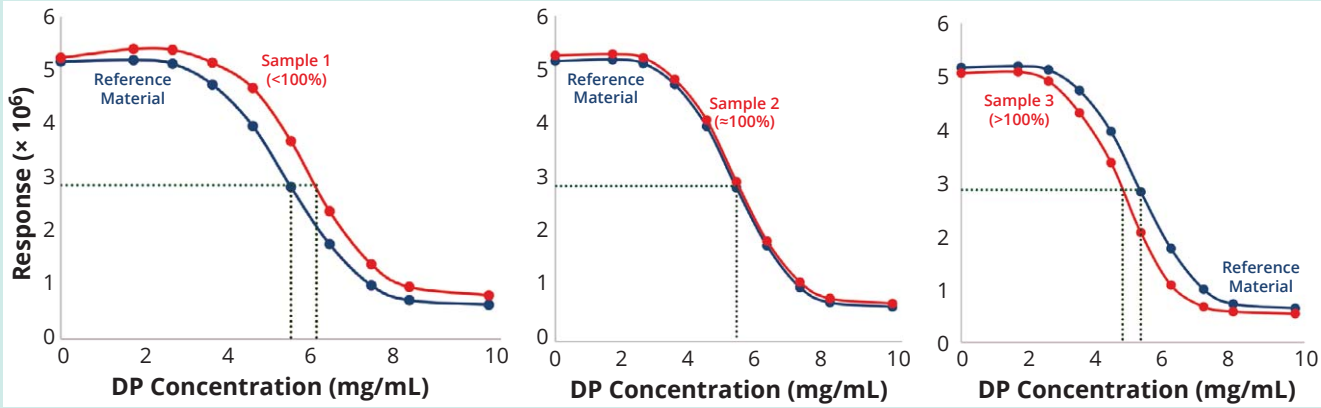
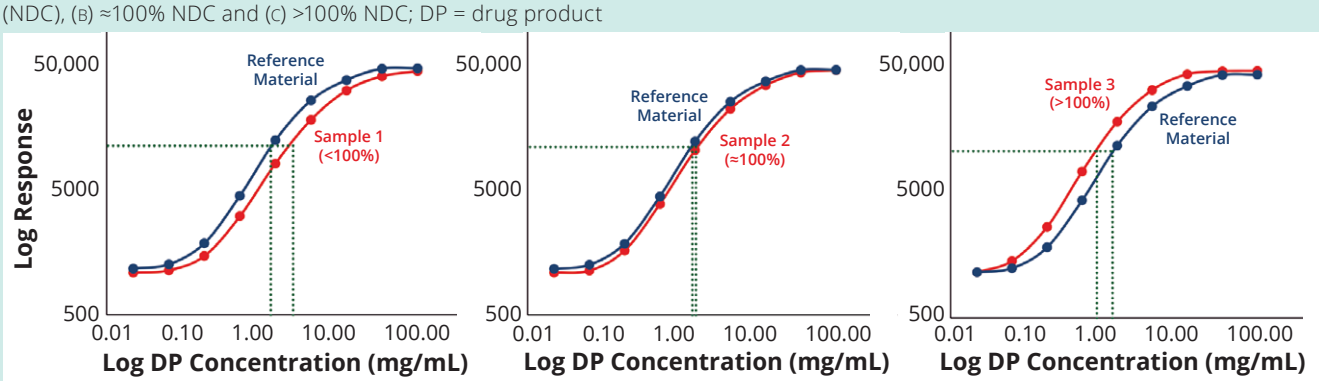


Figure 4: Representative data from an antibody-dependent cellular cytotoxicity (ADCC) assay; (A) <100% nominal dose concentration (NDC), (B) ≈100% NDC and (C) >100% NDC; DP = drug product



and for endocytic incorporation of the payload to induce the cytotoxic effect. That can be enhanced by using engineered test cell lines that overexpress the target protein.

We also analyzed a representative ADC product using an ADCC assay. Here, the antibody binds to one cell by recognizing the antigenic protein, and then the fragment crystallizable (Fc) portion of that antibody engages immune cells (e.g., effector and T cells) to facilitate killing of the target cells. We plated antigen-expressing cells, dosed them with the ADC through serial dilution, and then incubated them for a prescribed period. Afterward, we added engineered effector cells and continued the incubation before determining the viable-cell population, confirming that more drug induced higher relative cell death. The effector cells that we used have been engineered to contain the nuclear factor of activated T cells (NFAT) response element, which drives expression of firefly luciferase that can be quantified with a luminescence readout.

We averaged our triplicate determinations to create a dose–response curve, with repeated testing across an assay range of 50–150% of the NDC. Such analyses typically are performed with a relative error of 10–15%. The choice of cell-type also can be critical for this type of assay because cells must express the correct surface marker that engages the antibody to stimulate the effector-cell immune response to kill those target cells. The effector cells also must have the appropriate recognition elements. Both requirements can be enhanced by engineering cell lines to overexpress the target protein.

Other assay formats and readouts can be used as well, depending on an ADC's MoA, target cells, and related considerations. The intent is to demonstrate that the DS selectively attaches to a target antigen protein and induces an action (e.g., cell death) that is conducive to improving a patient's disease state.

Drug:Antibody Ratio

DAR typically is determined using high-performance liquid chromatography coupled with high-performance mass spectrometry (HPLC-HPMS). That combination provides for robust characterization and quantitation of a range of ADC attributes including

- distribution of payload drug molecules on the antibodies
- average number of drug molecules on those antibodies
- modification sites on the ADCs
- nonproductive couplings
- other by-products and impurities.

Developers also can use HPLC-HPMS to monitor attributes of the antibodies themselves throughout an ADC manufacturing process — e.g., degradation, glycoforms, free light chains, and other attributes. That can inform improvements in such processes. In some manufacturing settings, the HPLC-HPMS assay ultimately may be transferred to capillary electrophoresis (CE) or HPLC coupled with a less complex detection method such as UV-vis or fluorescence spectroscopy. That provides a method that is more appropriate for regular quality-control (QC) laboratory use.

To determine DAR average and distribution, we tested samples of a

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representative ADC to interrogate the molecules both "as is" and after subjecting them to stressors such as heat and agitation. To gain positional information about the payload distribution on the antibody molecules (and any changes therein), we further treated ADC samples with immunoglobulin-G–degrading enzyme (IdeS) or trypsin digestion or reduction/alkylation.

For these analyses we used an Orbitrap Exploris 240 mass spectrometer from Thermo Fisher Scientific set at R = 30,000, with HPLC using 0.1% formic acid in water and acetonitrile as an eluting system. Mass ranges and HPLC columns varied by experiment. We used BioPharma Finder software (Thermo Fisher Scientific) to process the data, supplemented by relational interrogations of the spectra, then evaluated for a number of attributes: DAR, sequence coverage and divergence, glycoform, free-payload and related impurity levels, and other drug-related materials. To evaluate the robustness of our determinations, we made multiple measurements over time using different setups. Figure 5 gives representative results from the DAR determination for intact ADC. Figures 6 and 7 provide information about antigen-binding fragment 2 from pepsin digestion (F(ab)′2) and the light and heavy-chain (LC, Fd) fragments.

The intact DAR was between zero and eight drug molecules per antibody molecule, with an unweighted average of about three. The complex spectrum clearly shows an intact glycoform distribution. (Note that deglycosylation with peptide-N-glycosidase F (PNGase F) can be used to "clean up" such spectra and simplify estimation, but information will be lost using such an approach.)

Analysis of the IdeS-treated sample shows that the majority of the drug is present on the F(ab)′2 structure with an average DAR of 2 (Figure 6) and up to five drug molecules per fragment. That can be broken down further (Figure 7) to distinguish between the light and heavy components of the F(ab)′2 fragment. The light chain shows up to two drug molecules, and heavy-chain components show no more than one.

Those data provide key insights into a number of critical attributes of the ADC:DAR and its distribution, payload localization and site-specific attachment, conjugation heterogeneity, antibody glycoforms, degradation and payload-related impurities, and stress-induced changes in drug loading. Collectively those data support process understanding, method robustness, and analytical control of the ADC.

In addition, our results provide critical evaluation of the HPLC-HPMS method as integral to the analysis and control of ADC drugs. We established the level of variance in the determination of attributes including DAR, sequence coverage and divergence, glycoform, payload and payload-related impurities, and other drug-related materials. Although not a DAR-evaluation method per se, our approach provides an orthogonal series of experiments for accurate interrogation and comparison of drug loading and position-dependent distribution, including subunit analysis and peptide mapping.

Figure 5: High-performance liquid chromatography and mass spectrometry (HPLC-HPMS) results for an antibody–drug conjugate (ADC) product; circle indicates detection of three glycoforms at each drug-conjugation ratio.

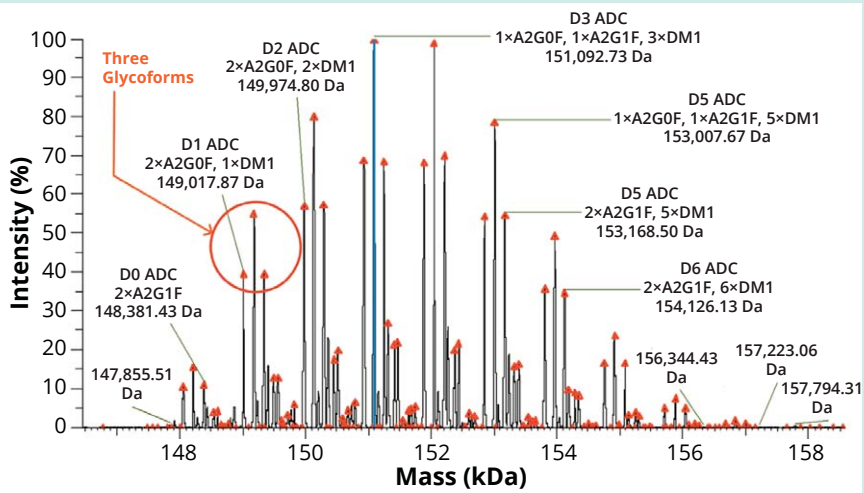
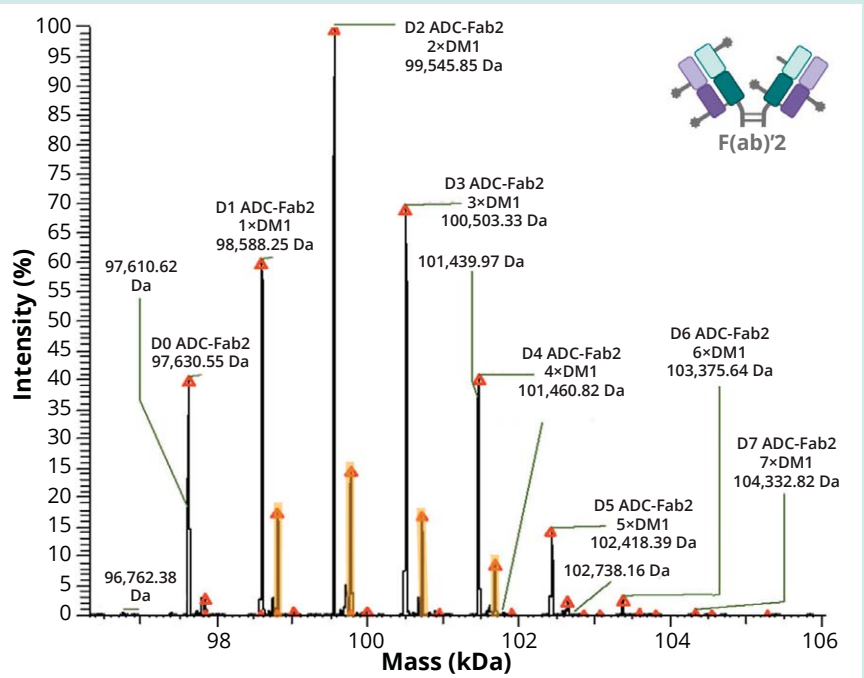


Figure 6: Immunoglobulin-G–degrading enzyme (IdeS) digestion of the same antibody–drug conjugate from Figure 5, with analysis of F(ab)′2 fragment; highlighted peaks indicate detection of attached linker without payload.



Collectively these data support process understanding, method robustness, and analytical **CONTROL** of the antibody–drug conjugate.

Figure 7: HPLC-HPMS analysis of reduced IdeS digestion indicates payload bound to the light-chain (LC) and heavy-chain (Fd) fragments.

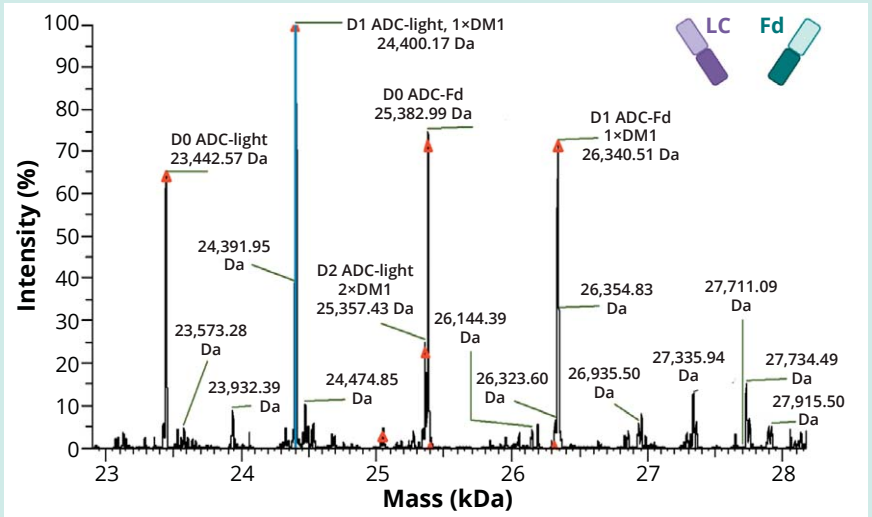
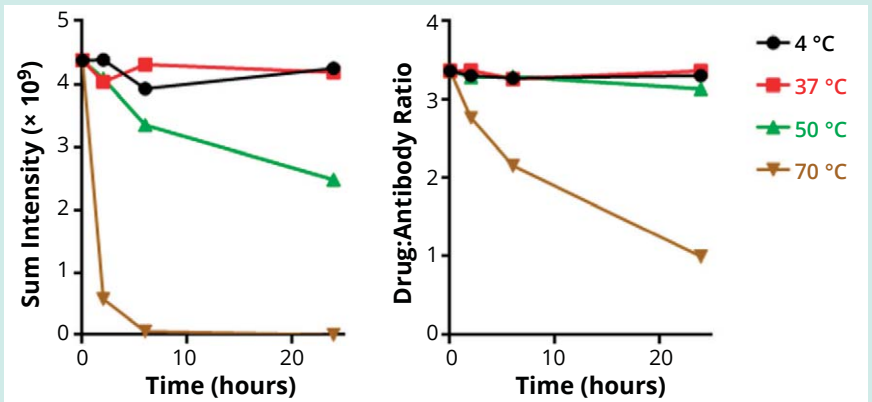


Figure 8: Tracking apparent loss of payload during thermal stress of the tested ADC



HPLC-HPMS also provides a mechanism by which to detect and measure the relative quantity of stress-related degradants and impurities associated with conjugation chemistry, process-related stress, and other modifications. For example, the presence of linkers without conjugated payloads is evident from measured masses in analysis of the F(ab)′2 subunit (Figure 6). Such spurious conjugation chemistry effects can increase immunogenic response and/or affect the pharmacokinetics of an ADC. Different forms of direct analysis provide comparable information with some variation.

By evaluating an ADC using the full spectral capabilities and resolving power of HPLC-HPMS, we can detect and identify multiple impurities and degradants for highly informative results. Note that variances in DAR, antibody identity (sequence coverage), glycoform distribution, and other attributes of <10% typically are suitable for early phase control of ADC products, if not for control throughout development. That is especially relevant when considering that other methods such as potency and purity assessments also will be performed to inform a holistic control and potency-assurance strategy.

In addition to the nascent ADC molecule, we analyzed other materials that were exposed to thermal stress using the same method to detect changes (degradation) in DAR. After material was stored at 4 °C, 37° C, 50° C, and 70° C, we estimated the total DAR and amount of antibody containing three drug molecules. Figure 8 shows the resulting trend with temperature over time for the loss (or lack thereof) in drug distribution and DAR. The three drugs exhibit a rapid and significant drop in their amount of free drug molecule and average DAR — an effect that is both time and temperature dependent.

We do not identify the exact nature of that degradation here, but it could be elucidated through a comprehensive control strategy — e.g., comparing results of charge-variant analysis (CE), size-variant analysis with size-exclusion chromatography (SEC), measurement of free drug and residual linkers, and so on — and considering whether the losses are chemical (linker hydrolysis) or physical (selective aggregate formation). The stresses applied here were extreme but intended to prove a specific point. Additional data and supporting information can be found in our other published work (**6**).

Controlling Quality Through Understanding

ADC analysis presents a number of challenges due to the complex nature of such molecules and the many attributes that need to be monitored. Here we have emphasized two of those attributes specifically, focusing on potency/cytotoxicity and DAR, which together form the foundation of ADC activity, control, and efficaciousness. Combining the rich information obtained from application of a typical control strategy provides a high level of assurance that both DS and DP will provide patients with necessary safety and activity.

The complexity of ADCs and the processes used to manufacture them demands the use of sophisticated tools and a deep understanding of the molecules to create and implement a control strategy, and then to interpret the data that result from it. As this drug modality evolves, new challenges will

arise in development of further control strategies.

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Continued from ***page 17***

The most successful innovators layer their protections — patents for genetic constructs and production technologies, trade secrets on proprietary refinements, and global filings aligned with commercialization plans. Such an integrated approach not only preserves competitive advantage, but also creates the clarity and confidence that investors and partners demand.

In the decade ahead, pharming could shift how we think about biomanufacturing. A vaccine grown in tomatoes or a therapeutic antibody expressed in a chicken's egg is more than a breakthrough product — it represents the convergence of agriculture, biotechnology, and pharmaceuticals. Those who cultivate innovation and strategically protect it will be best positioned to harvest the rewards.

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Unlocking Extended Fed-Batch Performance for CHO Cells

Jimmy Su and Xu (Penny) Peng

Biopharmaceuticals based on recombinant proteins offer clear advantages over many traditional small-molecule drugs. Biologics' mechanisms of action (MoAs) are often more specific, which can translate into fewer off-target effects and improved tolerability (**1**). Most recombinant-protein therapeutics are produced in mammalian cell culture, with Chinese hamster ovary (CHO) cells being the dominant production platform (**2**). Mammalian expression systems can produce complex proteins with proper folding and assembly, and the technology provides human-like posttranslational modifications that often are required for functionality and reduced immunogenicity (**3**).

Antibody-based products are the largest class of recombinant-protein therapeutics. Monospecific immunoglobulin G (IgG) molecules, which fall within the broader category of monoclonal antibodies (mAbs), account for about 74% of all approved antibody therapeutics (**2**).

Costs of Biomanufacturing

Despite the substantial success of antibody-based biotherapeutics, their widespread adoption and patient access are constrained by high prices (**4**). A prominent trend driving down both the cost of goods (CoG) and overall prices of mAbs is the growing biosimilars market, which is accelerating as originator biologic patents expire and allow for expanded competition (**5**).

In recent decades, upstream productivity gains have been a major contributor to significant reductions in CoG. For example, typical mAb expression titers in the late 1990s were often 1–2 g/L or less, whereas titers of 5–8 g/L are routine today (**2**). Among the levers now available to improve upstream process efficiency, optimization of cell-culture media and feed remains one of the most significant and scalable approaches.

Figure 1: Fed-batch results after 16-day process; (LEFT) viable-cell densities (VCDs, closed symbols, left axis) and cell viabilities (open symbols, right axis) obtained for the tested media combinations; (RIGHT) final volumetric titer measurements

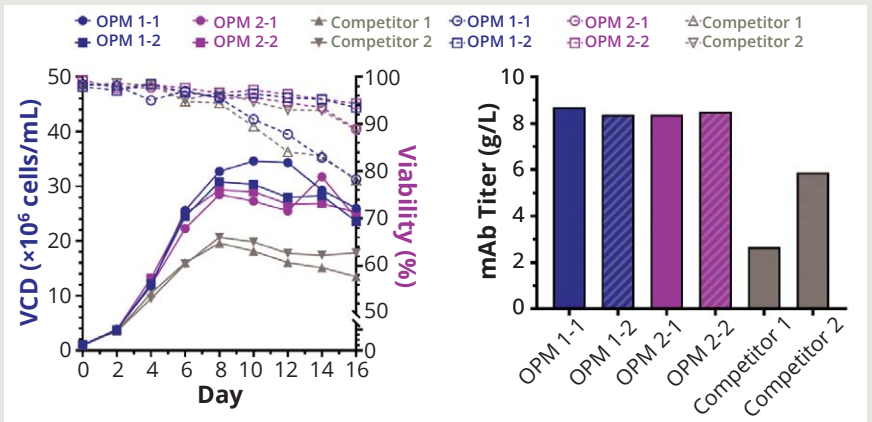


Table 1: Selected combinations of OPM basal media, feed media, and feed supplements tested

Group	Basal Medium	Feed Medium	Feed Supplement
OPM #1-1	StarCHO Medium	StarCHO Feed Plus medium	CDFS12
OPM #1-2	StarCHO Medium	VectorCHO Feed medium	CDFS12
OPM #2-1	StarCHO Plus Medium	StarCHO Feed Plus medium	CDFS12
OPM #2-2	StarCHO Plus Medium	VectorCHO Feed medium	CDFS12

Our Culture Media

OPM Biosciences is an integrated solutions provider of cell culture media products and services. We optimize cell culture processes to accelerate biotherapeutic discovery and manufacturing, helping to increase the affordability and accessibility of such therapeutics. Customers achieve that through using our high-performance, chemically defined media products, which are optimized specifically for upstream cell-culture process development and commercial biomanufacturing.

The OPM-CHO media portfolio is tailored for stable CHO cell protein expression. It can bolster cell-specific productivities that lead to volumetric product titer gains, reducing CoG for drug substances and ultimately drug products. Furthermore, our experience with biosimilar development gives us the expertise to help customer processes meet the critical quality attributes (CQAs)

Among the levers now available to improve upstream process efficiency, **OPTIMIZATION** of cell-culture media and feed remains one of the most significant and scalable approaches.

necessary for comparable clinical performance and regulatory approval.

Demonstrating Product Performance

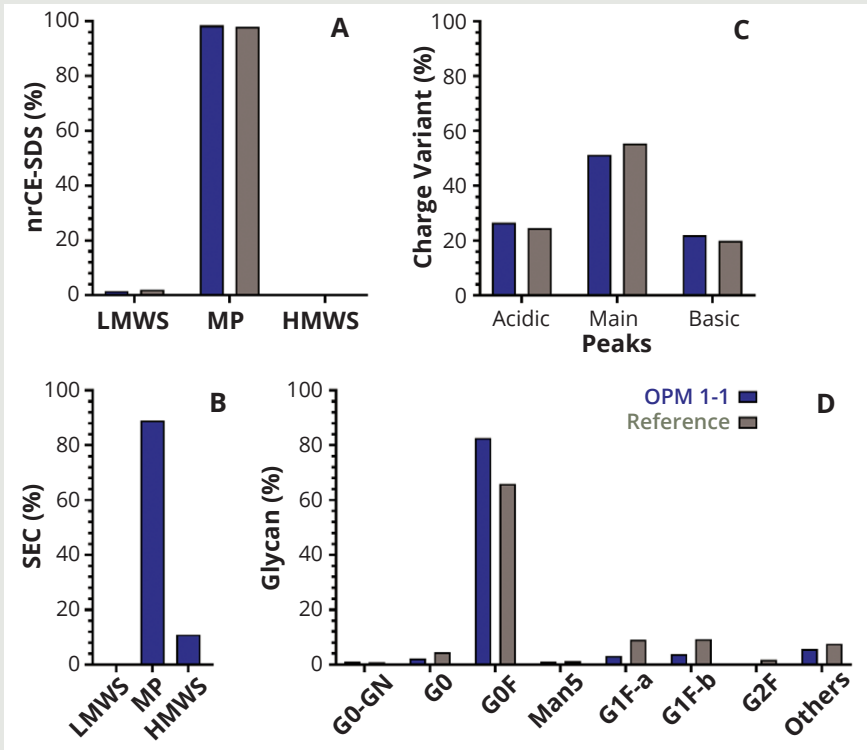
For a demonstration of OPM-CHO media performance, CHOZN GS$^{-/-}$ host cells were obtained from Merck KGaA. The GS$^{-/-}$ clone used in these experiments was engineered by the OPM cell-line development department for stable expression of a known IgG4 biosimilar mAb.

For the fed-batch cultures, we used 125-mL Erlenmeyer shake flasks maintained in an incubator shaker. Cells were inoculated at a density of 1.0×10^6 cells/mL in basal medium, then supplied with feed medium and feed supplement and given glucose to maintain concentrations >1 g/L.

Table 1 displays select combinations of OPM basal media, feed media, and feed supplements tested, with performance benchmarked against two global competitor products. To measure cell densities and viabilities, we used a Vi-CELL cell-viability analyzer instrument from Beckman Coulter; to measure mAb titers we used a Cedex Bio analyzer system from Roche CustomBiotech. The OPM analytical department evaluated product quality of a selected sample after a one-step purification and compared the results against those of a known reference molecule.

Combinations of OPM media and feeds supported higher growth profiles and peak VCDs than both competitor products did while maintaining similar or better cell viabilities (Figure 1, LEFT PANEL). Even with process extension to day 16, cell viabilities of OPM 1-2, 2-1, and 2-2 remained near or >90%.

Figure 2: Product quality characterization after 16-day fed-batch process with OPM 1-1 (blue) and a reference standard (gray); (A) size-variant analysis by nonreduced capillary electrophoresis sodium dodecyl sulfate (nrCE-SDS); (B) size-variant analysis by size-exclusion chromatography (SEC); (C) charge-variant analysis by imaged capillary isoelectric focusing (icIEF); (D) N-glycan profile analysis by ultraperformance liquid chromatography with fluorescence detection (UPLC/FLD); LWMS = low–molecular-weight species, MP = main peak, HMWS = high–molecular-weight species



Final titer measurements on day 16 demonstrate superior performance of OPM products over that of the competitor products, with >40% increase in volumetric productivity (Figure 1, RIGHT PANEL). Analytical characterization of product purified from group OPM 1-1 revealed limited product fragmentation and aggregation (Figure 2A, 2B) and similar charge-variant distribution to that of the reference molecule (Figure 2C).

N-glycan profile analysis revealed some variation from OPM 1-1 product compared with the reference molecule; however, it is important to note that glycan profile is not a CQA for IgG4 therapeutics such as the one here. That is because IgG4 isotype molecules do not rely on fragment crystallizable (Fc) region effector functions such as antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) as part of their MoA (**6**). Therefore, the molecule's therapeutic efficacy does not rely heavily on its glycosylation profile (**7**).

Conclusions and Future Directions

Here, we have demonstrated how a few products from our OPM-CHO media portfolio support high VCDs while sustaining high late-phase cell viabilities that provide for extended process durations. As a result, our products can bolster volumetric expression titers that are much higher than those obtained with leading global competitor products. Furthermore, product characterization revealed satisfactory quality attributes relative to the reference molecule.

Driven by continuous innovation, our team is advancing a strategy for intensified, extended fed-batch processes enabled by our media and feed platform. We look forward to sharing additional performance data

as investigational results become available. Whether your molecule of interest is an innovator biologic, biosimilar, or biobetter product, OPM offers solutions to help fulfill your productivity and CQA requirements. And because OPM products are specifically designed with the economics of production in mind, they offer substantial opportunity for biomanufacturers to reduce their overall CoG. Together, we can increase the global accessibility of these life-changing biotherapeutics.

Acknowledgments

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Critical Needs for Animal-Vaccine Manufacturing

An Expert Interview

Brian Gazaille and Sarah Stefancin, with Gustavo Mendes

Demand for vaccines has reached unprecedented levels, driven especially by the need to protect livestock. Yet such vaccines have distinctive production requirements compared with those of vaccines for human use. Corning Life Sciences offers its clients trusted products for success at every stage of development and manufacture. Speaking with BPI editors, Gustavo Mendes (senior cell biologist at Corning Life Sciences) discusses his company's work in the animal-vaccine industry and the workflows involved in such products' manufacture.

Historically, how has your company supported manufacturing for animal vaccines? Corning has worked closely with the animal-vaccine industry for many years, serving as a pioneer in both glass and plastic technologies that have enabled the industry's growth. For years, Corning has partnered with animal-vaccine manufacturers to develop and supply reliable, high-quality products essential for vaccine research, development, and production. Our organization has established a reputation for delivering dependable solutions that support the evolving needs of the vaccines market, helping manufacturers scale and innovate with confidence.

How would you describe current demand for animal vaccines? What types of vaccines are most in demand and for what species or applications? The global meat industry is expanding rapidly, with rising economic growth enabling more countries to increase animal-protein consumption than ever before. In regions where meat was once less accessible due to cultural or economic factors, increased wealth and market development have driven significant growth in animal production. As production methods have modernized to meet that demand, animals often are raised in well-managed, high-density environments. Although such advances support efficient food production, they also require careful attention to animal health. Vaccination therefore has become increasingly important as a proactive strategy to help maintain healthy herds and flocks. Demand for animal vaccines has reached unprecedented levels, driven by the need to protect livestock against diseases that threaten large-scale operations and food-supply stability. The most in-demand vaccines typically target major livestock species such as poultry, swine, and cattle.

How are such animal vaccines manufactured? What processes and equipment types are involved, and how significantly do those differ from processes and materials used to produce human vaccines? The production of animal and human viral vaccines shares a common foundation: Both typically begin with the cultivation of host cells to propagate viruses, which are then harvested, purified, and formulated. However, there are important distinctions in approach, technology, and regulatory requirements.

For animals, *attenuated* (live but weakened) vaccines are especially common. Their production usually involves cultivating large numbers of mammalian or avian cells, either in suspension cultures or, quite frequently, on adherent cell lines grown on surfaces such as roller bottles, multilayer vessels, and advanced systems that provide high

surface area per footprint. The virus is introduced to those cell cultures and allowed to replicate before being harvested and processed for vaccine formulation. Additionally, the US Department of Agriculture (USDA) is responsible for regulating animal vaccines. The overall workflow tends to be more streamlined and less complex compared with those for many human-vaccine processes, owing in part to less stringent regulatory oversight and a focus on established vaccine types.

Although similar vaccine modalities are used, human vaccine manufacturing requires an array of bioprocessing technologies, such as advanced bioreactors for large-scale cell culture, specialized purification systems, and complex downstream processing steps. Human-vaccine production is also subject to far more rigorous regulatory scrutiny compared with that of animal-vaccine production, with strict controls on safety, purity, and consistency at every stage. Such vaccines are regulated by the US Food and Drug Administration (FDA).

Key equipment types for both animal and human vaccine manufacturing include cell-culture vessels (roller bottles, multilayer flasks, modules that provide high surface area per footprint for adherent cells, or stirred-tank bioreactors for suspension cultures), systems for viral infection, and downstream purification equipment such as filtration and chromatography systems. However, human-vaccine production often leverages higher degrees of automation, closed systems, and single-use technologies to meet regulatory expectations for containment and sterility.

Do animal-vaccine processes raise distinctive requirements for sera, culture media, and so on? By contrast with the human-vaccine industry, which is moving rapidly toward alternatives to fetal bovine serum (FBS), the animal-vaccine sector still relies heavily on FBS and traditional basal media formulations. Roller bottles remain the most prevalent cell-culture platform for animal-vaccine production, followed by multilayer flasks and stirred-tank bioreactors.

Although all manufacturers must meet the same regulatory standards, the underlying needs of each company can **DIFFER SIGNIFICANTLY,** even when producing the same type of vaccine.

Because animal-vaccine manufacturers operate under tight cost constraints (often seeking to keep end vaccine prices below one dollar per dose), selection of reagents and consumables is driven first and foremost by cost. Such economic pressure influences choices in both cell-culture supplements and production platforms. However, there is growing recognition within the industry that investing in reliable, high-performance platforms — those that improve yield and process consistency — ultimately can deliver increased value, even if initial material cost is high. As a result, manufacturers are beginning to balance cost with attributes such as scalability, reproducibility, and supply assurance when selecting reagents and consumables for animal-vaccine production. Additionally, the adoption of closed-system solutions and single-use platforms is becoming prevalent. Such technologies help to mitigate contamination risks and enhance process safety, which further supports consistent, high-quality vaccine production.

What are some key challenges in animal vaccine production? How do those compare with challenges in producing human vaccines, and how does Corning help manufacturers to overcome those distinctive factors? Animal-vaccine manufacturers face the dual challenge of remaining competitive in a demanding market while embracing new technologies to improve efficiency and reduce contamination risks. With rising demand, producers are expanding capacity, often operating multiple vessels and scaling up production lines, to serve both livestock and domestic animal-vaccine markets. All of those activities must be accomplished under significant economic constraints; for example, poultry vaccines are typically priced between US$1 and $5 per 1000 doses, putting constant pressure on cost-effectiveness, quality, and innovation. Although the human-vaccine industry deals with many of the same issues, such as technology adoption and contamination control, it generally benefits from pricing flexibility and a different market dynamic, allowing for broad investment in complex manufacturing solutions.

At Corning, our commitment to customers extends well beyond supplying high-quality products. When our partners face process challenges (such as minimizing contamination risk, expanding production, or implementing new technologies), we provide practical, hands-on support. Such support includes technical guidance, sharing protocols, tailored training, and collaborative troubleshooting to address specific needs, including those related to domestic animal vaccines. Our goal is to work alongside our customers to help them achieve reliable, scalable, and efficient manufacturing outcomes. We believe that this approach — combining technical expertise with true partnership — creates lasting value in the evolving animal-vaccine industry.

What can manufacturers gain by leveraging customization services and related offerings (e.g., for custom culture media)? Although all manufacturers must meet the same regulatory standards, the underlying needs of each company can differ significantly, even when producing the same type of vaccine. For example, imagine two companies use

the same cell type to manufacture a vaccine. On paper, their processes might appear identical, but each cell line could come from a different source, be at a different passage number, or have unique growth characteristics. Those subtle differences can make a process require tailored supplementation, guidance, and optimization to achieve optimal results. Complexity increases further when factoring in the requirements of the specific virus or bacteria being produced. Customization services, such as designing specific culture media formulations or process adjustments, enable manufacturers to address those unique variables and ensure robust, efficient, and high-quality production that a standard solution might not deliver.

What are the most significant scalability barriers for animal-vaccine programs, and how do you support scale-up for such programs? A central challenge in animal-vaccine manufacturing today is the distinction between scaling up and scaling out. Historically, most production sites in the industry were designed decades ago around roller-bottle technology and can run thousands of bottles per batch. That approach made sense for the market at the time, but as demand has grown, those sites have reached their physical and operational limits. Scaling out, which adds production lines and/or space, has become increasingly difficult due to footprint limitations and infrastructure constraints.

Modern solutions focus on scaling up, which involves increasing the production capacity of each vessel to reduce the overall facility footprint and operational complexity. Technologies such as modules that provide significantly large surface areas within the same facility footprint, such as the Corning CellCube system, offer manufacturers the ability to consolidate production, increase yields per batch, and enable both efficient scale-up and manageable scale-out. By adopting such advanced systems, producers can overcome legacy barriers, meet growing market needs, and position themselves for flexible, future-ready manufacturing.

The animal-vaccine industry stands at a pivotal moment of opportunity, with modernization shaped by true **PARTNERSHIP** and practical **INNOVATION.**

What would you say are the biggest opportunities ahead for animal-vaccine manufacturing? In what ways will such processes continue to modernize, and how will Corning support that? The animal-vaccine industry stands at a pivotal moment of opportunity, with modernization shaped by true partnership and practical innovation. Corning is committed to supporting modernization efforts in a number of ways.

Communicating and Enabling Customization: Listening to manufacturers is essential. Customization services help companies to get more from their current sites and equipment, which can extend the lives of existing facilities.

Co-Creating the Next Generation: The most useful ideas for new products and methods come from conversations with people who work directly in production. By staying close to teams on the ground, we gain real insight into what's needed for the next generation of manufacturing and use that feedback to guide future development.

Supporting Practical Transitions: There is a clear need to move beyond roller bottles toward controlled and scalable systems, such as modules that provide significantly large surface areas within the same facility footprint. Not every technology fits every budget, so it's important to focus on practical improvements that make a real difference in day-to-day manufacturing. Through our commitment to listening to our customers, tailoring bespoke solutions, and support for stepwise modernization, Corning is dedicated to helping the animal-vaccine industry move forward with efficiency, flexibility, and readiness for future challenges.

What unique role do animal vaccines play within the broad vaccine industry? The needs, challenges, and realities of animal-vaccine producers are distinct, and those are important to consider when developing solutions. Real change comes from understanding those differences and offering support that improves what manufacturers can achieve.

From my years of experience in vaccine manufacturing, I have seen the value of introducing new technologies. Gradual adoption works best when it happens step by step, respecting the pace and priorities of each producer. Many Corning customers are not just clients, but also close collaborators. We work together to improve current processes, discuss future possibilities, and provide service as a team. Ongoing partnership is key to making meaningful progress in animal-vaccine manufacturing.

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Learn more about Corning's vaccine manufacturing workflows at https://www.corning.com/lifesciences.

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Engineering Outer-Membrane Vesicles

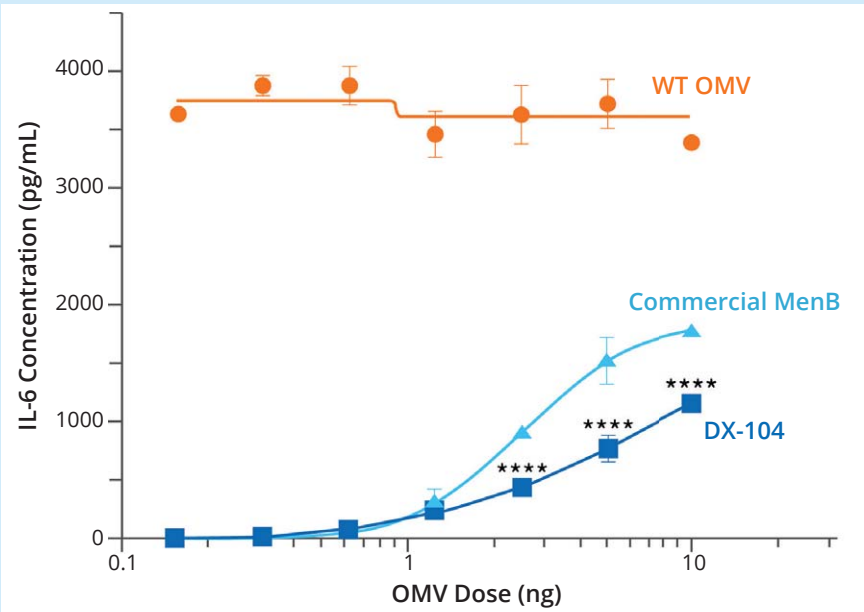
Leverage Rational Design To Shape the Future of Vaccine Development

Chen Dong, Miki Lee, Qiubin Lin, and Jason He

The shift from empirical discovery to rational, platform-enabled design is unlocking opportunities to develop safer, more effective vaccines for today's most challenging pathogens. As developers seek technologies that extend antigenic coverage, improve tolerability, and increase manufacturability, outer-membrane vesicles (OMVs) are reemerging as a solution. Once used primarily for regional outbreak control, OMVs have matured into a globally relevant, next-generation platform backed by extensive clinical evidence and expanding commercial adoption.

Among the three main categories of OMV-based vaccines, gOMVs stand out for their performance and represent the next logical step in the platform's evolution (Table 1) (**1**).

Figure 1: After incubation with outer-membrane vesicle (OMV) vaccine candidates, interleukin 6 (IL-6) release from Mono-Mac-6 cells shows that DX-104 induces much lower cytokine levels than wild-type (WT) OMVs. Activation is comparable with or below that of a commercial meningococcal B (MenB) vaccine.



Building a Versatile Vaccine Platform

OMVs are nonliving, nanosized (20–300 nm) particles that are released from the outer membranes of Gram-negative bacteria (**2**). Preserved outer-membrane antigens, intrinsic innate-immune stimulation, and an acellular safety profile provide a robust biological foundation for modern vaccines (**3**).

As the industry trends toward adaptable, multivalent vaccine platforms, the advantages of OMVs are clear. Their inability to replicate removes risks associated with using live or attenuated viruses while their diverse native antigens increase vaccine recipients' immune recognition beyond subunit constructs. Membrane-associated pattern-recognition signals provide natural adjuvant activity.

From Early Meningococcal Vaccines to Today's Pipeline

As understanding of OMV biology deepens, the platform has expanded from epidemic-focused meningococcal (Men) vaccines to an abundant and diverse pipeline. Since the first OMV vaccine was licensed in 1989 to suppress an epidemic in Cuba, clinical evidence consistently has demonstrated strong safety and immunogenicity, encouraging broad application.

Today, OMV-based candidates address many types of pathogens, including shigella, salmonella, pertussis, gonorrhea, and klebsiella bacteria — and even some viral and oncology targets. Such progress underscores an industry trend toward modular, strain-designable vaccine systems that are tunable to specific immune profiles, antigen compositions, and manufacturing requirements.

Table 1: Comparison of different outer-membrane vesicle (OMV) vaccine modalities

Type	Safety Risk	Antigen Preservation	Antigen Overexpression	Yield	Adjuvant Activity	Clinical Use
nOMV	High	High	Low	Low	Excessive	No
dOMV	Medium	Low	Low	High	Low	Yes
gOMV	Low	High	High	High	Optimal	Yes

Strain-Engineered OMV Vaccine Design

Strain-level modifications — including adjustments to lipopolysaccharide structure and vesicle biogenesis pathways — support gOMV performance by lowering pyrogenicity, increasing vesicle release, and maintaining key antigen profiles. Today, such strategies guide gOMV design generally.

DX-104, a next-generation candidate vaccine generated using Delonix Bioworks' OMV Plus platform, applies a defined combination of edits that have been optimized for MenB. Precision strain editing coordinates multigene changes that reduce endotoxic activity, widen strain coverage, raise OMV yield, and preserve and overexpress antigens that are key to protective immunity. This unified architecture displays protective antigens directly on the OMVs, creating potent, self-adjuvanted immunogens without need for detergent extraction, recombinant proteins, or added adjuvants. DX-104 more than triples the protective breadth of a commercially available vaccine, covering 71% of isolates compared with 21%. DX-104 also shows much lower reactogenicity (Figure 1). The candidate's high yield also supports large-scale production.

GMP Manufacturing for Scalable, Affordable Access

To make a global impact, gOMVs must be scalable and cost efficient. WuXi Vaccines brings the manufacturing capabilities needed to advance DX-104 from concept to clinic. Integrating upstream, downstream, and analytical development, WuXi Vaccines collaborated with Delonix to scale the DX-104 manufacturing process rapidly to 100 L with high batch-to-batch consistency and full good manufacturing practice (GMP) readiness.

Cryogenic transmission electron microscopy (cryo-TEM) and size-exclusion high-performance liquid chromatography (SEC-HPLC) confirm uniform vesicle morphology and 98% purity, indicating process robustness (Figure 2). Moreover, the process is projected to yield over 1 million doses per batch, reducing manufacturing costs and enabling increased access to the product.

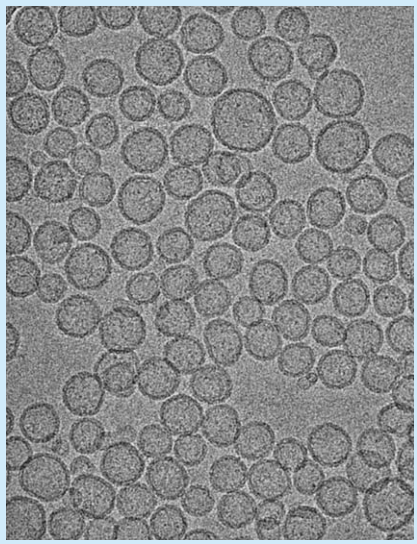
The Future of Engineered OMVs

With trials planned in China and Australia, DX-104 is poised to become the first gOMV vaccine to enter clinical study in China. The candidate's rapid advancement highlights how strain-designed OMVs can improve product safety, broaden coverage, and enable consistent, large-scale production to address complex and emerging pathogens.

As the pipeline continues expanding, gOMV-based products are positioned to support advances in vaccinology, drug delivery, and immunotherapy. To accelerate that progress and enhance real-world impact, Delonix and WuXi Vaccines are partnering with collaborators to advance gOMV technology worldwide.

As the pipeline continues expanding, gOMV-based products are positioned to **SUPPORT ADVANCES** in vaccinology, drug delivery, and immunotherapy.

Figure 2: Cryogenic transmission microscopy (cryo-TEM) analysis of DX-104 outer-membrane vesicle (OMV) morphology demonstrates a consistent and well controlled manufacturing process.



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How Can AI Speed Life-Saving Cures to Patients?

Chirantan Chatterjee and Tinglong Dai

For patients facing cancer, heart disease, diabetes, or Alzheimer's, the wait for new therapies can feel endless. A promising discovery in a laboratory today could take a decade or longer to become an approved treatment. Tragically, many people who could benefit from tomorrow's cures do not have the time to wait. Such bottlenecks are familiar: Researchers painstakingly test and try — running experiment after experiment, discarding countless dead leads before a viable pathway emerges. Data collection and analysis demand extraordinary attention to detail. Even once a breakthrough occurs, peer review and regulatory processes add years. That methodical system safeguards patients but slows progress.

Imagine if the entire process could be compressed. Possibilities and hypotheses could be tested against each other in seconds instead of months. Data analysis could be accelerated by orders of magnitude. And researchers could spend less time buried in paperwork and more time pursuing new ideas. That is the goal of an emerging array of artificial intelligence (AI) tools.

AI in Action

Major biopharmaceutical organizations and companies are investing accordingly into AI technology. Insilico Medicine has advanced an AI-designed small molecule into phase 2 trials for idiopathic pulmonary fibrosis (**1**). Sanofi's US$5.2 billion collaboration with Exscientia continues to hit milestones, and a three-way effort with Sanofi, OpenAI, and Formation Bio is producing concrete tools for drug development (**2, 3**). Meanwhile, the AI–infrastructure layer is maturing: Recursion's NVIDIA-powered stack and Generate:Biomedicines' first-in-human (FiH) AI-generated proteins compute meaningful biological insights (**4**). On the clinical-operations side, Sanofi, Formation Bio, and OpenAI have begun rolling out their Muse model to accelerate subject recruitment and streamline late-stage trial execution, including phase 3 multiple-sclerosis studies (**5**). The implications are profound. By compressing discovery cycles and lowering attrition, AI could shorten patent lives as competition arrives earlier than ever before, creating an access dividend for patients.

AI also can **reopen neglected frontiers**. In the antibiotics sector, AI-guided discovery recently surfaced *abaucin*, a narrow-spectrum compound active against *Acinetobacter baumannii* — a World Health Organization priority pathogen — reviving a field long considered to be uneconomical (**6**). And in protein therapeutics, generative models are informing FiH studies and facilitating billion-dollar partnerships, suggesting that design space — not just screening throughput — is changing (**7**).

But acceleration alone will not save lives. AI can **generate promising leads** faster than trial systems can validate them, risking an evidence backlog. Regulators are drawing the outlines of a remedy. A 2025 US Food and Drug Administration (FDA) draft guidance explains how AI-generated information can support decisions about the safety, efficacy, and quality of drugs and biologics (**8**). Similarly, the FDA's 2023 discussion paper on AI in drug manufacturing anticipated concerns for model validation, data provenance, and cloud oversight (**9**). Those materials mark the beginning of an AI-ready playbook, including expectations for model life-cycle management (e.g., documentation of training data, drift monitoring, and change control when algorithms and inputs evolve).

Biomanufacturing is **becoming AI native** as well (**10**). Cell-line development is shifting from incremental design of experiments to model-informed design. Process analytical technology combined with machine learning is moving monitoring toward prediction, and "digital twins" of upstream and downstream steps are starting to support real-time decision-making and accelerated deviation root-cause analysis (**11**). Real progress will be measured on factory floors: AI that can shorten technology transfer, flag scale-up risks before they can reduce yields, and accelerate batch-record deviation triage already is emerging. Such capabilities augment rather than replace quality systems.

Access to computing capability will matter as AI technologies become prevalent. Computing power is a new kind of scientific capital, but it should not be a gating factor. Philanthropic investments are helping: the Chan Zuckerberg Initiative is building one of the world's most powerful nonprofit graphics processing unit (GPU) clusters for life-science research, a step toward democratizing discovery tools (**12**). And the field is embracing open challenges: The 2025 Alzheimer's Insights AI Prize invited teams to build agentic AI that could turn existing data into new hypotheses — another way to accelerate breakthroughs without reinventing data collection from scratch (**13**).

The **future of AI in biopharmaceuticals** will not be about machines replacing humans. It will be about "centaurs," teams in which humans and AI work together, amplifying each other's strengths. Companies can treat every experiment as data for the next iteration. Regulators can codify standards for AI-native facilities and AI-assisted manufacturing. Funders can keep investing in shared computing power and open datasets. Clinicians can demand transparent tools that they can trust and explain.

AI already has delivered antibiotic candidates and FiH proteins — goals that were once thought to be unreachable. But the true promise lies in more than rapid discovery; it includes translating that speed into evidence, regulation, and adoption. The biopharmaceutical sector can transform from a business of serendipitous breakthroughs into one of systematic, global, and equitable innovation. For patients, that could mean something more important than price and profit: time.

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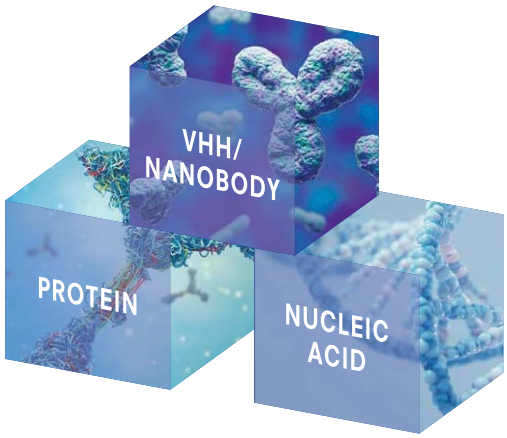

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