POTENCY MEASUREMENTS
for Cellular and Gene Therapy Products

By Maria Amaya, PhD, Andrew Chang, PhD, Keith Wonnacott, PhD, Derek T. Scholes, PhD, John MacNair, PhD, Lawrence C. Starke, PhD, and Lesbeth C. Rodriguez, MS

Cell and gene therapy (C&GT) products address various diseases at the cellular or genetic level, offer innovative treatment approaches, and represent a significant advancement in the field of medicine. However, developers of C&GT products face unique challenges due to their complexity, such as establishing assays that show a clear link between potency, mechanism of action (MoA), and clinical performance. Sponsors face a significant risk of a clinical hold if an adequate "potency assay" has not been established by the pivotal phase of clinical trials.

BACKGROUND ON GUIDANCE

The US Food and Drug Administration (FDA) has defined potency in the Code of Federal Regulations (CFR) Title 21 as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result" [1].

Similarly, ICH Q6B "Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products" describes potency as "the measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties" [2].

Furthermore, the FDA 2011 guidance on potency tests for C&GT products states that "potency measurements are a necessary part of product characterization testing, comparability studies, and stability protocols, which are used to establish that a consistently manufactured product is administered during all phases of clinical investigation" [3].

The 2011 guidance [3] emphasizes the importance of accurate measurement methods to ensure product quality and outlines specific considerations and methodologies for assessing potency. However, it acknowledges challenges to assay development for these products, such as the inherent variability of starting materials, limited lot size, material for testing, and stability; lack of appropriate reference standards; multiple active ingredients; the potential for interference or synergy between active ingredients; common MoA(s); and in vivo fate of the product. Consequently, it suggests that a single assay may not be sufficient to measure the product attributes, and multiple assays in combination may be required.

In December 2023, the FDA published "Draft Guidance for Industry: Potency Assurance for Cellular and Gene Therapy Products" [4], which provides recommendations for a science- and risk-based strategy to ensure the potency of human C&GT products. The potency assurance strategy proposed in this guidance document includes manufacturing process design, control, material management, in-process testing, and release potency assays to minimize risks and ensure the intended therapeutic effect for each product lot.

The draft guidance [4] advocates for the application of quality risk management principles throughout the product life cycle, which is similar to the recommendations outlined in ICH Q9(R1) [5]. These principles are adapted to C&GT products by using formal risk assessment tools to identify and mitigate factors affecting potency.

The 2023 guidance also addresses assay development [4]. However, it reduces rather than elaborates on principles related to assay selection and development. Though it provides a holistic overview and addresses assay development, it lacks depth when it comes to elucidating principles related to the selection and development of potency assays when compared to the 2011 guidance. Once the 2023 draft guidance becomes final, it will replace the 2011 guidance.

In Europe, the European Commission's 2017 guidelines on GMP for advanced therapy medicinal products (ATMPs) [6] address scenarios where traditional release testing may not be feasible due to various constraints. For investigational ATMPs, alternatives such as testing key intermediates or in-process controls, real-time testing for short shelf-life products, and increased reliance on process validation are suggested.

Moreover, as routine testing may be limited, the importance of process validation becomes heightened, though any adjustments to release testing strategies require approval from competent authorities. The European Medicines Agency guidelines, similar to the FDA guidance, offer concrete instances of adaptation strategies. Nevertheless, much like the 2023 FDA guidance, it falls short in delving into the foundational principles governing the selection and refinement of potency assays.

CURRENT CHALLENGES TO C&GT PRODUCT POTENCY TESTING

The challenges that manufacturers face in developing potency assays are described in the FDA's 2011 guidance and continue to be relevant. Acknowledging that the challenges will require creativity and flexibility, the guidance states that "FDA regulations allow for considerable flexibility in determining the appropriate measurements of potency for each product" [3].

To illustrate that flexibility, the guidance pointed out three ways in which potency could be measured: a biological assay, an analytical assay that is a surrogate for a biological assay, and a matrix of "complementary assays that measure different product attributes…that are correlated to a relevant biological activity" [3].

Despite the publication of the 2011 guidance, potency testing remained a significant challenge for the C&GT field. Sponsors have found that application of regulatory flexibility has been minimal and that regulators have linked the three different approaches to potency (bioassay, single surrogate, or matrixed surrogate) more frequently by an "and" statement rather than the "or" statement implied in the guidance.

This has created an expectation of adhering to all three methods simultaneously rather than choosing one based on context. When surrogate measures and matrix approaches have been tried, the pathway to establishing meaningful correlations between analytical assays and biological activities has proven difficult.

Additionally, the 2023 guidance emphasizes that "because C&GT products usually have multiple potency-related critical quality attributes (CQAs) that cannot be controlled adequately without release testing, your potency assurance strategy should typically include multiple release assays" [4]. Examples of the challenges identified for the development of C&GT products are described in Table 1.

The release of the 2023 Draft Guidance for Industry marks a continued evolution in the FDA's approach to potency for C&GT products. With the introduction of a potency assurance strategy, instead of viewing potency solely as a test, it is now recognized as a matrix and as a strategic imperative. However, due to the newness of the potency assurance strategy, and the uncertainty around FDA implementation, this article will not cover testing aspects of the potency assurance strategy.

Moreover, our accumulated knowledge on potency testing suggests that the 2023 guidance does not significantly change our understanding in this regard. The 2023 guidance eliminates the use of surrogate potency measures or potency matrices, emphasizing instead that potency testing usually involves a combination of multiple methods and expects sponsors to establish correlations between potency measures and proposed MoA.

Table 1: Challenges identified for the development of C&GT products.

Complex, multicomponent MoA	• Lack of one assay to cover all aspects of potency • Multiple MoA—matrix approach may result in redundant testing • Animal models may not be relevant or sufficiently robust [7] • The functional biological activity of a product may not be induced until engraftment and final maturation has taken place post administration • Certain elements of the MoA might serve solely for characterization purposes • As cell therapies progress, adaptability becomes crucial due to the evolving nature of understanding their MoA
Inherent variability of cell-based assays	• Complex sample matrix • Lack of reference materials and/or assay controls
Product-specific vs. patient-specific attributes may be difficult to predict and control	• Only product-specific attributes can be tested • Patient-specific attributes of the MoA cannot be measured a priori batch • Composition is not available until it is manufactured
Assays evolving as clinical development progresses	• Increased understanding of CQAs, product, and process

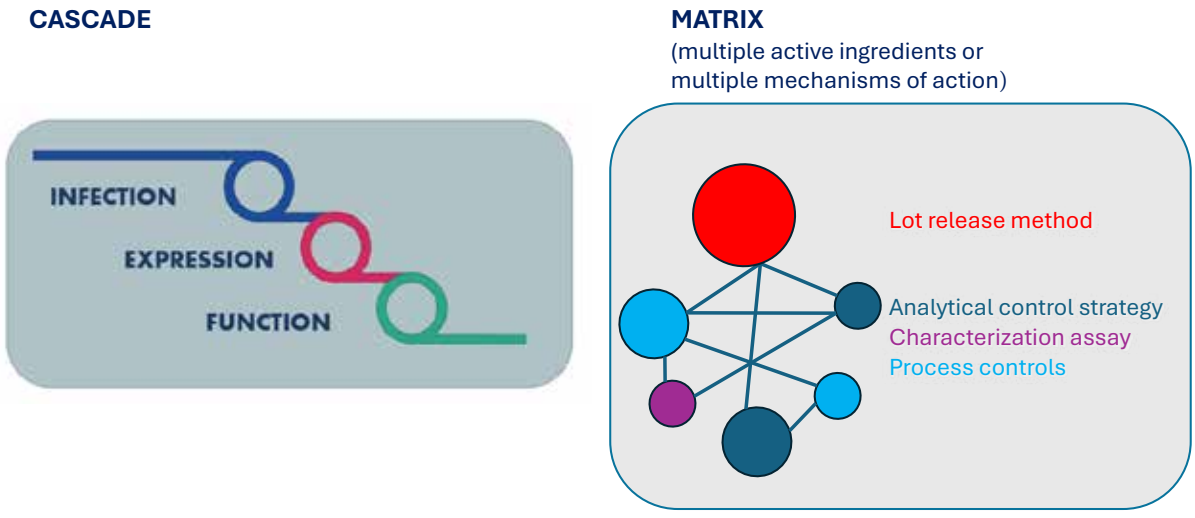
In this article, we introduce proposals to overcome some of the challenges identified. We expect that these proposals will help sponsors and regulatory agencies advance the discussion on the topic of potency for C&GT products.

POINTS TO CONSIDER

The ideal potency assay is relevant, reliable, and can quantify the functional biological activity related to the MoA. For a gene therapy, the ideal potency assay should be able to determine whether a particular environmental factor, storage condition/duration, presence of impurity(ies), post-translational modification of the product, or other such factors influence the biological activity associated with the MoA. This may, in turn, have an impact on clinical performance. In addition, for gene replacement therapy, the ideal potency assay should normally encompass an evaluation of efficiency of gene transfer (infectivity/transduction/delivery) and the levels of expression of the therapeutic sequence to its direct activity.

Potency assays should detect meaningful changes related to biological activity, have a defined product-specific acceptance criterion, have meaningful system suitability controls, and be stability-indicating. The ability to develop the ideal potency assay for C&GT products is variable and depends on the type of challenges

Figure 1: Primary strategies for potency assessment.



the developers face, which include but are not limited to complex and usually not fully known MoA, lack of assay sensitivity, and variability of cell-based assays (see Figure 1).

Potency Attributes

Potency is considered a CQA and the development of the relevant assays are the center of many challenges and discussions amongst developers of C&GT products and regulators. Measurement of potency plays an essential role not only for ensuring consistent bioactivity in each therapeutic dose administered to patients but also in quality control and batch release, product characterization, comparability, and stability.

An ideal potency assay's results should be quantitative, be stability-indicating, confirm lot-to-lot consistency, meet predefined acceptance and/or rejection criteria, and measure a biological activity that correlates with clinical function in the best-case scenario.

Fit-For-Purpose Solutions

As described in the 2011 FDA guidance "Potency Tests for Cellular and Gene Therapy Products" [3], the complexity and diversity of C&GT products can present significant challenges for the development of potency assays. Challenges such as variability of starting and raw materials, multiple active ingredients, complex mechanisms of action, variable CQAs, and complex manufacturing processes, make the development of fit-for purpose solutions a necessity.

As stated in the paper "Addressing Potency-Assay Related Development Delays for Cell and Gene Therapies" [8], "gene and cell therapies often undergo a series of processing events that ultimately result in the functional therapeutic entity. Further downstream events may be required to achieve the final therapeutic outcome, which itself may be another cascade. These biological steps are often referred to as a 'biological cascade.'"

As an alternative, the matrix approach to potency assessment recognizes that a single potency assay may not capture the full spectrum of therapeutic activity, and advocates for the integration of multiple assays tailored to the diverse MoA exhibited by these products. In essence, absent a singular potency assay, a matrix of assays is employed, each designed to reflect specific facets of the product's therapeutic potential. Figure 1 illustrates two primary strategies for potency assessment: the cascade approach and the matrix approach.

Potency is almost never univariate. In the cascade approach, as seen in Figure 1, potency assessment progresses through a series of assays, each addressing specific aspects of the product's MoA or biological activity. The matrix approach involves the simultaneous use of multiple assays, each capturing different dimensions of potency. These assays may encompass various methodologies, such as cell-based assays, biochemical assays, and molecular assays. Integration of data from these diverse assays provides a comprehensive understanding of the product's potency profile.

Biological Assays

Biological assays inherently exhibit variability, and in the context of cell therapies like chimeric antigen receptor (CAR) T cell therapy, this variability is compounded by differences in starting materials. These challenges underscore the difficulty in selecting the most suitable activity for validation and specification setting, especially concerning its proximity to drug delivery. Despite this, regulatory agencies often prioritize evaluating downstream protein activity

alongside gene activity. Consequently, developing assays that capture various levels of the activity cascade becomes imperative for product characterization and may even be mandated.

Multiple Distinct or Orthogonal Assays

Based on the MoA, the question of whether one assay is sufficient or if multiple assays will be needed is not always easily determined. When multiple components come together to make up a product, multiple potency measures may be needed. Examples might include when a lentiviral vector and a T cell are brought together to make a CAR T cell or when the various components (guide ribonucleic acid [RNA] and nuclease) of gene editing are brought together in a delivery vector. Multiple assays may also be needed if multiple MoAs are intended by the product either due to the presence of multiple transgenes or the presence of multifunctional cells.

The FDA strongly recommends [3] developing multiple assays in parallel during early clinical investigations. This may include the development of orthogonal methods that measure the same attribute of potency. Having multiple assays improves the likelihood that an acceptable assay can be found and agreed upon with the FDA and increases product characterization and understanding.

The 2023 guidance recognizes that redundant assays can be eliminated when multiple options are available [4]. It also states that one assay may be sufficient if it is a later step in the chain of biological activities that is completely dependent on the earlier steps. However, many sponsors have not experienced the stated flexibility that the draft guidance offers.

Surrogate Assays

Although the concept of surrogacy assays remains beneficial, the agency omitted the use of this term in the 2023 guidance [4]. The adequacy of a surrogate measure is often determined by the strength of the correlation to the proposed biological function. However, in some cases where the MoA is highly complex, conventional measurement methods may yield insensitive or highly variable results. In such scenarios, surrogate assays such as those quantifying clinically relevant biomarkers, may offer a more meaningful source of quantitative data.

A surrogate assay, instead of capturing the entire mechanism of "functional activity" in a single method, provides an alternative measure. Presumably, one that is physiologically and molecularly linked to functional activity and ideally whose outcome may be predictive of preclinical or clinical activity. This may include characterization of one molecular aspect of functional activity particularly when the functional activity of a transgene product is complex and involves multiple molecular events. The use of surrogate assays can strengthen the confidence of potency assessments for C&GT products, especially when there are limitations to establish a functional potency assay and/or the MoA is not clear.

Points to consider for establishing a surrogate potency assay follow. The extent of the linkage between a surrogate assay related to potency (e.g., T cell activation) and clinical performance should be established through bridging studies during product development. The surrogate assay may not be a bioassay: Surrogate assays may be less variable as compared to a bioassay, which provides an advantage for establishing product consistency and dosing recommendation.

Multiple assays may be needed, and, in some cases, a matrix approach could be considered. Different purposes may require different assays. For example: Bioassays are more meaningful and practical for product characterization, comparability, and stability studies. However, performing a cell-based functional biological assay for release of drug product (DP) is not practical due to limited time from formulation of the cell clusters to administration to the patients. Surrogate measurements (e.g., surface markers) for cellular products may be more appropriate and practical for DP lot release.

Example surrogate measures include biochemical, immunochemical, physical (e.g., phenotype, viable cell numbers), and assays that correlate with biological activity (e.g., messenger ribonucleic acid (mRNA) or protein expression, the activity measure of a single functional domain or protein binding).

Bridging Studies

Bridging studies are performed to build a relationship between a surrogate assay and the performance of the product. The purpose of bridging studies is to explore the relationship between product quality attributes (in this case, surrogate potency assays) and the clinical profile (efficacy). Some aspects of bridging could be done at the preclinical level rather than clinical and it could be used to demonstrate that certain iterations of the product (i.e., forced degradation, presence of certain post-translational modifications, impurities, etc.) do not have the intended functional impact.

Points to consider for the development of bridging studies include the following.

Quantitative linkage

If possible, sponsors should establish a quantitative linkage between the surrogate assay and the biological activity as measured using in vivo studies, in vitro studies, or other characterization of the products biological activity performed as part of the product development.

The FDA's 2011 potency guidance states that "the correlative relationship between the surrogate measurement and biological activity may be established using various approaches, including comparison to preclinical/proof of concept data, in vivo data (animal or clinical), or in vitro cellular or biochemical data" [3]. Previously established scientific knowledge or a platform approach could also provide good rationale for bridging a chosen surrogate measurement to the biological activity.

Processes and models

The outcome of the bridging studies and relevance of the correlation made will guide the decision-making process for choosing the right surrogate assay for potency. Bridging studies used to demonstrate that the product doesn't have the intended functional activity

Embracing this shift toward platform-based methodologies holds the potential to drive advancements across the entire C&GT field, ultimately enhancing product development and regulatory processes.

would be very challenging for indications that do not have reliable preclinical animal models. In some cases, extremely novel assays may require use of an assay bridging protocol/plan to ensure effective bridging to more conventional assays.

Changes over time and development

It should be recognized that potency assays may change over time and with stages of clinical development. With new scientific information, the test may be optimized, or new tests may be added. When a surrogate method(s) is introduced at a later phase of product development, a bridging study or head-to-head comparison is expected to ensure the adequacy of the replacement of the original test.

More versatile platform approaches

Furthermore, as collaboration between the FDA and developers progresses, there is a growing consensus on the need to transition from highly specialized, product-specific potency assessments to more versatile platform approaches. Analogous to the evolution seen in the monoclonal antibody field, where binding assays replaced highly specific biological assays, such platform approaches offer greater predictability and higher-quality data outputs. Embracing this shift toward platform-based methodologies holds the potential to drive advancements across the entire C&GT field, ultimately enhancing product development and regulatory processes.

CONCLUSION

Although there is a need for regulatory flexibility, quality standards should not be compromised for C&GT products and new approaches may be needed to ensure sufficiently high standards. Even though C&GT products pose many new challenges, they also bring new concepts and opportunities. In the case of the development of C&GT potency assays, there is no one-size-fits-all approach. Instead, fit-for-purpose approaches focused on developing activity-based methods or on the use of matrixed surrogate measures can provide a regulatory framework for potency assays that is flexible around some core requirements.

Specifically, we advocate for the following objectives. We encourage continued improvement of product and process understanding, as well as a subsequent evolution to the control strategy enabled through regulatory flexibility, e.g., persistent regulatory recognition that potency assays may change over time and with stage of clinical development given the limited materials available, often with high variability. We advocate for using surrogate measures, especially when the MoA is not clear. We recommend better understanding instances when regulatory flexibility can be applied. For example: a) where testing each component separately is necessary, particularly when the product comprises multiple components that function collectively as a final product; and b) assessing the final product without mandating multiple separate assays, enhancing efficiency without compromising the integrity of the assessment process.

Channels for enhanced communication and increased dialogue with health authorities regarding potency assay approaches will support the development of a roadmap for success. Finally, we encourage efforts for global harmonization on potency considerations for C&GT products and regulatory convergence in chemistry, manufacturing, and controls (CMC) development, including approaches for potency assays. Reliance and work-sharing approaches will facilitate timely access to safe, effective, and quality-assured medical products on a global scale.

Acknowledgments

We would like to acknowledge the contributions on this topic by the members of the Biotechnology Innovation Organization (BIO), a US trade association. We thank our colleagues and subject matter experts for their critical feedback and editorial input.

References

1. US Food and Drug Administration. "CFR - Code of Federal Regulations Title 21, Annex 11." March 1997. www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=11
2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. "ICH Harmonised Tripartite Guideline. ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for New Biotechnological/Biological Products." Published March 1999. database.ich.org/sites/default/files/Q6B_Guideline.pdf
3. US Food and Drug Administration. "Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products." January 2011. www.fda.gov/media/79856/download
4. US Food and Drug Administration. "Draft Guidance for Industry: Potency Assurance for Cellular and Gene Therapy Products." December 2023. www.fda.gov/media/175132/download
5. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. "ICH Harmonised Guideline Q9(R1): Quality Risk Management." Adopted January 2023. database.ich.org/sites/default/files/ICH_Q9%28R1%29_Guideline_Step4_2022_1219.pdf
6. European Commission. "EudraLex, Volume 4: Good Manufacturing Practice (GMP) Guidelines. Part II: Basic Requirements for Active Substances Used as Starting Materials." Published 2014. ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2014-08_gmp_part1.pdf
7. Gopinath, C., T. J. Nathar, A. Ghosh, D. D. Hickstein, and E. J. R. Nelson. "Contemporary Animal Models for Human Gene Therapy Applications." *Current Gene Therapy* 15, no. 6 (2015):531–40. www.ncbi.nlm.nih.gov/pmc/articles/PMC7709571/
8. Pfister, E. "Addressing Potency-Assay Related Development Delays for Cell and Gene Therapies: Results of a Scientific Exchange Between FDA and Developers." Alliance for Regenerative Medicines and the American Society of Gene and Cell Therapy. March 2023. alliancerm.org/wp-content/uploads/2023/03/Addressing-potency-assay-related-development-days-for-cell-and-gene-therapies-March-22.pdf

About the authors

Maria Amaya, PhD, is the Lead for External Advocacy, North America, in Quality Policy and Advocacy at Roche. In this position, Maria works within the Roche/Genentech Global External Advocacy community to develop and deliver innovative quality and current GMP regulatory pathways and collaborate with internal and external stakeholders, including support in harmonization and streamlining of regulations. Maria has more than 15 years of experience in the pharmaceutical industry working in product development, manufacturing technology, regulatory, and quality and

compliance. Maria holds a PhD in protein chemistry and a master's degree in protein engineering from the Paris-Sud University in France and a bachelor's degree in chemistry from the National University in Colombia. She joined ISPE in 2019.

Andrew Chang, PhD, is currently Vice President of Quality and Regulatory Compliance, Regulatory Policy and Intelligence, Global Regulatory Affairs, at Novo Nordisk, where he provides strategic leadership on regulatory- and quality-related policy, external affairs, strategic advice, and solutions to quality and regulatory challenges. He is a multifaceted quality and chemistry, manufacturing, and controls (CMC) leader with 28 years of medical product regulatory and industry experience. He is a board of director for Parenteral Drug Association (PDA) and CASSS-Sharing Science Solutions and the chair for PDA Biopharmaceutical Advisory Board. Andrew has substantial industry and ICH experience. He has represented Novo Nordisk at several work groups in industry trade organizations, such as Pharmaceutical Research and Manufacturers of America (PhRMA) and the BIO. He joined ISPE in 2019.

Keith Wonnacott, PhD, is the Vice President of Regulatory Affairs at Lexeo Therapeutics, a small gene therapy company based in New York that has multiple active information for human gene therapy investigational new drug applications (INDs) for adeno-associated virus (AAV) gene therapies to treat cardiac and neurologic disorders. He spent 13 years at the FDA working as a CMC reviewer and branch chief in the Cell Therapy Branch in the Division of C&GT. He joined Novartis in 2015 and was the Regulatory CMC for the first FDA-approved gene therapy, Kymriah. He also worked for several years at Pfizer, leading regulatory policy and strategy related to their AAV gene therapy programs. He holds a BS in microbiology from Brigham Young University and a PhD in immunology from the Pennsylvania State University.

Derek T. Scholes, PhD, serves as Vice President, Science and Regulatory Affairs, at the Biotechnology Innovation Organization (BIO). In this capacity, he leads BIO's team focused on regulatory oversight of the biotechnology industry. Scholes has worked for two decades in science and health policy in industry, the non-profit sector, the Executive Branch, and US Congress. Previously, he served as the first head of policy and advocacy at the American Society of Human Genetics, where he built the Society's policy and advocacy department and led the advancement of policies related to human genetics. He has also served as policy branch chief at the National Human Genome Research Institute and led advocacy efforts to advance federal legislation addressing heart disease at the American Heart Association. As a policy fellow on Capitol Hill, he handled a broad portfolio of health and science issues on the Senate HELP Committee.

John MacNair, PhD, is Senior Director, Head of Analytical Program Development and Management at Spark Therapeutics. Previously, over a period of 22 years, he progressed through positions of increasing responsibility in vaccine analytical development at Merck & Co., Inc. Over the last three years at Spark, John has formed a group, Analytical Program Leads, to guide leads as they design and facilitate execution of the analytical strategies associated with the gene therapy programs Spark has in development. He received a BA in chemistry from Binghamton University and a PhD in analytical chemistry from the University of North Carolina at Chapel Hill. He joined ISPE in 2022.

Lawrence C. Starke, PhD, is the Global Head of Regulatory CMC Policy and Intelligence for Cell and Gene Therapies at Novartis. In this role, he is responsible for developing regulatory strategies to expedite CMC regulatory approvals for cell and gene products. In addition to his regulatory activities at Novartis, he co-chairs the manufacturing subteam of the Bespoke Gene Therapies Consortium to facilitate first-in-human trials for ultra-rare genetic diseases and is a member of the ICH Discussion Group for C&GT products. Prior to this role, he led the C&GT CMC team at Novartis responsible for three global approvals. He has held regulatory leadership positions at Merck and Eli Lilly & Co and holds a BA from Colby College and a PhD in cell and molecular biology from Duke University.

Lesbeth C. Rodriguez, MS, is Director of Regulatory Policy and Innovation at Bayer, where she joined in 2003 as a Process Scientist in the Formulation and Freezing Drying team. She is responsible for driving the global regulatory policy strategy for C&GT and CMC (all modalities) at Bayer. She is a regulatory policy professional with over 20 years of experience in the biopharmaceutical industry with extensive knowledge of global regulatory frameworks. Prior to joining the Regulatory Policy and Innovation team, she held various roles, including Manager of the Manufacturing Sciences Final Product Manufacturing and Director of Regulatory CMC. She represents Bayer at BIO, PhRMA, and International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) C&GT committees/task forces. She is a member of the ICH Q13 Implementation Working Group (IWG) and the ICH C&GT Discussion Group (DG), representing PhRMA in both groups. She holds a MS in biochemistry from the University of Pennsylvania.

Please Join Us in Thanking ISPE's Corporate Partners

ISPE

Through the ISPE Corporate Partnership program, these companies have committed to supporting and contributing to ISPE's mission within the pharmaceutical industry.

PLATINUM

GOLD

SILVER