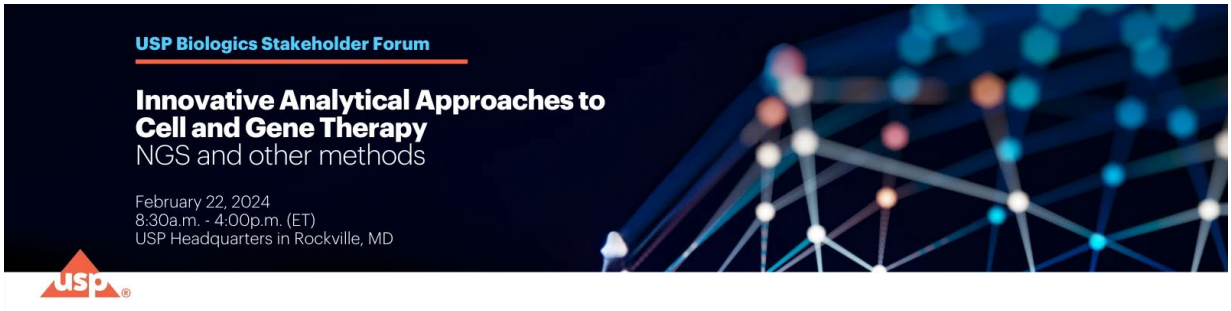

USP Biologics Stakeholder Forum 2024: Innovative Analytical Approaches to Cell and Gene Therapy

Executive Summary

In February 2024, USP held its fourth annual stakeholder forum, which brought together experts from industry, standards development organizations, and regulatory agencies at USP's headquarters in Rockville, Maryland. Participants shared their most pressing challenges to help direct USP's standards development efforts. USP standards accelerate the development of new medicines and promote a culture of quality across the industry. Presentations and discussions emphasized challenges related to next generation sequencing (NGS), also known as High-Throughput Sequencing (HTS), and its role in testing cell banks and qualifying raw materials.

Opening remarks were made by Fouad Atouf, Ph.D., Senior Vice President for Global Biologics at USP. He was followed by a presentation from Edward Chess, Ph.D., USP Biologics Stakeholder Forum Planning Committee Chair and event moderator. As an introduction to the day's events, Dr. Chess described the history and objectives of the stakeholder forum, highlighting how previous forums led to the development of new standards.

Session 1: Innovations to Overcome Limitations in an Evolving Regulatory Landscape

Speakers described the standards and guidance documents that are evolving in response to advancements in analytical technologies, such as NGS. The increasing inclusion of NGS in regulatory guidance reflects the readiness of the industry to supplement and replace conventional assays with validated NGS-based methods.

New and updated standards developed by the European Pharmacopeia (Ph. Eur.) include chapters 5.2.3 and 2.6.16. These chapters introduce testing strategies based

on risk assessments that allow for the use of broad molecular methods (e.g., NGS) and substitution for some in vivo test methods. All changes align with World Health Organization (WHO) recommendations. Ph. Eur. 5.2.14 elaborates on the transition from animal testing to in vitro methods, provides guidance for situations when direct comparison of the methods is not possible, and emphasizes the scientific rationale behind the in vitro methods and validation.

The ICH Q5A(R2) update that was finalized in 2023 reflects current knowledge and advances in viral safety evaluation. It provides new assays, alternative analytical methods (including NGS), and additional validation approaches for virus clearance in biotechnology products. Of note: direct head-to-head comparison with existing methods is generally not expected.

Speakers encouraged early interaction with the relevant regulatory agencies through transparent communication on filings. This open dialog gives agencies an opportunity to provide feedback on validation strategies and packages. Increased acceptance of NGS opens the possibility of establishing universal controls to support method development and validation.

Standards to Support Gene Therapy

Ben Clarke, Senior Scientist II, Global Biologics, USP

Dr. Clarke discussed the role of USP in the emerging cell and gene therapy landscape, as well as USP's current and planned documentary, physical standards, and reference materials. USP has developed best-practice informational chapters, physical reference standards and materials, validated compendial methods, training, and education. USP's newest chapter for CGT is ⟨1040⟩ *Quality Considerations of Plasmid DNA as a Starting Material for Cell and Gene Therapies* which covers manufacturing considerations, quality risk management, and DNA quality attributes.

Stakeholder input help prioritize the types of NGS standards that USP will develop. In the future, USP plans to develop reference standards and reference materials, as well as analytical procedure guidelines.

Minimizing the Impact of Stability Studies on Gene Therapy Batch Yield

Gaël Debauve, Head of Gene Therapeutics CMC Analytics, UCB Pharma S.A.

Dr. Debauve began by describing the impact of quality control (QC) and stability testing on batch yield for gene therapies and recommended strategies to reduce sample requirements by up to 50%. By implementing these mitigation strategies, products can reach more patients and at lower cost.

Dr. Debauve then explored in greater detail an emerging low sample volume technology, mass photometry (MP), for measuring capsid distribution that uses up to 50-fold less sample volume than the 'gold-standard' analytical ultra-centrifugation methods. Other advantages are shorter run times and easy data analysis and technology transferability. Next steps will be an in-depth comparison of MP to other methods, including assessments of precision and accuracy.

Dr. Debauve credited the BioPhorum position paper[1] on stability mitigation strategies as the source for much of the data shared in his presentation.

EDQM/Pharmacopeia Europe Activities in the Field of NGS/HTS
Gwenael Cirefice, Scientific Officer, EDQM, European Pharmacopeia
Dr. Cirefice discussed the evolution of the European Pharmacopeia's (Ph. Eur.) thinking on testing of extraneous agents as applied to vaccines and viral vectors used in gene therapies. He discussed the drivers for revisions and updates to two Ph. Eur. chapters, 5.2.3 *Cell substrates for production of vaccines for human use* and 2.6.16 *Tests for extraneous agents in viral vaccines*. He also discussed the replacement of in vivo methods for detecting extraneous agents by NGS as described in Ph. Eur. 5.2.14 *Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines* without the need for head-to-head comparisons. He went on to share perspectives on NGS, saying that the future chapter 2.6.41 *High throughput sequencing for the detection of viral extraneous agents* will provide a detailed description of NGS technology and validation guidelines[2].

FDA Perspectives on NGS in Adventitious Virus Testing
Arifa Khan, Senior Investigator, Center for Biologics Evaluation and Research (CBER), U.S. FDA
Dr. Khan recognized NGS as a powerful advanced technology for adventitious virus detection that can supplement or replace traditional techniques. Potential applications of NGS for safety testing and characterization of biologics include testing to mitigate risk of adventitious virus introduction through raw materials or manufacturing operations, characterization of cell banks, and replacement of animal assays.
Current regulatory documents provide flexibility for using alternative approaches that have broad virus detection capabilities and are fit-for-purpose. Going forward, work remains to develop standardized protocols and test datasets, as well as other types of standard materials. CBER will soon publish the results of a collaborative, head-to-head study evaluating NGS with in vivo and in vitro adventitious virus detection assays.

Session 1 Panel Discussion

Moderator: Kok-Seong (K.S.) Lim, Biologics Stakeholder Forum Planning Committee, Vice Chair.

Dr. Lim led the panel of speakers in a moderated question and answer session. The first set of questions related to USP standard development and how USP is innovating to support CGT stakeholders. Dr. Clarke explained that a panel of expert volunteers typically requires about two years to draft a new general chapter, though the time varies. He also explained that USP is supporting CGT stakeholders with solutions, such as white papers, online courses, and analytical reference materials. Raw material

[1] https://www.biophorum.com/download/minimizing-the-impact-of-stability-testing-on-gene-therapy-batch-yield/
[2] https://pharmeuropa.edqm.eu/app/phpa/search/

qualification standards and solutions have the potential to support the broadest range of CGT stakeholders.

Dr. Debauve addressed the next set of questions related to AAV stability studies. Stakeholders with unconventional approaches, such as limiting AAV stability studies to the intended storage temperature, should communicate with regulatory authorities prior to starting. Elevated temperatures or other stress conditions are often incorporated into biologics stability studies, as robustness to stress can be predictive of storage stability. However, AAV behavior evolves differently during storage versus under stress. The sensitivity of an AAV product to a particular stress condition may not be predictive of storage stability. Future guidance from working groups at BioPhorum, ICH Q1, and the USP AAV expert panel will support stakeholders performing AAV stability studies.

Dr. Cerefice was asked to expand on the drivers for developing the new Ph. Eur. draft chapter, 2.6.41. The strongest driver for NGS-based adventitious agent tests are reports of NGS-based virus tests that have identified viral contaminants that escaped detection by traditional Ph. Eur. methods. This indicates that there is a gap in the adventitious virus safety net. The other drivers for the chapter are the maturing viral standards at WHO, new molecular methods and risk assessment approaches, and commitments to reduce animal use in testing. Dr. Khan noted that the ICH Q5A(R2) working group and IABS have been supporting drivers for NGS-based adventitious virus testing approaches. The confluence of these efforts has produced a regulatory environment that supports stakeholders in substituting a conventional test(s) for an NGS-based test, without the need for head-to-head comparison.

Also discussed were questions of NGS-based method validation, reference standards, reference viruses, read depth/coverage, sensitivity, sequence databases, and application of NGS to microbial sterility assurance.

Session 2: NGS: Improvement, Replacement, or New Standard Approach?

In the second session of the day, industry experts discussed the use of NGS as an analytical tool for identity testing and product characterization. NGS is poised to be a faster, safer, and more ethical testing strategy. Other advantages include its speed, efficiency, and potential to eliminate animal testing.

The inclusion of NGS in updated guidance documents indicates that the current regulatory environment is supportive of using NGS as a replacement for traditional assays. Of particular note, a flurry of new guidance documents has been developed to support the use of NGS in the replacement of in vivo and in vitro adventitious virus detection tests.

NGS Transcriptome Analysis in Cell Banking

Colette Cote, Chief Scientific and Portfolio Officer, U.S. General Manager, Pathoquest

Dr. Cote described NGS transcriptome analysis as a technique for screening and characterizing cell banks. Looking at the RNA expressed in a cell is a rapid and simple way to detect and identify viral activity. Assay sensitivity is similar to PCR-based techniques and compares favorably to the capabilities of in vivo assays (MAP/HAP/RAP).

The validation and GMP-compliance of NGS-based methods has been a concern for industry. Dr. Cote recommended a modular 'system validation' approach that individually assesses the different aspects of the assay to build a full validation package. Likewise, each method step can have independent quality acceptance criteria.

Off-Target Analysis: Identification, Verification and Compliant Testing
Aaron Zhang-Chen, CTO, GeneGoCell
Dr. Zhang-Chen began by describing sources of off-target editing, including partial sequence homology to the guide RNA, specificity and purity of the nuclease, errors introduced into the guide RNA during chemical synthesis, and cross-contamination of guide RNAs by residual material from previous synthesis batches. Once high risk sequences are identified using broad in vivo, in vitro cellular, in vitro biochemical, or in silico prediction techniques, off-target editing can be verified using powerful and specific amplicon-based target enrichment, probe-based target enrichment, or deep whole genome sequencing (WGS).
Important considerations for qualification and validation include: linearity; limits of detection and quantitation (limit of detection/lower limit of detection and limit of quantitation/lower limit of quantitation); accuracy (sensitivity and specificity); precision (intra-run and inter-run repeatability). For the latter considerations, additional guidance from regulatory bodies and standards would be beneficial.

Recommendations for the Validation of rAAV Identity by Next Generation Sequencing
Jarrod Dean, Associate Director, Sanofi, Genomic Medicine Unit BioAnalytics, NGS CMC
Mr. Dean, presenting on behalf of the BioPhorum NGS subgroup, described a whitepaper on the collective knowledge and best practices for establishing a validated NGS method for rAAV vector identity testing. The two biggest shortcomings of historical methods for rAAV genomic identity testing (e.g., qPCR/dPCR, restriction enzyme digest with PCR, Sanger sequencing) are the difficultly in providing a full-length sequence readout and the insufficiency of the techniques for robust variant analysis. The BioPhorum white paper describes a modular NGS workflow based on short read sequencing. It considers method development, control strategies for each workflow module, system suitability, and sample acceptance criteria that ensure method performance and facilitate validation. In closing, Mr. Dean provided information and perspectives on long-read sequencing technologies, such as PacBio- and Nanopore-based solutions. Long-read sequencing can also be applied to identity testing and is a highly performant rAAV characterization approach. The BioPhorum whitepaper is currently in draft and includes input from 29 subject matter experts across 21 member companies.

Session 2 Panel Discussion

Moderator: Michael Jesudoss, Senior Scientist, NGS, BioMarin Pharmaceuticals
Dr. Jesudoss led the panel of speakers in a moderated question and answer session. The initial questions related to the progress of NGS-based method adoption in the

industry. Mr. Dean noted that NGS adoption can happen rapidly with the dedicated efforts of empowered subject matter experts and broad support from leadership across an organization. The versatility of NGS-based methods will give adopting organizations the ability to ask questions and address issues throughout the product lifecycle. The guidance from EDQM is an important step for improving adoption and alignment of best industry practices.

On the topic of reference standards, the panel noted the importance of versatility, ensuring accessibility through at most a BSL-2 requirement, and the necessity to support modular assay development, qualification, and validation. A reference standard that addresses issues with sgRNA purity would benefit genome editing product developers.

The panel agreed that assay sensitivity is an area that requires additional consensus building and regulatory guidance. It is straight-forward to design an NGS-based assay with either a very low risk of false positives or a very low risk of false negatives, but doing both is unachievable. The design decisions for the assay include the amount of material (population sampling), the number of reads, the complexity of the target sequence, and the sequencing technology. These decisions should be made in the context of the assay's purpose and the product-specific risk profile.

Also discussed were questions of traditional molecular method replacement by NGS-based methods, infrastructure requirements for NGS, in silico models, AAV ITR sequencing, structural heterogeneity of product nucleic acids, validation of a bioinformatic pipeline, and emerging innovations.

Conclusion

As many innovative medicines do, cell and gene therapies have stretched the limits of traditional analytical approaches and spurred the development of new analytical technologies. As an organization at the intersection of regulatory, manufacturing, and product development stakeholder, USP is positioned to convene industry leaders of diverse backgrounds to address salient shared challenges. This Biologics Stakeholder Forum exemplifies USP's collaborative approach to finding consensus and developing impactful standards. Speakers and audience members from across the world gathered to reflect on the unique requirements of CGT products and related regulatory guidance, scientific knowledge, and analytical method performance.

Participants came away from the event better informed on how to validate and implement NGS and other innovative analytical approaches for their products. They learned about the regulatory expectations of the US FDA and EMA, as well as the newest developments in the industry to find best practices. The recommendations for new standards for NGS that were shared will form the core of USP's standards development work in the area. Critically, stakeholders connected to like-minded individuals to continue the conversation on converging best practices.

For over 200 years, USP has gathered medical and pharmaceutical experts to establish the standards of medicine. Each of USP standards is approved by a committee of expert volunteers that donate their time and expertise to safeguarding the quality, safety, and accessibility of medicine. Consider joining them by applying to be an expert volunteer during the 2025-2030 cycle. https://callforcandidates.usp.org/node