Project A-Gene

A case study-based approach to integrating QbD principles in Gene Therapy CMC programs

Presented by

Alliance *for* Regenerative Medicine

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Contributors

CHAPTER LEADS / CONTRIBUTING MEMBERS

Greg MacMichael
CMC Bioservices

Saroj Ramdas
Amicus

Mo Heidaran
Parexcel

Paul McCormac
Pfizer

Iryna Sanders
Pfizer

Aili Cheng
Pfizer

Cirelli David
Pfizer

Hone Megan
Pfizer

Baldus Phoebe
Pfizer

Banbula Agnieszka
Pfizer

Lavoie Janelle
Pfizer

McEnroe Janet
Pfizer

Micklewright Althea
Pfizer

Alphonse Ignatius Arun
Pfizer

Furcolo Patrick
Pfizer

Parker Joann
Pfizer

Dawn Henke
Standards Coordinating Body (SCB)

James Warren
Ultragenyx

Jessie Sun
Ultragenyx

Jim Richardson
United State Pharmacopia (USP)

Jeffrey Hung
Vigene Biosciences

Alexandra Beumer-Sassi
Voisin Consulting Life Sciences

Luis Maranga
Voyager Therapeutics

Shamik Sharma
Voyager Therapeutics

Nripen Singh
Voyager Therapeutics

Harvir Grewal
Voyager Therapeutics

Krishanu Mathur
Voyager Therapeutics

REVIEWERS

Note: All chapters were reviewed, but individual reviewers did not review every chapter.
Reviewers volunteered to provide editorial review for select chapters according to their expertise and interest.

Tayler Renshaw
Audentes Therapeutics

Malou Gemeniano
Audentes Therapeutics

William Werner
Audentes Therapeutics

David Gray
Audentes Therapeutics

John Grunkemeier
Audentes Therapeutics

Dominic DeMuro
Audentes Therapeutics

Isabella Palazzolo
Biogen

Stuart Beattie
Biogen

Clare Blue
Biogen

Nicole Faust
Cevec

Nadine Sandhöfer
Cevec

Tim Farries
ERA Consulting

Raj Puri
(Regulatory chapter only)
FDA

Tim Miller
Forge Biologics

Michele Meyers
GSK

Steven Weisser
GSK

Michael Mercaldi
Homology Medicines

Maria Lobikin
Homology Medicines

James McGivney IV
Homology Medicines

Eric Faulkner
Homology Medicines

Jean Stanton
Johnson & Johnson (J&J)

Kelvin Lee
National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)

Hardeep Samra
Neurogene

Tina Parikh
Neurogene

David Litwack
Prevail Therapeutics

Michael Boychyn
REGENEXBIO

Mike Byrne
REGENEXBIO

Tristan Marshall
REGENEXBIO

Don Startt
REGENEXBIO

Mike Winkler
REGENEXBIO

Justin Horvath
REGENEXBIO

Rob McCombie
Sangamo Therapeutics

Robert Shaw
SCB

Sven Kili
Sven Kili Consulting

Khandan Baradaran
Ultragenyx

Jeffrey Hung
Vigene

Erica Giordano
Voisin Consulting Life Sciences

Emmanuelle Sabbah-Petrover
Voisin Consulting Life Sciences

PRODUCTION

Medical/Technical Writers
Darshana Gupte
Cecelia Wall
Amr Eissa
Julia Grant

Project Management
Michael Lehmicke
Adam Roose

Editor
Marie Daghlian

Graphic Design (select figures)
Michaela Flatly

Overall Graphic Design and Layout
Carol Collier

Project A-Gene

Introduction

The effort that has ultimately resulted in the document below began with a short, yet complex question posed to an audience of cell and gene therapy (CGTx) CMC experts. The Alliance for Regenerative Medicine (ARM) convened its first of a now annual series of CMC Summits in December of 2017, inviting individuals from across the industry to join in a conversation on manufacturing. As part of this discussion, the question of "What is the biggest challenge to cell and gene CMC? How can ARM help to address it?" was raised to the audience and, across the numerous responses and specific examples given, there emerged a common complaint related to the heterogeneity of the process. After exploring this further, there was general agreement that many of the issues driving the challenges to streamlined, cost effective manufacture of CGTx products derive from a lack of standardized methodologies and training around CMC programs. It was further suggested that this phenomenon was not new, and that we should look to the past for inspiration on how to address this challenge for the future. Therefore, it was decided to embark on a mission to recreate 'A-Mab' for the CGTx industry.

In previous years, the broader life sciences industry has encountered systemic barriers to the continued development of promising technologies. In both the monoclonal antibody industry and the vaccine development world, the whirl of energy around scientific discovery was stalled by the realities of manufacturing. While a small team of well-trained experts can produce high quality batches of drug product for use in process development and early clinical trials, it is inevitable that this process will need to be dramatically increased in scale, and the process transferred to other parties for commercial production. To help lower the barrier to this necessary tech transfer, and to better prepare new entrants to the industry, the leading developers of monoclonal antibodies and vaccines have produced a consolidated set

of recommendations for implementation of Quality by Design (QbD). A-Mab and A-Vax, respective to each industry, have played a significant role in elevating best practices within their industries, and have been effective in continued workforce development efforts. Borrowing from this model, the members of the Alliance for Regenerative Medicine have worked to produce a similar document for use by the burgeoning gene therapy sector.

In order to make A-Gene an effective resource, and reflecting the ongoing innovation in the sector, we sought to:

1) draw from as wide a set of expertise as possible,
2) specify our area of focus to direct gene therapy, and
3) focus on AAV as the case study.

This effort to catalogue expertise in gene therapy development occurred in parallel to approval of the first cohort of human gene therapies, which have shown the value and clinical relevance of such programs. As the field continues to develop it has been recognized that the future of cost effective gene therapy relies on implementation of common practices, development of specialized technologies, and above all else, standardization of methods. Given the wide scope of innovation underway in each of these areas of focus, ARM and the A-Gene team sought to bring in as much thought leadership as possible to ensure that what was recorded was truly a representation of best practices in the industry. Reflecting on this, A-Gene is a truly collaborative effort that has been crafted with contributions from more than 50 industry experts from more than 20 leading therapeutics developers.

To further maximize the utility of A-Gene, the drafting team decided to focus on *in vivo* gene therapy. Current FDA language around Gene Therapy encompasses *in vivo* gene therapy, gene editing/manipulation, and gene modified cell therapy. Each application carries a wide array of specific manufacturing steps and considerations, and so in order to draft a cohesive and informative narrative, the A-Gene team decided to focus on *in vivo* gene therapy. *Ex vivo* gene-modified cell therapies will be the subject of a future case study.

Finally, the A-Gene team deliberated on what the underlying case study would be for this document. As each chapter is meant to operate as both an independent resource, as well as part of a comprehensive narrative, we felt it was necessary to focus on one specific use case to facilitate the utility of A-Gene. Lentiviral and AAV vectors are the two most frequently used viral vector platforms used in gene therapies, and the approach to producing these two vectors are similar. While lentiviral vectors are predominantly used for *ex vivo* cell modification for gene modified cell therapies, AAV is the major vector type for *in vivo* applications. Given our previous decision

to draw from the widest set of expertise possible on direct gene therapies and the focus on *in vivo* gene therapy approaches, it naturally made sense to focus our case study on human rAAV therapeutic development. This is not to imply either a relative value in rAAV vs other viral platforms for direct gene therapy, but is, in general, recognition of the number of companies pursuing rAAV applications. We deliberated on inclusion of parallel tracks throughout the document, comparing and contrasting rAAV to lentivirus, but in the end decided this would be too cumbersome. Beyond this, there is a great deal of overlap in thought process and methodology between the two approaches, and so in lieu of a running comparison we elected to spend some time in the introduction addressing differences in CMC methods between the two viral platforms.

A-Gene is not intended to represent a standard to be rigidly applied. It is a hypothetical case study representing an archetype of an AAV vector for gene therapy. Therefore, it is a snapshot in time of current best principles in a rapidly evolving field. The data cited in the document are non-proprietary, and are intended to be for illustrative purposes only. Where appropriate the authors have borrowed formatting and structure from the A-Mab case study. While we have attempted to be as comprehensive as possible, and have subjected the document to rigorous review, it is not a "recipe book" for AAV manufacture. Some aspects of process development (e.g., facility design), were deliberately omitted for the sake of brevity. Importantly, A-Gene is not an example of a mock regulatory submission, nor should it be interpreted as regulatory advice, or cited as regulatory guidance.

As a final point, we wish to thank those who contributed to this effort. The Alliance for Regenerative Medicine is grateful to the innumerable thought leaders, subject matter experts, and researchers who have helped to make this project a reality. We also wish to extend our deep appreciation to the National Institute for Innovation in Manufacturing Biopharmaceuticals for their support and contributions to this effort, and for working with ARM to make this project a reality. We look forward to continuing our work with our members, key opinion leaders, and the numerous innovators who have made gene therapies a reality for the thousands of patients who rely on biotherapeutic developments to improve their quality of life. We intend to continue our work with these groups to maintain the relevance and accuracy of this document as the industry advances.

This work was performed under financial assistance award 70NANB17H002 from the U. S. Department of Commerce, National Institute of Standards and Technology.

Chapter 1

Regulatory Considerations

Alliance *for* Regenerative Medicine

NIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 1 | Contents

Introduction

Gene therapies, a subset of regenerative medicine therapies, include plasmid DNA and RNA, viral vectors, bacterial vectors, products incorporating human gene editing technology, and patient-derived cellular gene therapy products. These are transformative therapies addressing conditions such as cancer and genetic and infectious diseases.[1] Few commercial assets have been approved for patient use globally, reflecting the infancy of this modality. Currently, there are pathways for regulatory review and approval in three major markets: United States (U.S.), European Union (EU), and Japan. This chapter summarizes (1) the regulatory frameworks in these three major markets, (2) expedited regulatory pathways, (3) Health Authority (HA) innovation teams, (4) HA and sponsor meetings, (5) gene therapy specific information to be included in the electronic Common Technical Document (eCTD) format for the license applications, and (6) lifecycle change management.

Regulatory Framework in the U.S., EU, and Japan

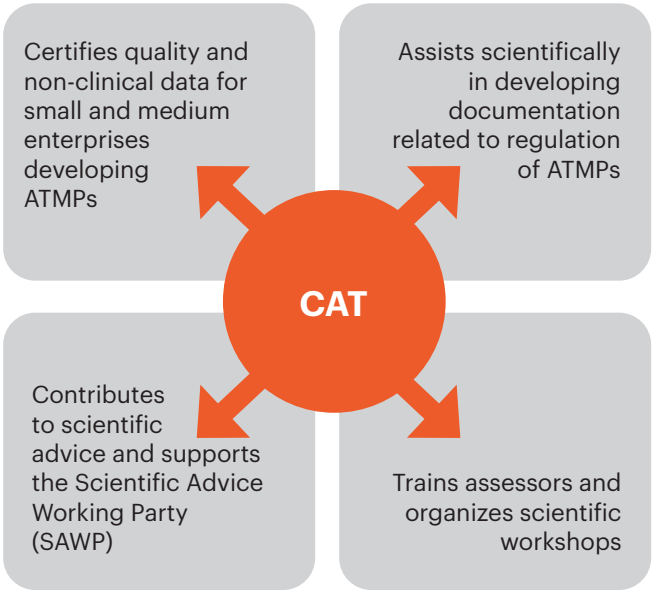
The novel and diverse nature of gene therapies has resulted in evolving regulatory frameworks specified to support these products in markets such as the U.S., EU, and Japan. With increased experience with this broad family of products, regulatory agencies will have the opportunity to further define guidance that will facilitate their development to address patients' unmet needs.

UNITED STATES

In the U.S., regenerative medicine therapies are regulated by the Food and Drug Administration's (FDA's) Office of Tissues and Advanced Therapies (OTAT) within the Center for Biologics Evaluation and Research (CBER). OTAT oversight ensures the safety, purity, potency, and effectiveness of gene therapy products. CBER has released guidance documents addressing the following gene therapy CMC topics:

- Chemistry, Manufacturing, and Controls (CMC) Information for Investigational New Drug Applications (INDs)[2]

Figure 1-1. *CAT role in ATMP review process in EU*



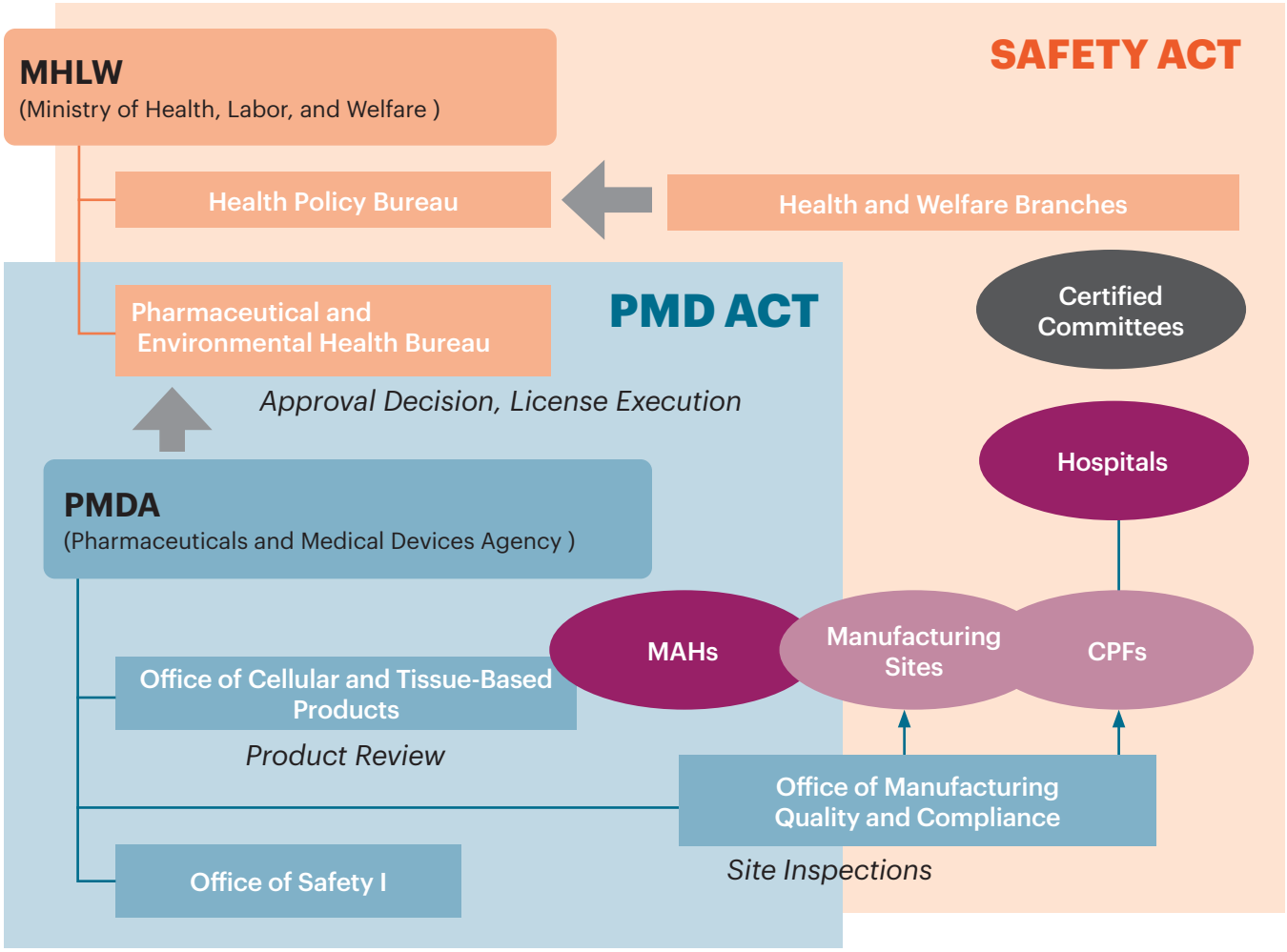
- Testing of retroviral vector-based human gene therapy products[3]
- Devices used with regenerative medicines advanced therapies[4]
- Microbial vectors used for gene therapy[5]
- Potency tests for cellular and gene therapy products[6]

To provide additional support to developers, CBER has established OTAT Learn as an educational resource for industry and includes several course listings led by OTAT staff.[7]

EUROPEAN UNION

In the EU, gene therapy products are included under the umbrella of Advanced Therapy Medicinal Products (ATMPs) and are regulated by the European Medicines Agency's (EMA's) Committee for Advanced Therapies (CAT), which is tasked to assess the quality, safety, and efficacy of medicinal products. There may be instances where classification of medicinal products as ATMPs may be borderline with respect to other areas (e.g., medical devices); thus, EMA has established an ATMP classification process[8] further described in ATMP classification. CATs primary responsibility in ATMP regulation is to provide draft opinions regarding approval to The Committee for Medicinal Products for Human Use

Figure 1-2. *Japan Regulatory Framework for Regenerative Medicines* [12]



(CHMP). In addition to drafting recommendations, CAT is integral in the following activities summarized in Figure 1.[9]

The EMA has released guidance documents addressing the following gene therapy CMC topics:

- Quality, nonclinical and clinical aspects of gene therapy medicinal products
- Scientific requirements for the environmental risk assessment of gene therapy medicinal products
- Quality, nonclinical and clinical aspects of medicinal products containing genetically modified cells

In addition to guidelines related to several aspects of gene therapy development, the EMA has established guidance regarding marketing authorization procedures for ATMPs on the following topics:[10]

- Procedural advice
- Dossier requirements and submission dates
- Guidelines for risk-based approaches

JAPAN

Japan has two main regulatory authorities that are independent agencies with distinct roles during the drug approval process: (1) The Ministry of Health, Labor, and Welfare (MHLW), which is responsible for publishing regulatory guidelines, managing advisory committees, and providing final authorizations for applications and (2) the Pharmaceuticals and Medical Devices Agency (PMDA), which is responsible for regulatory and scientific review (i.e., Office of Cellular and Tissue based Products), and Good Manufacturing Practice (GMP)

compliance and inspections (i.e., Office of Manufacturing Quality and Compliance). Japan's regulatory framework for regenerative medicines, including production of therapeutic products by industry, is based on "The Act on Pharmaceuticals and Medical Devices (PMD Act)," which underpins activities within the PMDA regarding gene therapy regulatory evaluation (see Figure 2[11]).

Designations to Expedite Development

The U.S., EU, and Japan have established expedited pathways to support accelerated development and regulatory approval for medicinal products that have the potential to address unmet medical needs. These pathways provide opportunities for developers to engage with regulators

Table 1-1. *Summary of Expedited Pathways (U.S.)*

	Fast Track (FT)	Breakthrough Therapy (BTD)	Regenerative Medicine Advanced Therapy (RMAT)	Priority Review	Accelerated Approval
Date Established	1997	2012	2017	1992	1992
Qualifying Criteria	Must treat serious condition. Clinical or nonclinical data demonstrates that the therapy has the potential to address unmet medical needs for such disease or condition. Must be designated as a qualified infectious disease product	Chemical, Biological and Regenerative Medicines. Must treat serious condition. Preliminary clinical evidence indicates that the therapy may demonstrate substantial improvement on a clinically significant endpoint(s) over available therapies	Regenerative Medicines. Must treat serious or life-threatening disease/ condition. Preliminary clinical evidence indicates that the therapy has the potential to address unmet medical needs for such disease or condition	Must treat a serious condition. Must provide a significant improvement in safety or effectiveness. Any supplement that proposes a labeling change pursuant to a report on a pediatric study under 505A. An application for a drug that has been designated as a qualified infectious disease product. Any application or supplement for a drug submitted with a priority review voucher	Must treat a serious condition. Provides a meaningful advantage over available therapies. Demonstrates an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality (IMM) that is reasonably likely to predict an effect on IMM or other clinical benefit (i.e., an intermediate clinical endpoint)
Key Program Features	Frequent written communication. Actions to expedite development and review. Rolling review	Same as FT, plus: Early and more frequent communications with FDA during development. Rolling submission and review. Priority Review	Same as BTD, plus: Early discussion of potential surrogate or intermediate clinical endpoint	Shorter review of marketing application (6 months compared with the 10-month standard review)	Approval based on the effect on a surrogate endpoint or an intermediate clinical endpoint

Table 1-2. *Summary of Expedited Pathways EU*

	Accelerated Assessment	Conditional Marketing Authorization [20,21]	Exceptional Circumstances	PRIME
Date Established	2004	2004	2004	2016
Qualifying Criteria	Request should be made at least two to three months before submitting a makreting-authorisation application. Important in terms of public health and innovation. Fulfills an unmet need. Strong evidence.	Filling an unmet medical need. Pertaining to life-threatening, serious, or emergency disease, or orphan products. Company must be able to provide clinical data comprehensively. Positive benefit/risk balance.	Applicants are not able to provide clinical data comprehensively because of rarity of the disease for example. Applicable to life-threatening or serious disease.	Address unmet medical need. Provide a major therapeutic advantage over existing treatments. Based on early clinical data.
Key Program Features	Reduce the timeframe for marketing authorisation to 150 days.	Active for one year only with an annual renewal of the approval until the EMA converts the approval to standard authorization. Enables early approval while confirmatory.	Applicants do not need to submit comprehensive data.	Enhanced interaction and early communication with sponsors. Accelerated assessment and scientific advice.

during the development process and participate in accelerated review programs within each agency.

In the U.S., the FDA has developed five designations for expedited pathways that are relevant for gene therapies: Fast Track designation, Breakthrough Therapy designation, Regenerative Medicine Advanced Therapy designation, priority review designation, and accelerated approval. The fast track designation provides advantages for facilitating development and expediting review of the product. The Breakthrough Therapy designation (BTD) is an expedited pathway available for all treatment modalities, including gene therapies, and incorporates all the benefits of fast track designation and more. This pathway was followed by a regenerative medicine pathway known as Regenerative Medicine Advanced Therapy (RMAT) designation in 2017. Gene therapy products, including those that received fast track designation, BTD, or RMAT designation, may also be eligible for priority review designation and accelerated approval.

In the EU, there are three expedited pathways that are relevant for gene therapies: Accelerated Assessment (AA), Conditional Marketing Authorization, and Authorization under Exceptional Circumstances. In addition, there is also the Priority Medicine (PRIME) scheme. The Accelerated Assessment reduces the time of assessment by the EU from the 210-day maximum to 150-day maximum. The Conditional Marketing Authorization (CMA) is a temporary authorization for medications filling an unmet medical need. The Authorization under Exceptional Circumstances (AEC) is a temporary authorization awarded for medications dealing with very rare diseases. The PRIME scheme was introduced in 2016 to support accelerated development of clinical programs to facilitate earlier patient access for unmet, serious medical needs. The PRIME scheme leverages on existing procedures and tools provided by the EMA with a commitment to engage more closely. Of the requests submitted, 81 products have been granted the PRIME scheme, while 239 have been denied.

In Japan, expedited pathways that are relevant for gene therapies are: Priority Review, Conditional and Term-Limited Approval, Conditional Approval, and

Table 1-3. *Summary of Expedited Pathways (Japan)*

	Priority Review	Conditional and Term-Limited Approval [24]	Conditional Approval [25]	Sakigake [26]
Date Established	2004	2004	2004	2016
Qualifying Criteria	No standard existing therapy or superior clinical usefulness as compared with the existing products in terms of quality of life of patients, efficacy, or safety Applicable to serious disease	Promising results of early-phase I/II registration trials in terms of efficacy and safety Sponsors must conduct postmarketing clinical studies and so on to confirm the efficacy and safety and resubmit applications for regular approval within a predetermined period Only for regenerative medicines	No standard therapy exists or superior clinical usefulness is demonstrated as compared with the existing products in terms of quality of life of patients, efficacy, or safety Applicable to serious disease It is difficult or would take too long to conduct a confirmatory study Exploratory clinical studies show efficacy and safety Surveillance or clinical studies must be conducted as a post-marketing requirement	Products for diseases in dire need of innovative therapy Applied for approval firstly or simultaneously (defined as submissions within 30 days of each other) in Japan Prominent effectiveness can be expected based on nonclinical and early phase trials
Key Program Features	Target total review time is nine months	Valid for no more than seven years	Conditional approval for drugs Priority Review	Prioritized consultation Prioritized review Review partner Substantial post-marketing safety measures Rolling submission and review

Sakigake. Priority Review lessens the target review date to nine months and is available for medications that fulfill an unmet need. The Conditional and Term-Limited Approval pathway is for regenerative medicines that show promising early phase results. The Conditional Approval pathway is targeted for highly useful and effective drugs treating serious diseases. The Sakigake early access scheme was introduced in 2014 to expedite innovative assets and was implemented in 2015.

Table 1, Table 2, and Table 3 summarize the criteria for expedited pathways in the U.S., EU, and Japan that can be utilized for gene therapy medicinal products.[13]

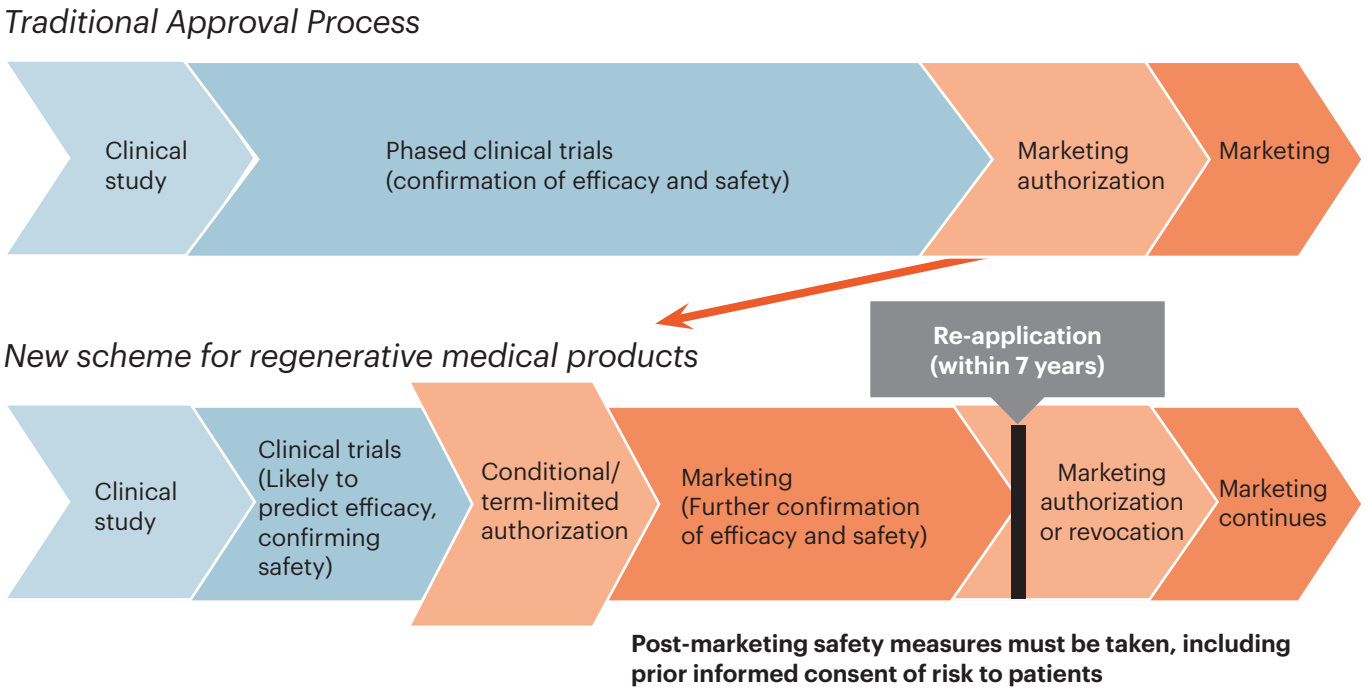
In addition to Sakigake, the PMDA Act also provided a new scheme for expediting regenerative medical products. Differences between the traditional approval process and the new scheme for regenerative medical products are presented in Figure 3.

Health Authority Innovation Teams

Health Authorities (HAs) such as the FDA and EMA are eager to support innovation as gene therapies mature and aim to ensure patient safety and efficacy while increasing patient access. However, the biopharmaceutical industry has been slow in adopting innovative manufacturing technologies due to concerns regarding regulatory acceptance and the impact on global supply chains due to the difficulty in post-approval change processes. Thus, engagement with HAs via innovation teams within the U.S. and EU agencies can facilitate dialogue between a sponsor and the HA, and support progression of innovative approaches.

The FDA's CBER Advanced Technologies Team (CATT) was recently established to promote engagement with prospective innovators and developers and sponsors regarding advanced manufacturing technologies. This

Figure 1-3. *Expedited Approval System under PMDA Act. Sato*



team serves as a resource that provides early engagement during development of an innovative technology through opportunities for feedback from CBER regarding issues related to implementation of advanced manufacturing and testing technologies (in addition to facilitating logistics to support the discussion). Industry applicants should submit requests electronically to *Industry.Biologics@fda.hhs.gov* and include the following:[27]

- Brief description of the technology
- Explanation of why the technology is novel and unique
- Description of the impact of the technology in terms of improved product manufacturing, product safety and efficacy
- Summary of the development plan and questions to be addressed

In the EU, the Innovation Task Force (ITF) in the EMA provides an opportunity for sponsor and HA engagement regarding emerging therapies and technologies, which include gene therapies. In contrast to the FDA's CATT, the ITF helps EMA to (1) clarify questions regarding the pathway for emerging therapies and technologies and (2) ensure EMA readiness for evaluation of developments in innovative medicines and technologies. The ITF is a multidisciplinary team that includes scientific, regulatory, and legal competencies.[28] To apply to the ITF, sponsors should complete the ITF briefing meeting request form (link to the form found here: *https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines*) and submit via email to *itfsecretariat@ema.europa.eu*. In addition to the centralized EU ITF, national innovation offices are another resource for engagement (e.g. PEI Innovation Office for ATMPs in Germany[29]).

Health Authorities and Sponsor Meetings

Development of innovative investigational products, such as gene therapy products, can introduce unique challenges due to unknown safety profiles, complex manufacturing technologies, incorporation of innovative devices, and the use of cutting-edge testing methodologies. In recognition of the complex nature of gene therapy products, HAs in the United States and Europe have introduced preliminary informal consultations to

allow sponsors to obtain feedback from the HAs to assist on the product development and clinical planning. These early meetings are in addition to the conventional HA/sponsor meetings.

As each HA has its own pathways, so does each have its own expectations on engagement by sponsors. During the life cycle of drug development, sponsors may seek advice from the FDA regarding several topics, including, but not limited to, the following: regulatory, clinical pharmacology, safety, product quality, and nonclinical matters.

FDA

Meetings between FDA and sponsors occur at critical junctures during the life cycle of product development and are aimed at minimizing wasteful expenditures of time and resources. In addition to INTERACT meetings, other particularly important milestone meetings under Prescription Drug User Fee Act (PDUFA) include: pre-IND, end-of-phase 1 (EOP1), EOP2, and pre-Biologics License Application (pre-BLA) meetings. Additional details on the available meetings between HAs and sponsors are described below and summarized in Figure 4.

The FDA offers four types of meetings related to the development and review of investigational new drugs and biologics under the PDUFA: Type A, Type B, Type B (end of phase (EOP)), and Type C, as further described below.[30] During the preclinical and early clinical stages, most of the in-person meetings with the agency are not guaranteed, and the agency might respond to a sponsor's inquiry for advice in writing or via teleconference.

- **Type A meetings:** necessary for an otherwise stalled product development program to proceed or to address an important safety issue. It is important to point out that Type A meetings are only granted for stalled product development due to an action taken by the FDA, and not for an issue from the developer side. Topics that are often covered by Type A meetings include dispute resolutions as described in 21 CFR 10.75, 312.48, and 314.103, clinical holds, receipt of an FDA Nonagreement Special Protocol Assessment letter, FDA regulatory action other than an approval, and FDA issuance of a refuse-to-file letter.
- **Type B meetings:** cover pre-investigational new drug applications (pre-INDs), pre-BLAs, pre-emergency use authorization, FDA regulatory actions other than approval, risk evaluation and mitigation strategies, post-marketing requirements outside the context of the review of a marketing application, and development programs for products granted BTD and/or RMAT designation status.
- **Type B (EOP) meetings:** include certain end-of-phase 1 meetings for products considered for marketing approval under 21 CFR part 312, subpart E, or 21 CFR part 314, subpart H, or similar products and end-of-phase 2 or pre-phase 3 meetings (21 CFR 312.47).

Figure 1-4. *Interactions with FDA[31] for Regenerative Medicine*

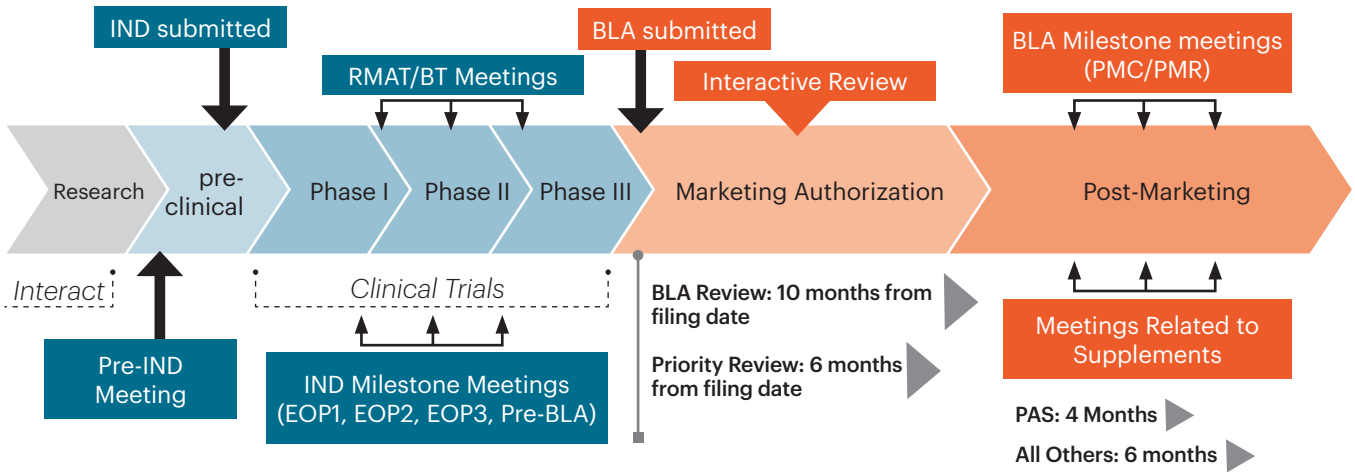


Table 1-4. *Dos and Don'ts for INTERACT Meeting Package*

INTERACT Meeting Package Content	Dos	Don'ts
Description of the product and the disease or condition being treated or prevented. Summary of information about the product development to date and future development plans, if appropriate. Brief statement summarizing the purpose of the meeting. List of questions for discussion, grouped by topic. Summary of the data to support a discussion organized by topic and question. List of all participants, with their titles and affiliations, who will attend the meeting from the sponsor's organization, including consultants and interpreters. Suggested dates and times for the meeting.	Submit the package together with the meeting request. Package should be no more than 50 pages. Identify the specific investigational product to be evaluated in a clinical study. Define key strategic product development activities such as manufacturing process, starting materials, cell sources, critical components, and use of devices prior to the INTERACT meeting. Ask specific, targeted questions.	Include questions regarding candidate selection. Request an INTERACT meeting if the sponsor has already requested and obtained formal regulatory advice about a similar product/indication from the FDA. Include questions regarding the adequacy and design of toxicology studies that have been completed–these should be submitted as pre-IND. Include requests for pre-review of completed proof-of-concept–these should be submitted as pre-IND. Include review of clinical study designs or protocols–these should be submitted as pre-IND. Ask questions that are not necessarily product-specific, such as those about novel technologies that can significantly impact on a product class.

- **Type C meetings:** any meeting other than a Type A, Type B, or Type B (EOP) meeting regarding the development and review of a product, including meetings to discuss adequacy of facility design and establishment issues, and to facilitate early consultations on the use of a biomarker as a new surrogate endpoint that has never been previously used as the primary basis for product approval in the proposed context of use.

Preclinical Meetings: (INTERACT)

Sponsors applying to the FDA can obtain a preliminary informal non-binding consultation with the Agency through the INTERACT meeting[32,33] prior to a pre-IND meeting. Some sponsors are already familiar with this type of early meeting, as it replaces the pre-pre-IND meeting that was in place until 2018. It is important to note that the INTERACT meeting is available for innovative investigational products at an early stage of development on issues that are not yet at the pre-IND meeting phase, validating FDA's recognition of the complexity of such products. The INTERACT meeting is not mandatory, but may be highly valuable to developers. This meeting is non-binding in nature, which means that a sponsor is not bound to pursue a particular regulatory pathway. This also means that the FDA feedback can change depending on information/updates the sponsor provides in the future.

Sponsors can obtain non-binding advice regarding different aspects of the development process, such as:

- Planning initial clinical development strategies
- Chemistry, manufacturing, and controls
- Pharmacology/Toxicology development
- Clinical aspects of the product development program

Identifying the optimal time of the meeting relative to product development might be the sponsor's greatest challenge when seeking an INTERACT meeting. The meeting might be declined if it is requested too early in the process at a point when a clear design has not been established, or when it is considered too late, after a clinical protocol has already been developed. At the same time, sponsors are

advised to apply for the INTERACT meeting early rather than late, as this meeting is the only opportunity to engage with the FDA during the pre-IND process. CBER strives to schedule INTERACT meetings within 21 calendar days and hold the meeting within 90 calendar days of receipt of the request. INTERACT meetings are held via teleconference only, and generally last for one hour.

The INTERACT meeting requests and packages are submitted to CBER by email to INTERACT-*CBER@fda.hhs.gov*. The INTERACT meeting package should not exceed 50 pages. As with other FDA/sponsor meetings, it is expected that the sponsor will provide the scientific rationale to support each question in the meeting package. Table 4 provides tips on the Dos and Don'ts for the meeting package content.

Pre-IND

Pre-IND meetings are Type B meetings and are meant to initiate or continue the dialogue regarding product development in its early stages, with the aim of understanding the mechanism of action of the drug and possible study designs. These meetings are valuable to anticipate and potentially prevent clinical hold issues from arising and aid sponsors in developing a complete IND.[34]

FDA encourages sponsors to request a pre-IND meeting for gene therapy products for the following topics: a product not previously approved or licensed; a new active pharmaceutical ingredient (API) with a novel pharmacologic mechanism; products for which it is critical to public health to have an effective and efficient drug development plan (drugs to treat life-threatening or severely debilitating illnesses); drugs with substantial early development outside the United States; and drugs with adequate and well-controlled trials to support a new indication.[35]

The broad range of topics that can be discussed during a pre-IND meeting may appear overwhelming at first. Instead, it should be seen as an opportunity to obtain feedback from the Agency on several topics that can propel the clinical development of a product.[36]

Once the meeting request is granted, the sponsor must provide the meeting package at least 30 days prior to the meeting date. Preparation for the pre-IND meeting, and other FDA/sponsor meetings, is critical for achieving a productive discussion. The meeting package should provide information relevant to the discussion topics and enable the FDA to prepare adequately for the meeting.[37] It is highly recommended that sponsors initiate the briefing package draft at the same time as the meeting request. This strategy can facilitate the definition of the questions in the meeting request and help the sponsor to avoid delays in getting the final meeting package ready. A failure to deliver the meeting package 30 days prior to

Table 1-5. *Dos and Don'ts for Pre-IND meeting package*

Pre-IND Meeting Package Content	Dos	Don'ts
Description of product manufacturing and testing. Completed and planned preclinical study summaries. Phase 1 clinical study design or protocol.	Submit the package 30 days prior to the scheduled meeting. There is no page limit, but it is recommended to be around 100-150 pages. Include relevant CMC information. At a minimum, include a description of the manufacturing scheme for drug substance (DS) and drug product (DP), quality of the starting materials, release specifications, and a stability plan. Include information for device and if the product will be a combination product.	Ask questions for answers that are already available in FDA guidance documents. Ask open-ended questions. Exceed more than 12 questions (including sub-questions). Present new data/information or alternate approaches during the meeting in response to the preliminary FDA feedback.

Table 1-6. *Summary of Meeting Management Procedural Goals*

Meeting Type	FDA Response to Request	FDA Receipt of Meeting Package	FDA Preliminary Responses to Requester (if applicable†)	Requester Response to FDA Preliminary Responses (if applicable†)	FDA Scheduled Meeting Date (days from receipt of request)	FDA Meeting Minutes to Requester (if applicable†)
A	14 days	With meeting request	No later than 2 days before meeting	--	Within 30 days	30 days after meeting
B	21 days	No later than 30 days before meeting	No later than 2 days before meeting	--	Within 60 days	30 days after meeting
B (EOP)	14 days	No later than 50 days before meeting	No later than 5 days before meeting	No later than 3 days after receipt of preliminary responses	Within 70 days	30 days after meeting
C	21 days	No later than 47 days before meeting	No later than 5 days before meeting	No later than 3 days after receipt of preliminary responses	Within 75 days	30 days after meeting

† Not applicable to written response only.

the meeting can result in the FDA cancelling the meeting.

While these meetings are free of charge, sponsors should approach them with deliberate purpose. Typically, sponsors should ask no more than 10 to 12 questions, which should, naturally, be specific to the sponsor's product and process. Table 5 summarizes the "Dos" and "Don'ts" for the pre-IND meeting package, with a focus on CMC.

Clinical Meetings — End of Phase 1, 2, and 3

End of Phase (EOP) meetings serve to evaluate the next clinical phase plan and protocols, the adequacy of current studies and plans to assess safety and efficacy, and the adequacy of manufacturing and testing plans to support the next clinical phase studies. In particular, EOP2 meetings allow for preparation for commercial manufacturing, evaluation of the human factors validation plan if a device is used for administration of the gene therapy product, and identification of any additional information necessary to support a marketing application for the uses under investigation.

All EOP meetings are Type B meetings and subject to different timelines as summarized in Table 6.[38] From a CMC perspective, by the time of the end of the phase 2 clinical studies, the sponsor should have a very robust knowledge of the manufacturing process and have started preparing for the phase 3 clinical materials that will be representative of the commercial product. For gene therapy products, this timeline is not straightforward. As previously discussed, the clinical results in gene therapy products is often ahead of the CMC development. Clinical phase 2 and phase 3 timelines are condensed and, as a result, the CMC development must be expedited. Therefore, these meetings can often be almost overlapping with pre-BLA meetings and preparation is key for obtaining the right feedback from the Agency.

In preparation for the EOP2 meeting, the sponsor should take the opportunity to seek advice from the FDA that the current data package, in addition to the potential planned studies, will be sufficient for a BLA submission.[39] It is recommended that sponsors request a CMC-focused EOP2 meeting to ensure that there is sufficient time dedicated to CMC discussions. Typical topics discussed during the EOP2 meetings include, but are not limited to: release specifications and justifications, overall control strategy with definitions of critical quality attributes (CQAs) and critical process parameters (CPPs), manufacturing process and analytical assay validation plans, and stability data to support product storage and shelf-life. At such a meeting, sponsors should also discuss readiness/plans for the device (used for product administration) and/or companion diagnostics that will be part of the marketed product.

EMA

Early engagement and scientific advice with the EMA are key drivers of faster, and, more often, successful registration. Similar to the FDA, the EMA offers several opportunities for a sponsor to start an early conversation with the agency to seek scientific, technical, and regulatory feedback. Sponsors can request meetings with the EMA for overall advice and there is no limit to the number of scientific advice meetings (or protocol assistance as it is called for products with orphan drug status) that can be requested during the development of a given gene therapy. However, clinical trials are still in the remit of national competent authorities.

Developers of ATMPs are mandated to seek marketing application authorization under the centralized procedure, along with specific therapeutics of certain modalities and for certain indications.[40]

In the centralized procedure, the CHMP plays a vital role in the authorization of medicines in the EU. The CHMP also evaluate medicines authorized at a national level in a harmonized procedure. In addition, the CHMP and its working parties contribute to the development of medicines and medicine regulations by providing scientific advice to develop new medicines, prepare guidance, and cooperate on harmonization of international regulatory requirements.[41] As mentioned previously, the CAT is the "central" committee for all procedures (including marketing application) for gene (and cell) therapies, even though formal recommendation is still issued by the CHMP.

ATMP classification

In Europe, ATMPs are governed under the ATMP Regulation (Directive 2001/83/EC, as amended by Regulation [EC] 1394/2007). In case of "borderline" product, if the developer is unsure if its product falls in the ATMP category, the developer can request a formal classification to the EMA. Although the scientific recommendation on classification of ATMPs is an optional procedure, there are advantages of requesting one.[42] The purpose of this request is to allow sponsors to "clarify the classification whether a given product based on genes, cells, or tissues meets the scientific criteria that define ATMPs, in order to address, as early as possible, questions of borderline with other areas such as cosmetics or medical devices, which may arise as science develops."[43] Though it is advised to request this classification before submission of other requests, including scientific advice, Pediatric Investigation Plan (PIP) evaluation, certification of quality and nonclinical data for Small and Medium-Sized Enterprises (SMEs) developing ATMPs, orphan drug designation, and Marketing Authorization Application (MAA), it can be submitted at any time during the product development. The CAT, after consultation with the European Commission (EC), delivers the ATMP classification recommendation within 60 calendar days following receipt of the request.

EMA scientific advice

Scientific advice can be requested to the EMA at any stage of the product's development, although information on the target indication is preferred for the Agency to provide advice as accurately as possible. There is no limit to the number of advices that can be requested; nor the number of the questions that can be on CMC/quality as well as nonclinical and clinical topics. For products that have been granted the orphan drug status, the scientific advice procedure is called "protocol assistance" and can also include questions related to the "significant benefit" and/or "clinical superiority" of the product.

Questions in the briefing package should be detailed and precise and should, in all cases, be followed by a

justification of the company's planned strategy with regard to the question, all relevant information about the topic, and cross-references to any relevant annexes. It is highly recommended that the sponsors provide a clear strategy, compelling argument, and well-rehearsed preparation, as these are key to having a successful EMA meeting.[44]

The briefing package should contain the following:

- Background information about the product and its mechanism of action
- CMC and quality data
- Preclinical data
- Clinical data
- Intended indications
- Regulatory status
- Stage of program development
- Stage of clinical study development
- Questions for the reviewers and applicant's justification ("position")

The level of information included will vary depending on the stage of development of the product and the topics of the questions. Annexes can be included in the package, if deemed appropriate to provide further information.

Scientific advice/protocol assistance can be a written procedure only (40 days) or include a discussion meeting with the Agency (70 days procedure). Of note, the decision of a discussion meeting is at the Agency's discretion.

Additionally, the applicant can request a "preparatory meeting" that will take place before the submission of the final package and will help to refine the format of the document. No assessment or in-depth review will take place; however, it might be relevant for an applicant submitting for the first time to the EMA that wants to maximize the chance of getting appropriate advice. Applicants should give a presentation at the meeting and submit a draft version of the briefing document a few days ahead that includes questions based upon what EMA staff will review, including, but not limited to the following: overall compliance of the intended submission package with applicable regulatory requirements, possible gaps in knowledge that could be useful to discuss, documentation against relevant scientific and regulatory guidelines about products in the same class, and relevant guidance.[45]

Following completion of the procedure and receipt of the final advice letter (FAL), the requester may ask for clarification if it disagrees with, suspects a misunderstanding, or spots contradictions or imprecisions in the advice given by CHMP. This will not lead to a new discussion and is purely in writing, but may help clarify some wording in the FAL, for example.

Submission deadlines for scientific advice/protocol assistance are published every year for the following calendar year.

National Competent Authorities

In the European regulatory ecosystem, sponsors can interact not only with the centralized EMA, but also with national agencies, which will give specific recommendations on the clinical trials. National Competent Authorities (NCAs) often offer significant contributions to the product's development plans, including critical points on how to define the starting materials, and how to define drug substance (DS) and drug product (DP), as these can be not well-defined during development in a continuous manufacturing process. The EMA works closely with the NCAs of the Member States of the EU and the European Economic Area (EEA) responsible for human medicines.[46]

For gene therapy products specifically, it is recommended to engage discussions with NCAs early in the development process, especially if the developer plans to conduct the clinical development (i.e., clinical trials) in one or several EU member states. Though this does not rule out validation or questions following clinical trial applications (CTAs), it will enable the NCAs to be aware of the development of the product and upcoming CTA.

Parallel Consultation

In addition, early discussion with health technology assessment (HTA) bodies and other stakeholders can be critical towards early patient access and commercial success in Europe. Some initiatives have been implemented over the last years to facilitate such dialogue, via, for instance, the parallel consultation where EMA and HTA provide simultaneous advice to a developer. The importance of seeking parallel HTA-EMA advice and being well-prepared for the meeting are critical to ensure successful development, followed with registration and commercial success.[47]

The main benefits of the parallel consultation procedure include:

- Streamlined procedure
- Increased mutual understanding and problem-solving ability between EMA and HTA bodies
- Improved coordination with HTA bodies and greater participation of HTA bodies in parallel consultations through EUnetHTA's Early Dialogue Working Party (EDWP) and the EUnetHTA early dialogue (ED) secretariat.[48]

EMA-FDA PROGRAMS

Parallel Scientific Advice

In addition to the separate interactions with the EMA and FDA, these two major agencies offer the Parallel Scientific Advice (PSA) program in order to provide a mechanism for staff from both EMA and FDA to concurrently convey to sponsors their views on scientific issues during the development phase of new medicinal products. These interactions are meant to increase dialogue between the two agencies and sponsors from the beginning of the lifecycle of a new product, provide a deeper understanding of the basis of regulatory decisions, optimize product development, and avoid unnecessary testing replication or unnecessary diverse testing methodologies. The agencies conduct PSA procedures according to the confidentiality arrangement between the European Commission, EMA, and FDA.[49]

PSA procedures usually occur at the request of the sponsor, though in special circumstances, EMA or FDA may also initiate the PSA process in full cooperation with the sponsor. PSA requests should focus on specific questions or issues involving the development of a medicinal product for which the sponsor desires to gain further scientific input from both EMA and FDA. The PSA procedures should focus on sharing information and perspectives. Following PSA meetings, sponsors should have a clearer understanding of the agencies' respective requirements and perspectives regarding the development program discussed, and if divergent, the reasons for the divergence. FDA and EMA consider the best candidates for PSA to be important medicinal products being developed for indications lacking development guidelines or for those indications for which existing EMA and FDA guidelines differ significantly.

Sponsors wishing to nominate a product for PSA should address a single "Request for PSA" letter to both *emainternational@ema.europa.eu* and *OC-OIPEurope@fda.hhs.gov*. In this letter, the sponsor should provide information about the following: the product in development; why a discussion with EMA and FDA staff would be beneficial to the product's development; specific questions requiring clarification; the desired goals for the meeting; and an explicit authorization for the agencies' comprehensive exchange of all information relevant to the product, including trade secret information. Any fees applicable for scientific advice at either agency are unaffected by PSA status. If both agencies grant the PSA request, the sponsor will receive an email from each agency acknowledging the agreement and indicating the primary contact person at each agency. The PSA process generally corresponds to the 70-day timeline of SAWP at EMA and the timeline for a Type B meeting at FDA. The designated primary contact for each agency will coordinate with the sponsor regarding final meeting logistics, including timelines for submission of pre-meeting background information to both agencies. The two agencies will conduct a pre-sponsor meeting tele- or video conference (usually around day 60 of the 70-day timeframe) to discuss the sponsor's questions prior to the meeting. The two agencies may also conduct a post-sponsor tele- or video conference if needed.[50]

If a sponsor's request for PSA is not granted, the sponsor is free to pursue a scientific advice procedure with each agency individually, following each agency's normal procedural rules. Both agencies may also engage in a Consultative Advice procedure, as described below.

Consultative Advice

The Consultative Advice procedure allows sponsors to request scientific advice from one regulatory agency and concurrently notify the other regulatory agency of the request. At the invitation of the first agency, the second will participate in the sponsor meetings or teleconferences, as able. Unlike the PSA process, the second agency will be expected to only engage on top level issues. The review and sponsor meeting will follow the

timelines of the regulatory agency with whom the sponsor initially seeks scientific advice. Only the initially contacted regulatory agency will provide written scientific advice in accordance with standard agency meeting procedures.

PMDA

In Japan, the PMDA provides opportunities for meetings between sponsors and the Agency to allow for feedback and guidance during clinical development.[51] In clinical trial consultations for new drugs, PMDA checks whether a proposed clinical trial complies with the requirements for regulatory submission, taking into consideration the ethical and scientific aspects of the development program, the reliability of the clinical trial, as well as the safety of trial subjects. The PMDA also gives advice to facilitate the improvement of the clinical trial.

Since 2009, PMDA started providing prior assessment consultations, which adds value to the development process through feedback from reviewers on CMC data, in addition to efficacy and safety feedback on the product. This consultation process constitutes part of the review of the product once the application is submitted.

For sponsors that are located outside of Japan, it is recommended to appoint a Japanese Marketing Authorization Holder (MAH). The sponsor can request meetings with the PMDA through the MAH, who can also assist with translation and interpretation, since all communications and submission forms are in Japanese.

Preclinical and clinical meetings

The pre-phase 1 study consultation is an opportunity for sponsors to obtain guidance from the PMDA prior to initiation of the clinical study in Japan. The goal of the early phase consultation is to solve potential issues in clinical development, identify tests that will be needed in the early product development stage, and shorten the time before the application, saving time and costs by avoiding critical issues during development, as well as during NDA review.

The process for obtaining a meeting with the PMDA can take eight weeks from the acceptance of the meeting request to the face to face or online meeting. Five weeks prior to the meeting, the briefing package is submitted. During the review of the briefing package, PMDA may ask questions to which the sponsor must respond in a timely manner. PDMA provides the opinion four days prior to the meeting. Meeting minutes from the PMDA are provided 30 days after the meeting.

In addition to the pre-phase 1 consultation, the PMDA has implemented new consultations as of 2011 to promote the practical application of innovative drugs, medical devices, and regenerative medical products originating in Japan. These consultations provide significant benefit for universities, research institutions, and venture companies that are involved in the discovery of promising "seed-stage" technologies. These early consultations have been very beneficial to developers of gene therapy products.

More recently, in April 2018, PMDA started to provide the Collaborative Consultation on Practical Application of Innovative Products while sharing information with the Medical Innovation Support. The consultation service also provides guidance and advice on the quality and safety of regenerative medical products (including gene therapy products intended for transgene expression in the human body and used to prevent diseases (e.g., live recombinant vaccines)) at an early development stage.[52]

Additional consultations with the PMDA for times in the development process applicable to CMC development are available and include:[53]

- Before start of early phase 2 study
- Before start of late phase 2 study
- After completion of phase 2 study
- Prior Assessment Quality consultation

REGISTRATION MEETINGS

FDA: Pre-BLA

Pre-BLA meetings are meant for FDA reviewers to provide advice to the sponsor regarding the format and content of the planned marketing application, including labeling and risk management activities, presentation and organization of data, dataset structure, acceptability of data for submission, and the projected submission date of the marketing application. They are also intended to uncover major issues, identify studies intended to establish the drug's safety and efficacy, discuss the status of pediatric studies, and discuss statistical analysis methods and results. FDA encourages sponsors to request pre-BLA meetings for all planned marketing applications.[54] Sponsors should plan for a single-multidisciplinary

pre-BLA meeting because the FDA only grants one pre-BLA meeting.

The timelines for meeting request and submission of the meeting packages follow the Type B meeting requirements. Once the meeting request is granted, the sponsor must provide the meeting package at least 30 days prior to the meeting date. The meeting package should provide information relevant to the discussion topics and enable the FDA to adequately prepare for the meeting. A failure to deliver the meeting package 30 days prior to the meeting can result in the FDA cancelling the meeting.

The briefing package for a pre-BLA meeting, or any other pre-marketing authorization meeting, should be adequately prepared to ensure that there are no surprises during the review of the BLA. Sponsors may provide summaries of their stability and process validation studies for comment on adequacy, but the FDA will not comment on final product specifications, shelf life, or actual sufficiency of process validation in a pre-BLA meeting. Sometimes, the timing for the pre-BLA meeting needs to align with the timing of availability of data to be presented. If the meeting is done too early, the FDA will not be able to provide full feedback on questions and will postpone decisions to the review of the BLA. If the meeting is done too late, and too close to the BLA submission, there is not sufficient time to generate more data prior to submission. To maximize the chance for success, requests for pre-BLA meetings should be planned together with the overall regulatory strategy and timelines for product development. This approach is applicable not only to meetings with the FDA, but also other HAs.

EMA: presubmission meeting and rapporteur/co-rapporteur meeting

Under the centralized authorization procedure, pharmaceutical companies submit a single MAA to the EMA. This allows the MAH to market the medicine and make it available to patients and healthcare professionals throughout the EU on the basis of a single marketing authorization.

CHMP carries out a scientific assessment of the application and gives a recommendation on whether the medicine should be marketed or not.

However, under EU law, the EMA has no authority to permit marketing in the different EU countries. The EC is the authorizing body for all centrally authorized products, and makes a legally binding decision based on EMA's recommendations. This decision is issued within 67 days of receipt of EMA's recommendations. Once granted by the European Commission, the centralized marketing authorization is valid in all EU Member States, as well as in the EEA countries of Iceland, Liechtenstein and Norway.

Commission decisions are published in the Community Register of medicinal products for human use. The national competent authorities are primarily responsible for the authorization of medicines available in the EU that do not pass through the centralized procedure. They also supply thousands of European experts who serve as members of the Agency's scientific committees, working parties, or in assessment teams supporting their members.

Once the eligibility to the centralized procedure at the EMA has been confirmed (a mandatory step even for gene therapies) and the rapporteur and co-rapporteur of the procedure have been appointed, the future marketing authorization holder (MAH) can request two different meetings prior to the submission of the MAA dossier.

EMA: presubmission meeting

Presubmission meetings offered by the EMA are intended to address product-specific legal, regulatory, and scientific issues, to facilitate the validation of the MAA, and to support applicants in submitting applications for smooth evaluation. Sponsors may discuss final practical and regulatory aspects of their upcoming application and clarify application-specific issues not addressed on the EMA website.

In addition to the presubmission meeting held with the EMA, the applicant of an MAA can request a meeting with the appointed rapporteur and co-rapporteur, the key experts who will drive the assessment.

This meeting is not meant to discuss procedural aspects but to present the product and key information, as well as discuss questions and potential issues. Importantly, the advice given during this meeting is non-binding and informal; it does not preclude the outcome of the MAA but will provide some insights on potential "weaknesses."

POTENTIAL CMC TOPICS TO BE COVERED IN HA/SPONSOR MEETINGS

Different elements in gene therapy development that are considered essential and typical topics of interest are usually raised during these interactions with HAs that start with early product development. The types of potential questions asked during these meetings between HAs and sponsors may or may not be evident to sponsors when developing their gene therapy products.

A key challenge is the selection and the quality of the raw materials. Often, the early development process of gene therapy products will utilize research grade materials that could compromise the quality of the product when progressing into clinical manufacturing. Another challenge regarding starting materials is the utilization of materials of human or animal origin that can be of insufficient quality for clinical studies. Engaging in early discussions with the Agency can positively impact decisions made in the product development process.

Another key challenge is the proper development of a potency assay. Although the requirements for a potency assay in the U.S. and EU differ during early stage development, it is highly encouraged that sponsors start the development of one or more potency assays that can successfully demonstrate the mechanism of action of the product. Even if the method is still undergoing optimization, early discussions with the Agency can help with the proper design and phase-appropriate implementation of the assay.

A few questions that are commonly asked during early interactions with the three agencies are listed below. As expected, these questions should not be followed as a recipe, but are intended to provide some guidance on points to consider prior to requesting a meeting.

- Does the Agency agree on the proposed quality and selection criteria (including testing scheme) for starting materials and raw materials used in DS and subsequent DP manufacturing?
- Does the Agency agree on the suitability of the characterization studies and proposed specification for DS and DP release testing?
- Does the Agency agree on the stability plan for DS and DP?
- Does the Agency agree on the comparability plan proposed for the nonclinical and clinical batches?

Submission Content

ELECTRONIC COMMON TECHNICAL DOCUMENT (ECTD)

The eCTD, and electronic submission structure developed by the ICH, provides the backbone for providing information regarding Chemistry, Manufacturing, and Controls (CMC) in Module 3, with a summary in Module 2.3 (Quality Overall Summary).[55] These two modules include sections for DS and DP. Information to be provided about the DS includes the proper identification, quality, purity, and strength of the active ingredient, with an emphasis on the identification and control of raw materials and the new drug substance. Information to be provided about the DP is similar to that required for the DS section, with information about the assays and acceptable results for assessing identity, strength, quality, and purity. Additionally, information about stability for at least the duration of the clinical trial, with the purpose of establishing the drug product shelf-life and recommended storage conditions, are expected.[56]

It should be noted that these data are expected to evolve over time as the sponsor optimizes production processes, analytical methods, and formulation of the drug product. It can be challenging to fit information about gene therapy products into the eCTD structure due to the lack of clear delineation between DS and DP, as well as the specific need to control and release critical starting materials.

Both IND and clinical trial application (CTA) submission content directly relating to CMC is to be submitted in documentation structured according to the heading of the corresponding sections of Module 3 of the eCTD.

IND SUBMISSIONS TO FDA

Sponsors who wish to conduct a clinical trial in the U.S. must submit an IND. The FDA's review of the IND takes 30 days. From the perspective of CMC, FDA will focus on determining if there are any reasons to believe the manufacturing or controls for the clinical trial product present unreasonable health risks to the subjects in the initial IND trials; as always, safety is the first priority.

When filing an initial IND submission, details about the following CMC information are presented

Table 1-7. *Summary of CMC information for Module 3 (Drug Substance)*

Main Sections	Gene Therapy Recommended Content
3.2.S.2.2 Description of Manufacturing Process and Process Controls	Manufacturing process description and process controls should include the following, as applicable: cell culture; transduction; cell expansion; harvest(s); purification; filling; and storage and shipping conditions. The description of your manufacturing process should include a process flow diagram(s) and a detailed narrative. A description of how you define each manufacturing run (i.e., batch, lot, other) should be submitted with an explanation of the batch numbering system. Indicate whether any pooling of harvests or intermediates occurs during manufacturing. Pooling may be needed as some gene therapy batches are made at a low scale. Any reprocessing during manufacture of the active substance should be described and justified. Your description should clearly identify any process controls and in-process testing (e.g., titer, bioburden, viability, impurities) as well as acceptable operating parameters (e.g., process times, temperature ranges, cell passage number, pH, CO2, dissolved O2, glucose level). Even in early stages, monitoring process performance parameters is recommended. Describe any controls for cleaning and changeover as well as tracking and segregation procedures that are in place to prevent cross-contamination. For the IMPD, in addition to the items listed above, the guidelines specify that the rationale for the use of a particular cell substrate should be provided. Additionally, a purification process should be in place to reduce impurities. This is also expected by the FDA. Impurities include hybrid viruses in the case of virus vector production, host cell-DNA and protein, residual plasmid DNA, lipids and polysaccharides in the case of production systems which involve bacterial fermentations, and RNA and chromosomal DNA in the case of plasmid purification. For the IMPD, for non-replication competent viral vectors and conditionally replicating viral vectors, information should be provided on process parameters, and controls conducted to prevent contamination of the packaging cell line by wild-type, helper, or hybrid viruses that might lead to the formation of replication-competent recombinant viruses during production. For the FDA, this is expected in the IND submission, as this is a safety issue.
3.2.S.2.3 Control of Materials	In this section, you must provide a list of all materials used in manufacturing and a description of the quality or grade of these materials. Typically, this is presented in a tabular format, including the identity of the material, the supplier, the quality, the source of material, and the stage at which each material is used in the manufacturing process. It is recommended that for all materials used, the acceptance criteria be included, or at a minimum, the Certificate of Analysis (CoA) for each material be included. This includes information on components, such as cells, cell and viral banking systems, and reagents, and also includes raw materials and equipment that come into contact with the product, such as culture bags, culture flasks, chromatography matrices, and tubing. Sponsor should note equipment that is single use, product-dedicated or intended for multiproduct use. It is important to note that the terminology used in the U.S. and EU for materials is slightly different. Therefore, when preparing a global dossier to support submission for the IND and IMPD, these differences should be taken into consideration. According to the FDA, materials used for manufacturing (e.g., cell growth, differentiation, selection, purification, or other critical manufacturing steps) that are not intended to be part of the final product are called reagents or ancillary materials, while according to the EMA, these are called raw materials. For biologically sourced reagents, the FDA guidance for industry "Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications" is applicable. Specific recommendations for each type of biological material is presented in the following: *https://www.fda.gov/media/113760/download.* For the IMPD, specific guidance is provided for all raw materials of biological origin in Ph.Eur. (5.2.12) "Raw Materials for the Production of Cell based and Gene Therapy Medicinal Products." *continued on next page*

Table 1-7. *Summary of CMC information for Module 3 (Drug Substance)*

continued from previous page

Main Sections	Gene Therapy Recommended Content
	Information for starting materials should be organized to include description of the cell source, collection procedure, and any related handling, culturing, processing, storage, shipping, and testing performed prior to use in manufacture. When using allogeneic cells or tissues, you must perform donor screening and testing, as required in 21 CFR Part 1271, Subpart C, except for those cells and tissues that meet the exceptions in 21 CFR 1271.90(a) for the IND. For the IMPD, donation, procurement and testing of human cell-based products need to comply with the requirements of Directive 2004/23/EC or, where applicable, Directive 2002/98/EC. The cell banking systems should be presented at a minimum for the Master Cell Bank (MCB) and, if applicable, the Working Cell Bank (WCB). Information is expected on the cell banking and characterization and testing of the established cell banks, as well as available information on cell substrate stability. For both the IND and IMPD, the safety assessment for adventitious viruses should be presented in 3.2.A.2. Additional sources of information regarding qualification of cell substrates can be found in ICH Q5D.
3.2.S.2.6 Manufacturing Process Development	You should provide a description and discussion of the developmental history of the manufacturing process as described in 3.2.S.2.2. For early stage INDs, there may be differences between the manufacturing and testing of the nonclinical and clinical batches. It is crucial to clearly present, typically in a tabular format, the changes made in the process with an assessment of the impact of these changes on the quality of the product, as well as a rationale for the change. This provides a clear view for the reviewer and allows for a better understanding of the process development. If you make significant manufacturing changes, then comparability studies may be necessary to determine the impact of these changes on the identity, purity, potency, and safety of the product. Comparability reports including information on the changes put in place, the risk assessments performed, the testing strategy, and the supporting data collected to demonstrate comparability should be submitted.
3.2.S.3.1 Elucidation of Structure and other Characteristics	Characterization studies will provide a comprehensive picture and knowledge of the gene therapy product. The knowledge will evolve as the product development progresses. In this section, include annotated sequence analysis for your vector in the original IND submission and any additional sequence information gathered during the course of product development in subsequent submissions. Provide any further information confirming the primary, secondary, or higher order structure; post-translational modifications; and/or distribution of cell types for the DS if it has not already been described in 3.2.S.1.2. For the IMPD, it is clearly stated that reference to the literature data alone is not acceptable. Basically, the characterization of a gene therapy active substance is necessary to allow relevant specifications to be established. Tests should be included to show integrity and homogeneity of the recombinant viral genome or plasmid and the genetic stability of the vector and therapeutic sequence.
3.2.S.3.2 Impurities	Information on process-related and product-related impurities should be provided. Process-related impurities (e.g., media residues, growth factors, host cell proteins, host cell DNA, column leachables) and product-related impurities (e.g., cell types not linked to the therapeutic effect, cell fragments or non-viable cells, precursors, degradation products, and aggregates) should be kept to the minimum or a risk assessment should be provided. Based on the risks identified, consideration should be given to the maximum amount for the highest clinical dose and an estimation of the clearance should also be provided. Overall, the manufacturing process should be designed to remove the impurities to levels that are acceptable and justifiable.

continued on next page

Table 1-7. *Summary of CMC information for Module 3 (Drug Substance)*

continued from previous page

Main Sections	Gene Therapy Recommended Content
3.2.S.4.1 Specifications	You should list DS specifications in your original IND/IMPD submission. Specifications are defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria used to assess safety and quality. Since the acceptance criteria are normally based on a limited number of development and nonclinical batches, it is understandable that they are preliminary and need to be optimized during development. It is important to emphasize that it is better to present a wider range for acceptance criteria than to not present one at all. Even at early stages, presenting acceptance criteria as "report results" are often questioned by the Health Authorities, as these specifications do not demonstrate that you will have control of the quality of your product.
3.2.S.7 Stability	Describe in your original IND submission the types of stability studies (either conducted or planned) to demonstrate that the DS is within acceptable limits. The protocol should describe the storage container, formulation, storage conditions, testing frequency, and specifications (i.e., test methodologies and acceptance criteria). As is the case for several gene therapy products, if the DS is immediately processed into a DP, long term DS stability data may not be needed. In the stability protocol, it is often helpful to demonstrate that at least one or more of the test methods in your stability analysis are stability-indicating. Although this is not required for early clinical trials, you can demonstrate a test is stability-indicating by using forced degradation studies, accelerated stability studies, or another type of experimental system that demonstrates product deterioration. When sufficient stability data is not available for the clinical batch, stability data for at least one batch representative of the manufacturing process of the clinical trial material should be included. Any other stability data relevant from development and nonclinical batches can be provided as supportive data. For the IMPD, vector integrity, biological activity (including transduction capacity) and strength are critical product attributes that should always be included in stability studies. In addition, if a shelf-life extension is planned, the applicant should commit to performing the proposed stability program according to the presented protocol and inform the Competent Authorities in the event of unexpected issues.

in an eCTD structure and should include: (1) DS; (2) DP; (3) placebo formulation, if applicable; (4) labeling information for the labeled products relevant to the investigational drug; and (5) an environmental analysis for assessment of the effects of the investigational new drug or biological product on the environment (though many gene therapy products qualify for an exemption from this assessment, some may not).[57,58]

If the FDA identifies an unresolved safety issue (CMC, clinical, or nonclinical) in the IND, or if FDA identifies such an issue arising during development, the Agency will issue a clinical hold on the application. Regulations require the FDA to attempt to discuss and satisfactorily resolve any resolvable issue that may prevent the clinical hold with the sponsor before issuing the clinical hold.

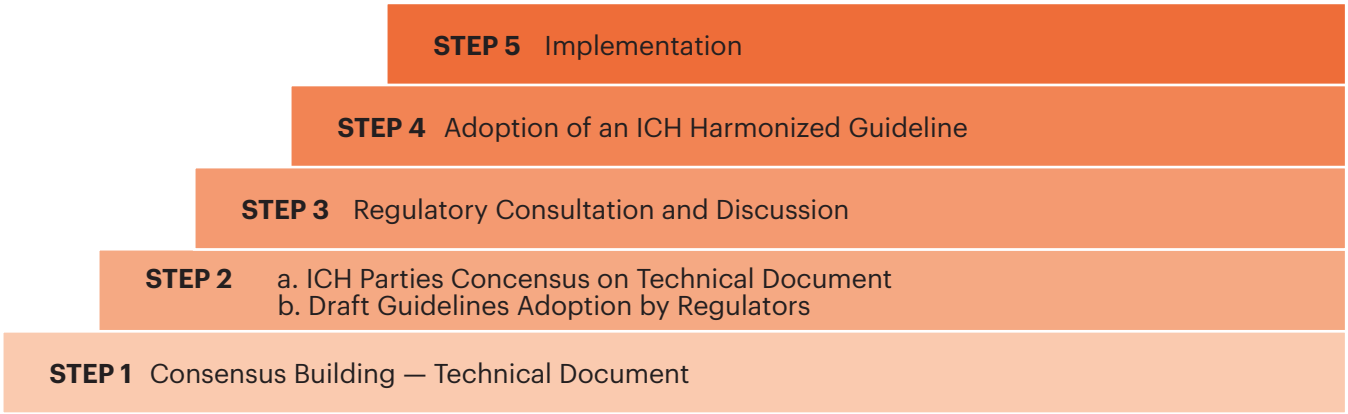
FDA also acknowledges there can be specific challenges for applications that have received (or are likely to receive) expedited designations. These challenges include possible difficulties in aligning CMC and clinical development and possible difficulties in making risk/benefit assessments (with particular regard to patient benefit) in situations in which there may be a relative lack of CMC information. These situations are considered on a case-by-case basis.

Once an IND has been deemed safe to proceed by the FDA, multiple studies can be conducted under the same IND, as per the FDA's legal requirements described in 21

Table 1-8. *Summary of CMC information for Module 3 (Drug Product)*

Main Sections	Gene Therapy Recommended Content
3.2.P.1 Drug Product Description and Composition	You should provide a description of the DP and its composition (21 CFR 312.23(a)(7)(iv)(b)). This includes a description of the dosage form and a list of all of its components (active and inactive), the amount on a per unit basis, the function, and a reference to quality standards for each component (e.g., compendial monograph or manufacturers' specifications). If a placebo treatment is used in the clinical trial, a separate DP section should be provided for the placebo. In addition, you should provide a description of any accompanying reconstitution diluents and a description of the container and closure used for the dosage form and accompanying reconstitution diluent in a separate DP section (3.2.P. Diluent), if applicable. The 3.2.P. Diluent section should contain all the information for DP diluent manufacturing, testing, and stability.
3.2.P.2 Pharmaceutical Development	The Pharmaceutical Development section should contain information on the development studies conducted to establish that product formulation, manufacturing process, container closure system, microbiological attributes, and instructions for use are appropriate for the stage of clinical development. In early stages of development, it is acceptable that limited information is available. Most importantly, and similarly to 3.2.S.2.6, any changes in the process and formulation of the drug product from the nonclinical to the clinical batches should be clearly identified. Compatibility studies (or in-use stability studies) should be included to support recommended hold times and conditions outlined in the clinical protocol for patient administration. It should be demonstrated that the specified reconstitution or preparation process is sufficiently robust and consistent to ensure that the product fulfils the specifications and can be administrated without negative impact on quality/safety/clinical properties.
3.2.P.5.1 Specifications	DP specifications should be listed. Your testing plan should be adequate to describe the physical, chemical, or biological characteristics of the DP necessary to ensure that the DP meets acceptable limits for identity, strength (potency), quality, and purity (21 CFR 312.23(a)(7)(iv)(b)). For IND and IMPD, tests for contents, identity and purity are mandatory. Tests for sterility and endotoxin are mandatory for sterile products. For the IMPD: a potency test should be included unless otherwise justified. For the IND, some measure of potency is required but a biologically relevant potency test is not mandatory. Although typically not required, the need for a biologically relevant potency test is highly dependent on the patient population and type of clinical trial proposed.
3.2.P.8 Stability	You should summarize the types of studies conducted, protocols used, and the results of the studies. Your summary should include, for example, conclusions regarding storage conditions and shelf-life, as well as in-use and in-device storage conditions. If a short-term clinical investigation is proposed, or if a DP manufacturing process has limited product hold times, stability data submitted may be correspondingly limited. Early in development, stability data for the gene therapy product may not be available to support the entire duration of the proposed clinical investigation. Therefore, we recommend that you submit a prospective plan to collect stability information and update this information to the IND in a timely manner (e.g., in an annual IND update).

Figure 1-5. *ICH Guideline Development Process*



CFR 312.22. These studies must use the same investigational drug but do not require the same indication. After the initial clearance, subsequent protocols can be initiated immediately after submission of the IND for agency review without a statutory waiting period as long as appropriate supporting documents are also submitted.

CLINICAL TRIAL APPLICATION (CTA)

Sponsors who wish to conduct clinical trials in the EU must do so by submitting a CTA. The authorization and oversight of clinical trials remains the responsibility of Member States until full implementation of regulation N°536/2014 and the Clinical Trials Information System (CTIS).

The central document required for the CTA is the investigational medicinal product dossier (IMPD), which contains the quality information. The quality data are presented according to the heading structure of the eCTD. It should be noted that CMC information in the IMPD is subject to specifications not only issued by EMA, but also to European Pharmacopoeia (Ph Eur) monographs and the European Directorate of the Quality of Medicines (EDQM) standard terms database.

Data requirements are known to evolve as development progresses from exploratory to confirmatory clinical trials. As such, quality data compiled in the IMPD are to reflect increasing knowledge and experience during product development.

Unlike INDs, CTAs are not subject to clinical holds; the CTA is either approved (perhaps with mandatory changes) or rejected.

MODULE 3 CONTENT

The CMC content for the Module 3-structured IND and IMPD will highly depend on the specificities of the gene therapy product in terms of the level of information provided. Table 7 and Table 8 summarize recommendations for the main Module 3 sections for Drug Substance and Drug Product, respectively,[59,60] on how to provide sufficient CMC information required to assure product safety, identity, quality, purity, and strength of the investigational product for IND and IMPD submissions. The tables combine the recommendations from EMA and FDA. Recommendations applicable to one HA only are clearly identified as such. Overall recommendations on manufacturing process information to be included in the sections for DP are similar to the ones recommended for the DS and therefore have not been repeated.

Lifecycle Management

Novel gene therapies currently being commercialized represent a new genre of medicinal products. As noted earlier, Health Authorities are interested in advancing manufacturing technologies among other innovations. One of the challenges to innovation is the burden of global change management. Thus, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) developed the ICH Q12 Lifecycle Management Guidance: "Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management,"[63] which provides a framework to facilitate management of post-approval changes in CMC. The guidance has achieved Step 4 in

the ICH process and is pending adoption and publication in various regulatory regions. The guidance development process is depicted in Figure 5.

Once adopted, this guidance will provide a global framework to further innovation and implement changes during the lifecycle of the product.

The scope of the guidance includes biologics, even if it does not specifically reference gene therapies. Tools within this guidance can be used to support post-approval changes for gene therapy medicinal products. These tools include:

- Established Conditions
- Post Approval Change Management Protocols (PACMPs)
- Product Lifecycle Management Strategy (PLCM)

ESTABLISHED CONDITIONS

Established Conditions are legally binding information also referenced as "registered details" considered necessary to assure process performance and desired quality in the product. Changes to Established Conditions require regulatory action and the classification of the regulatory change is driven by risk-to-product quality and control. Regulatory action is defined by two categories: (1) Prior: approval that requires regulatory authority review and approval prior to implementation of the change; and (2) Notification High/Low: does not require prior approval for implementation. Non-established conditions do not require regulatory action and are managed internally within the sponsor's Product Quality System (PQS).

POST APPROVAL CHANGE MANAGEMENT PROTOCOLS (PACMP)

A Post-Approval Change Management Protocol (PACMP), as defined in ICH Q12, is a two-step process that allows (1) the sponsor to define the specific change they would like to implement, assessment of the change, and how the change will be managed, reviewed, and approved by HA prior to execution; and (2) a proposed reduced reporting category for the change when all criteria are met. This process can accelerate implementation of a change by leading to approval for a reduced reporting category. Currently, this tool is acceptable in the U.S. and EU; however, upon adoption of Q12 among all ICH countries, this tool will lead to global simplification of management of changes and acceptance.

PRODUCT LIFECYCLE MANAGEMENT DOCUMENT (PLCM)

Product Lifecycle Management (PLCM) is a new concept for industry and Health Authorities. The PLCM document is a living document that will be a central repository, preferably in tabular format, for Established Conditions, PACMPs and Post Approval Commitments in each region. This document is to be submitted in the regional section of Module 3.

Conclusion

The regulatory framework governing gene therapy products can pose a large degree of complexity for developers. In addition, in comparison to more traditional biopharmaceutical products, the field is relatively immature. Therefore, both development efforts and regulatory guidelines are evolving. The FDA, EMA, and PMDA provide a range of opportunities for developers operating in the U.S., EU, and Japan markets to meet, discuss, and gain clarification on various aspects of the gene therapy product development process, including topics relating to early phases of development, CMC, and clinical trials. In addition, references specific to gene therapy products are starting to be addressed within the ICH. Materials developed and released by the ICH will provide guidance on a more general level and can be referenced as development progresses. Furthermore, the FDA, EMA and PMDA offer a number of expedited regulatory pathways to support and facilitate the innovation and therapeutic value promised by gene therapy products. As the gene therapy field continues to mature, it is expected that the corresponding regulatory structures and systems will continue to gain knowledge and experience from accumulated data, which will, in turn, allow both developers and regulators to move forward with ensuring that patients are given access to the safest and most efficacious products possible.

Appendix

Abbreviations

API	active pharmaceutical ingredient
ATMP	Advanced Therapy Medicinal Products
BTD	Breakthrough Therapy
CAT	Committee for Advanced Therapies
CATT	CBER Advanced Technologies Team
CBER	Center for Biologics Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
CMC	Chemistry, Manufacturing, and Controls
CPP	critical process parameters
CQAs	critical quality attributes
CTA	clinical trial application
CTIS	Clinical Trials Information System
DNA	Deoxyribonucleic acid
DP	drug product
DS	drug substance
EC	European Commission
eCTD	electronic Common Technical Document
ED	early dialogue
EDQM	European Directorate of the Quality of Medicines
EDWP	Early Dialogue Working Party
EMA	European Medicines Agency
EOP	End of Phase
EOP1	end-of-phase 1
EOP2	end-of-phase 2
EOP3	end-of-phase 3
EU	European Union
FAL	final advice letter
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
HA	Health Authority
ICH	International Council for Harmonisation
IMPD	investigational medicinal product dossier
IND	New Drug Application
INTERACT	Initial Targeted Engagement for Regulatory Advice
ITF	Innovation Task Force
MAA	Marketing Authorization Application
MAH	Marketing Authorization Holder
MHLW	Ministry of Health, Labor, and Welfare
OTAT	Office of Tissues and Advanced Therapies
PACMP	Post Approval Change Management Protocol
PDUFA	Prescription Drug User Fee Act
Ph Eur	European Pharmacopoeia
PIP	Pediatric Investigation Plan
PLCM	Product Lifecycle Management Strategy
PMD Act	The Act on Pharmaceuticals and Medical Devices
PMDA	Pharmaceuticals and Medical Devices Agency
pre-BLA	pre-Biologics License Application
pre-IND	pre-investigational new drug application
PRIME	Priority Medicines
PSA	Parallel Scientific Advice
RMAT	Regenerative Medicine Advanced Therapy
RNA	Ribonucleic Acid
SME	Small and Medium-Sized Enterprises
U.S.	United States

Endnotes

1. *U.S. Food and Drug Administration. What is Gene Therapy? https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/what-gene-therapy. July 2018.*
2. *U.S. Food and Drug Administration. Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug. January 2020.*
3. *U.S. Food and Drug Administration. Guidance for Industry: Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/testing-retroviral-vector-based-human-gene-therapy-products-replication-competent-retrovirus-during. January 2020.*
4. *U.S. Food and Drug Administration. Guidance for Industry: Evaluation of Devices Used with Regenerative Medicine Advanced Therapies. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/evaluation-devices-used-regenerative-medicine-advanced-therapies. February 2019.*
5. *U.S. Food and Drug Administration. Guidance for Industry: Recommendations for Microbial Vectors Used for Gene Therapy. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/recommendations-microbial-vectors-used-gene-therapy. September 2016.*
6. *U.S. Food and Drug Administration. Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/potency-tests-cellular-and-gene-therapy-products. January 2011.*
7. *U.S. Food and Drug Administration. OTAT Learn. https://www.fda.gov/vaccines-blood-biologics/news-events-biologics/otat-learn. March 2018.*
8. *European Medicines Agency. Advanced therapy classification. https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/advanced-therapies/advanced-therapy-classification.*
9. *European Medicines Agency. Committee for Advanced Therapies (CAT). https://www.ema.europa.eu/en/committees/committee-advanced-therapies-cat.*
10. *European Medicines Agency.Marketing-authorisation procedures for advanced-therapy medicinal products. https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/advanced-therapies/marketing-authorisation-procedures-advanced-therapy-medicinal-products.*
11. *Pharmaceuticals and Medical Devices Agency. PMDA presentation on Regulation of Regenerative Medicine in Japan. https://www.pmda.go.jp/files/000219466.pdf. May 2017.*
12. *Pharmaceuticals and Medical Devices Agency. PMDA presentation on Regulation of Regenerative Medicine in Japan. https://www.pmda.go.jp/files/000219466.pdf. May 2017.*
13. *Detela G, Lodge A. EU Regulatory Pathways for ATMPs: Standard, Accelerated and Adaptive Pathways to Marketing Authorisation. Mol Ther Methods Clin Dev. 2019 Jan 29;13:205-232. doi: 10.1016/j.omtm.2019.01.010. PMID: 30815512; PMCID: PMC6378853. https://www.sciencedirect.com/science/article/pii/S2329050119300130#fig2.*
14. *U.S. Food and Drug Administration. Fast Track. https://www.fda.gov/patients/fast-track-breakthrough-therapy-accelerated-approval-priority-review/fast-track.*
15. *U.S. Food and Drug Administration. Breakthrough Therapy. https://www.fda.gov/patients/fast-track-breakthrough-therapy-accelerated-approval-priority-review/breakthrough-therapy.*
16. *U.S. Food and Drug Administration. Guidance for Industry: Expedited Programs for Regenerative Medicine Therapies for Serious Conditions. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expedited-programs-regenerative-medicine-therapies-serious-conditions. February 2019.*
17. *U.S. Food and Drug Administration. Priority Review. https://www.fda.gov/patients/fast-track-breakthrough-therapy-accelerated-approval-priority-review/priority-review.*
18. *U.S. Food and Drug Administration. Accelerated Approval. https://www.fda.gov/patients/fast-track-breakthrough-therapy-accelerated-approval-priority-review/accelerated-approval.*
19. *European Medicines Agency. Accelerated assessment. https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/accelerated-assessment.*
20. *European Medicines Agency. Accelerated assessment. https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/accelerated-assessment.*
21. *Ministry of Health, Labour and Welfare. Strategy of SAKIGAKE. https://www.mhlw.go.jp/english/policy/health-medical/pharmaceuticals/140729-01.html.*
22. *European Medicines Agency. Accelerated assessment. https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/accelerated-assessment.*
23. *Nagai S. Flexible and Expedited Regulatory Review Processes for Innovative Medicines and Regenerative Medical Products in the U.S., the EU, and Japan. Int J Mol Sci. 2019 Aug 3;20(15):3801. doi: 10.3390/ijms20153801. PMID: 31382625; PMCID: PMC6696404.*
24. *Nagai S. Flexible and Expedited Regulatory Review Processes for Innovative Medicines and Regenerative Medical Products in the U.S., the EU, and Japan. Int J Mol Sci. 2019 Aug 3;20(15):3801. doi: 10.3390/ijms20153801. PMID: 31382625; PMCID: PMC6696404.*
25. *Nagai S. Flexible and Expedited Regulatory Review Processes for Innovative Medicines and Regenerative Medical Products in the U.S., the EU, and Japan. Int J Mol Sci. 2019 Aug 3;20(15):3801. doi: 10.3390/ijms20153801. PMID: 31382625; PMCID: PMC6696404.*
26. *Ministry of Health, Labour and Welfare. Strategy of SAKIGAKE. https://www.mhlw.go.jp/english/policy/health-medical/pharmaceuticals/140729-01.html.*
27. *U.S. Food and Drug Administration. CBER Advanced Technologies Team (CATT). https://www.fda.gov/vaccines-blood-biologics/industry-biologics/cber-advanced-technologies-team-catt.*
28. *European Medicines Agency. Innovation in medicines. https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines.*
29. *Paul-Ehrlich-Institut, Agency of the German Federal Ministry of Health. Innovation Office. https://www.pei.de/EN/regulation/advice/innovation-office/innovation-office-node.html.*
30. *U.S. Food and Drug Administration. Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/formal-meetings-between-fda-and-sponsors-or-applicants-pdufa-products-guidance-industry. December 2017.*
31. *U.S. Food and Drug Administration. FDA presentation: Facilitating Expedited Development of Advanced Therapy Products. https://ipq.org/wp-content/uploads/2019/09/Facilitating-Expedited-Development_Oh.pdf. June 2019.*
32. *U.S. Food and Drug Administration. CBER SOPP 8214: INTERACT Meetings with Sponsors for Drugs and Biological Products. https://www.fda.*

Endnotes, continued

gov/media/124044/download. June 2020.

33. *U.S. Food and Drug Administration. INTERACT Meetings. https://www.fda.gov/vaccines-blood-biologics/industry-biologics/interact-meetings.*
34. *U.S. Food and Drug Administration. Investigational New Drug (IND) Application. https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application#preIND.*
35. *U.S. Food and Drug Administration. Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-design-early-phase-clinical-trials-cellular-and-gene-therapy-products. June 2015.*
36. *U.S. Food and Drug Administration.End of Phase 2 Meeting. https://www.accessdata.fda.gov/cder/sb-navigate/topic3/topic3/da_01_03_0186.htm.*
37. *U.S. Food and Drug Administration. FDA presentation: Meetings with CDER. https://www.fda.gov/media/89217/download.*
38. *U.S. Food and Drug Administration. End of Phase 2 Meeting. https://www.accessdata.fda.gov/cder/sb-navigate/topic3/topic3/da_01_03_0186.htm.*
39. *U.S. Food and Drug Administration. Questions to Ask FDA during Formal Meetings. https://www.accessdata.fda.gov/cder/sb-navigate/topic3/topic3/da_01_03_0180.htm.*
40. *European Medicines Agency. Authorisation of medicines. https://www.ema.europa.eu/en/about-us/what-we-do/authorisation-medicines#scope-of-the-centralised-procedure-section.*
41. *European Medicines Agency. EMEA/45110/2007: Committee for Medicinal Product for Human Use Rules of Procedure. https://www.ema.europa.eu/en/documents/other/chmp-rules-procedure_en.pdf. March 2020.*
42. *European Medicines Agency. EMA/CAT/600280/2010: Committee for Advanced Therapies (CAT) Reflection paper on classification of advanced therapy medicinal products. https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-classification-advanced-therapy-medicinal-products_en-0.pdf. May 2015.*
43. *European Medicines Agency. EMA/CAT/99623/2009: Committee for Advanced Therapies (CAT) Procedural advice on the provision of scientific recommendation on classification of advanced therapy medicinal products in accordance with article 17 of regulation (EC) no 1394/2007. https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/procedural-advice-provision-scientific-recommendation-classification-advanced-therapy-medicinal/2007_en.pdf. London, December 2013.*
44. *Voisin Life Sciences Consulting. Unlocking The EMA Meetings: The Process And How To Prepare Adequately. https://voisinconsulting.com/resources/webinars/unlocking-the-ema-meetings-the-process-and-how-to-prepare-adequately/.*
45. *European Medicines Agency. EMA/4260/2001: European Medicines Agency guidance for applicants seeking scientific advice and protocol assistance. https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/european-medicines-agency-guidance-applicants-seeking-scientific-advice-protocol-assistance_en.pdf. October 2020.*
46. *European Medicines Agency. National competent authorities (human). https://www.ema.europa.eu/en/partners-networks/eu-partners/eu-member-states/national-competent-authorities-human.*
47. *Voison Life Sciences Consulting. Opportunities For Early Dialogue With The EMA And HTA Bodies. Optimizing Product Development To Ensure Commercial Success. https://voisinconsulting.com/resources/webinars/opportunities-for-early-dialogue-with-the-ema-and-hta-bodies-optimizing-product-development-to-ensure-commercial-success/.*
48. *European Medicines Agency. Parallel consultation with regulators and health technology assessment bodies. https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-advice-protocol-assistance/parallel-consultation-regulators-health-technology-assessment-bodies.*
49. *European Medicines Agency. EMA/309801/2017: General Principles EMA-FDA Parallel Scientific Advice (Human Medicinal Products). https://www.ema.europa.eu/en/documents/other/general-principles-european-medicines-agency-food-drug-administration-parallel-scientific-advice_en.pdf. April 2017.*
50. *U.S. Food and Drug Administration. CDER SOPP 8001.6: Procedures for Parallel Scientific Advice with European Medicines Agency (EMA). https://www.fda.gov/media/87478/download. October 2019.*
51. *Pharmaceuticals and Medical Devices Agency. Consultations. https://www.pmda.go.jp/english/review-services/consultations/0002.html.*
52. *Pharmaceuticals and Medical Devices Agency. Profile of Services. https://www.pmda.go.jp/files/000221139.pdf.*
53. *Pharmaceuticals and Medical Devices Agency. PMDA presentation: Clinical trial notifications and scientific consultation system in Japan. https://www.pmda.go.jp/files/000152326.pdf.*
54. *U.S. Food and Drug Administration. Draft Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products. https://www.fda.gov/media/109951/download. December 2017.*
55. *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH M2 EWG Electronic Common Technical Document Specification. https://admin.ich.org/sites/default/files/inline-files/eCTD_Specification_v3_2_2_0.pdf. July 2008.*
56. *U.S. Food and Drug Administration. Guidance for Industry: M4Q: The CTD — Quality. https://www.fda.gov/media/71581/download. August 2001.*
57. *U.S. Food and Drug Administration. IND Applications for Clinical Investigations: Chemistry, Manufacturing, and Control (CMC) Information. https://www.fda.gov/drugs/investigational-new-drug-ind-application/ind-applications-clinical-investigations-chemistry-manufacturing-and-control-cmc-information.*
58. *U.S. Food and Drug Administration. Guidance for Industry: Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/determining-need-and-content-environmental-assessments-gene-therapies-vectored-vaccines-and-related.*
59. *U.S. Food and Drug Administration. Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). https://www.fda.gov/media/113760/download. January 2020.*
60. *European Medicines Agency. EMA/CAT/852602/2018: Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy_en.pdf. January 2019.*
61. *European Medicines Agency. EMEA CPMP/ICH/294/95: ICH Topic Q 5 D Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-5-d-derivation-characterisation-cell-substrates-used-production-biotechnological/biological-products-step-5_en.pdf. March 1998.*
62. *U.S. Food and Drug Administration. Federal Register / Vol. 63, No. 182. https://www.fda.gov/media/71463/download. September 1998.*
63. *International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Quality Guidelines. https://www.ich.org/page/quality-guidelines.*

Chapter 2

Standards in Gene Therapy

Alliance *for* Regenerative Medicine

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 2 | Standards in Gene Therapy

Introduction

Gene therapy is an emerging field of medicine, with many promising products in development that could help manage and potentially cure conditions and diseases that are intractable, chronic, and even terminal. Given that the gene therapy field is currently at a key tipping point, with disruptive innovation pushing the boundaries of science and a number of products poised for commercialization (with a few already on the market), robust standards that can support developers in ensuring the safety, efficacy, and quality of products must be established. This chapter will provide an overview of why standardization in the field of gene therapy is needed, as well as an examination of the current landscape of standards development in gene therapy.

Benefits of Voluntary Consensus Standards in Gene Therapy

Standards are considered to be voluntary rules, conditions, characteristics, or physical materials that an organization can adopt to make a process safer, more efficient, or better aligned with the practices of other organizations in their industry. In general, standards can be considered to be either documentary or non-documentary.

Gene therapy is one of the key modalities of newly emerging regenerative therapeutics. The number and diversity of the product platforms in current use has exploded over the last ten years. However, given that relatively little experience has accumulated in the years that gene therapy has been a part of the therapeutic landscape, there are relatively few ways for developers across the research and industrial sectors to achieve consistency in areas such as protocol development, process infrastructure, and product quality testing and assurance. This lack of consistency and experience has made it challenging for stakeholders involved in gene therapy development and manufacturing to operate with a sense of certainty and provide patients in need of these essential and groundbreaking therapies with the confidence that products are of the maximum possible quality. The ongoing absence of standards to support safe and efficient practices that reduce the burden on companies seeking regulatory approval for their products may result in promising therapies being unable to successfully transition from the clinical development phase to being commercially available for the benefit of patients. The appropriately targeted, field-wide development of standards will help to address the previously tolerated shortcomings.

Specifically, standardizing equipment, methodologies, and testing methods will result in a number of benefits for industry stakeholders. Standards establish a base of legitimacy on which patients, regulators, and investors can rely—standards development will instill the public with confidence that development of gene therapy is adequately informed by thoroughly researched best practices and is, therefore, a safe and effective option for treating a wide range of diseases. In addition, from an operational standpoint, standardization can help industry stakeholders streamline business practices by allowing for more efficient coordination of efforts throughout the entire supply chain, as well as improving the predictability of costs and resource management. In effect, barriers to entry into the clinical development space may be lowered for smaller companies or academic researchers, thus facilitating the delivery of therapies to patients. Standards can assure regulators that the fundamental processes underlying the development of a new therapy are sound. This assurance, in turn, allows regulators to more rapidly review a product. A smoother, less uncertain regulatory review process increases industry stability, lowers perceived risk to investors, and accelerates market availability of products, all of which serve to increase access and options for patients. Lastly, standards can greatly aid the regenerative medicine therapy community as a whole by enhancing the ability of developers to collaborate and share knowledge with others. This can reduce the potential for redundant efforts to be undertaken and serve to patch up the relatively fragmented state of knowledge that characterizes the emerging state of this field.

Documentary Standards

WHAT ARE DOCUMENTARY STANDARDS?

Documentary standards set guidelines, protocols, procedures, methods, technical specifications, and terminology in the form of consensus-based documents that developers can use to ensure a high level of product quality and safety. These standards can apply to any step

of development, from the evaluation of source materials, the manufacturing of products, and assessment of safety and quality attributes of final products, to the transport, storage, and commercial clinical use of products.

Documentary standards are developed by standards developing organizations (SDOs), accreditation bodies, and professional societies, including (but not limited to):

- ASTM International
- The British Standards Institution (BSI)
- The Clinical and Laboratory Standards Institute (CLSI)
- The Foundation for the Accreditation of Cellular Therapy (FACT)
- The International Organization for Standardization (ISO)
- The United States Pharmacopeia (USP)

HOW ARE DOCUMENTARY STANDARDS, REGULATIONS, GUIDANCE, AND BEST PRACTICES DIFFERENT?

Documentary standards can be contrasted with regulations, guidance, and best practices. Regulations have the force and effect of law and are usually mandatory, setting out specific requirements, which products and organizations must meet. In the United States, regulations are written in the Code of Federal Regulations and published in the Federal Register. Guidances are formal documents issued by a government agency to clarify the regulatory body's thinking on existing laws or regulations and offer guidelines for how industry can comply with these regulations. Best practices are the informal methods most people in a field agree are the best way of accomplishing a goal. These are sometimes published by professional societies in academic journals. Guidances and best practices, for example, may include expectations that may be considered to be documentary standards.

HOW DOCUMENTARY STANDARDS CAN BE USED IN GENE THERAPY

Documentary standards development can benefit the gene therapy community by:

- Replacing costly time-intensive trial-and-error processes with proven best practices
- Increasing product safety by defining testing and processing parameters throughout the product life cycle
- Speeding review processes by eliminating the need to re-evaluate common operational steps for each new product
- Decreasing costs of therapies by increasing testing and process efficiency
- Decreasing time to market by minimizing time required for implementation of common operational steps and validation of unique manufacturing processes
- Increasing quality of raw materials and final products by standardizing reporting requirements and quality assays.

HOW DOCUMENTARY STANDARDS ARE DEVELOPED

Consensus-based standards are standards developed following a consensus-based process, which means an organization uses practices that are fair, open, balanced, equitable, accessible, and responsive to stakeholder needs. The American National Standards Institute (ANSI) accredits U.S. standards developing organizations (SDOs) that follow consensus-based processes.

Non-consensus-based standards are standards created by organizations that do not follow consensus-based processes. With respect to biopharmaceutical products, some of the most important of these standards are published in compendiums known as pharmacopoeia.

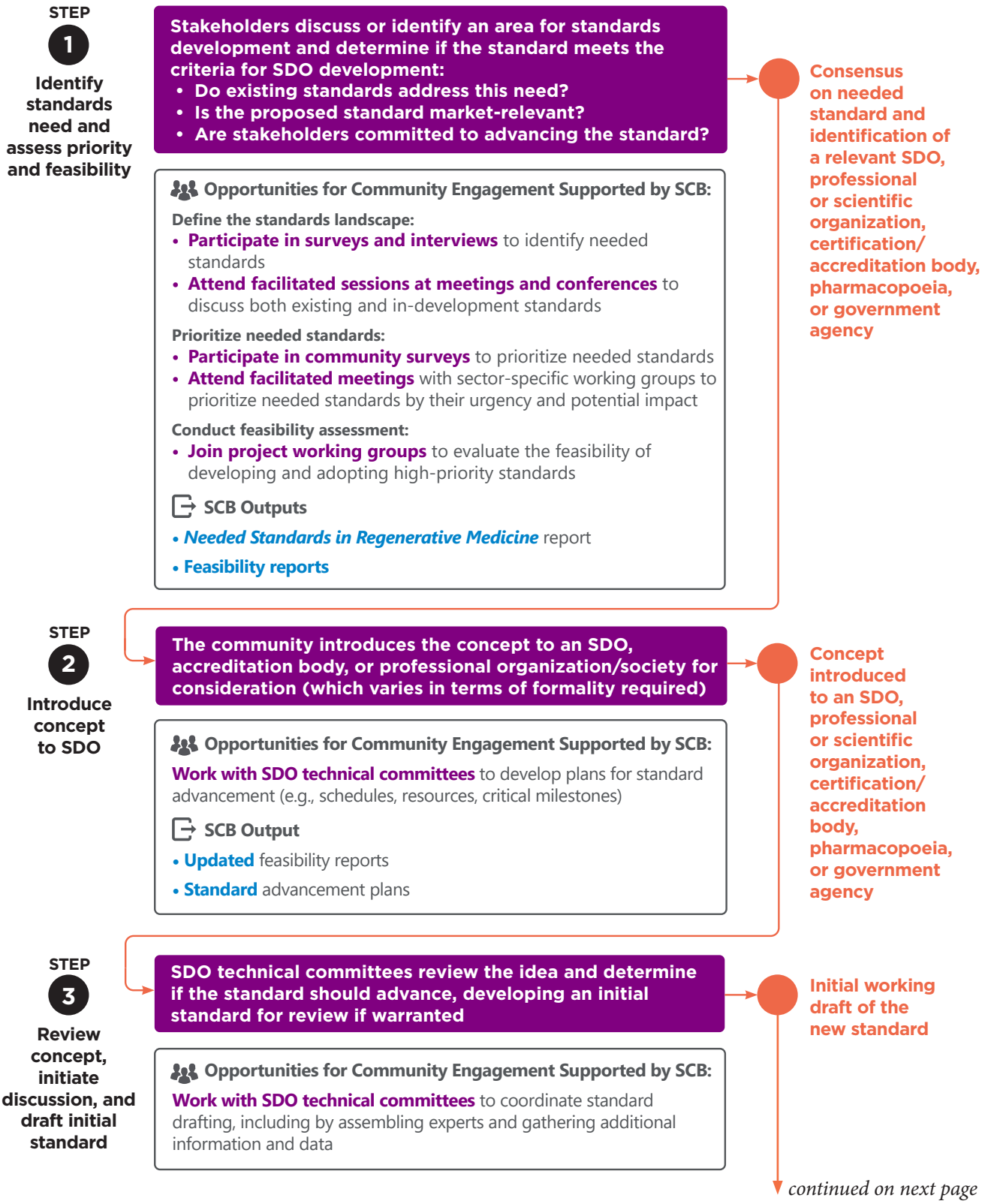
THE STANDARDS DEVELOPMENT PROCESS

The documentary standards development process:

- Brings together experts throughout the community to share pre-competitive knowledge
- Makes research results more readily available to the public to drive the entire field forward
- Gives stakeholders a voice in defining the standards that will best support their work

Figure 1 illustrates the development of documentary standards from the perspective of one particular non-profit organization, the Standards Coordinating Body for Gene, Cell, and Regenerative Medicines and Cell-Based Drug Discovery (SCB), which complements current SDO processes for standards development by engaging regenerative medicine stakeholders to ensure that new or revised standards provide the greatest benefits to the broad regenerative medicine community.

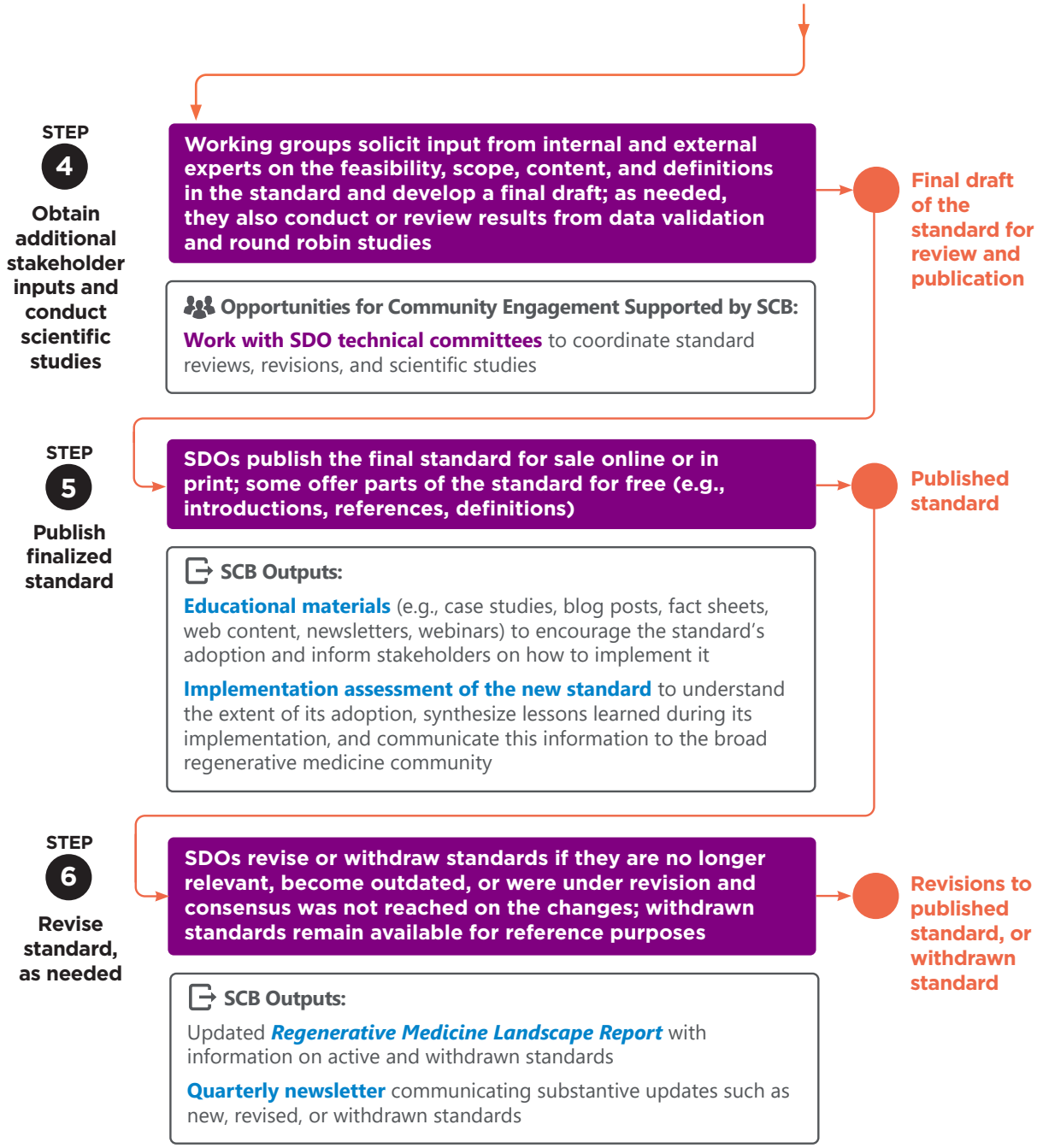
Figure 2-1. *Standards Coordinating Body—Process for Development of Documentary Standards*



continued on next page

Figure 2-1. *Standards Coordinating Body—Process for Development of Documentary Standards*

continued from previous page



source: Standards Coordinating Body; Documentary Standards for Regenerative Medicine

DOCUMENTARY STANDARDS CASE STUDIES

Pre-existing AAV Immunity

Patients who have pre-existing immunity to the vectors used in gene therapy products such as AAVs may experience suboptimal treatment outcomes. However, the gene therapy community currently lacks standards for evaluating pre-existing AAV immunity.

SCB is coordinating the development of a consensus-based documentary standard that defines common language for evaluating pre-existing immunity to AAVs. Defining basic language and concepts in the field of pre-existing immunity is needed to inform the development of standards in this area and facilitate pre-competitive dialogue among stakeholders. This documentary standard is anticipated to be available sometime between 2023 and 2025.

Nucleic Acid Target Sequence Quantification

Accurate quantification of nucleic acid target sequences allows developers and manufacturers of regenerative therapy products to monitor factors such as process controls, efficiency, and quality attributes. However, performing the required assays at a high enough degree of quality so as to effectively demonstrate product safety and/or efficacy can be challenging. ISO has developed the ISO DNA Quantitation Standard (ISO 20395) that can help manufacturers achieve high quality measurements for the quantification of nucleic acid sequences.

The document provides requirements and guidelines for ensuring the quality of methods used for the quantification of specific nucleic acid sequences. The main methods covered are based on digital (dPCR) and quantitative real-time PCR (qPCR) amplification technologies, which can be applied to target sequences present in nucleic acid molecules including double-stranded DNA (dsDNA) such as genomic DNA (gDNA) and plasmid DNA, single stranded DNA (ssDNA), complementary DNA (cDNA), and single stranded RNA (ssRNA) including ribosomal RNA (rRNA), messenger RNA (mRNA), and long and short non-coding RNA [microRNAs (miRNAs) and short interfering RNAs (siRNAs)], as well as double-stranded RNA (dsRNA). Specific topics covered include (but are not limited to) analytical design, assay design and optimization, data quality controls and analysis, and method validation.

The standard can be purchased here: *https://www.iso.org/standard/67893.html*

Reference Material Standards

WHAT ARE REFERENCE MATERIAL STANDARDS?

Reference materials are considered to be non-documentary standards and are typically highly characterized reagents or substances that are distributed to enable researchers and developers to assure consistency and quality in measurement processes intended to report on the safety and potency of a manufactured product. These materials can be linked to specific manufacturing processes and can be used for product development purposes such as the calibration of analytical equipment or the comparison of therapeutic products or product components to similar reference materials with known quality attributes. Reliable reference materials are of particular importance to the gene therapy community since many current testing methods and equipment are not yet standardized.

Reference materials development benefits the regenerative medicine community as a whole by:

- Enabling analytical equipment calibration to ensure valid results
- Defining safe, reliable baseline materials
- Speeding review processes for researchers using non-standard testing equipment and test methods
- Decreasing time and money lost to repetitive, inconsistent tests
- Improving coordination, community engagement, and education
- Increasing consistency through standardization of testing results and reporting

In 2015, FDA (Gavin, HGTM 26:3) and NIBSC/Medicines and Healthcare Products Regulatory Agency (Werling et al. HGTM 26:82) encouraged the use of reference standard materials as benchmarking tools for qualifying in-house reference materials and controls, and for demonstrating that assay methods are appropriately controlled.

For additional information and definitions, see: *https://www.nist.gov/srm/srm-definitions*

REFERENCE MATERIAL CASE STUDIES FOR GENE THERAPY — CASE STUDY #1

Gene therapy involves modifying the expression of a patient's genes and/or repairing abnormal genes. Since genes within cells cannot be changed without a way to administer the genetic changes to the cell itself, scientists produce delivery tools known as vectors that are capable of administering specific nucleic acids (DNA or RNA) into the cell for expression and replication. Vectors are frequently of viral origin and insertion of the desired nucleic acid into cells can occur either *ex vivo* or *in vivo*.

Following the establishment of the Adenovirus Type 5 reference material (ATCC VR-1516) close to 20 years ago, discussions were held to facilitate the preparation of reference standard materials for AAV vectors. A major goal was to be able to use the resulting reference standards to validate each laboratory's internal standards and methods. This would enable comparisons among studies conducted by different laboratories and aid in the manufacturing of higher quality vectors.

Since it was the primary serotype in use, the initial focus was on AAV2. The AAV Reference Standards Working Group (AAVRSWG) was formed to include members from industry, academia, government, and regulatory bodies. Early discussions finalized the profile of the material with regard to concentration and testing requirements. The reference material was produced, purified, formulated, and filled by a group of organizations that donated substantial time and materials to the effort.

A few years after the AAV2 effort, another working group was formed to facilitate the production of a reference standard for AAV8. Information regarding the production and characterization, as well as ordering information, can be found here: *https://www.atcc.org/en/Standards/Standards_Programs/ATCC_Virus_Reference_Materials.aspx*

These efforts were successful in highlighting the lack of standardization within the gene therapy community. To this day, organizations developing gene therapy products continue to work in relative isolation with respect to solving issues with variability in the analytical methods used to characterize AAV and other gene therapy vectors. The laboratory studies conducted using the original AAV reference standard materials resulted in a large data set that showed a high degree of inter-laboratory variability using similar methods. Even when the protocols and associated reagents were provided, there were sometimes large variances observed. For the AAV2 material, the mean titer was 3.28×10^{10} vector genomes/ml and the 95% confidence intervals were 2.70×10^{10} to 4.75×10^{10} vector genomes/ml. For a dose-determining assay, such as the assessment of genome titer, such variances can cause difficulties for developers and regulatory bodies with regard to being able to reliably cross-compare data across the field. In 2018, the FDA hosted a workshop entitled: "Quantitation of AAV-based gene therapy products." In this workshop, presentations covered both the biology of AAV, as well as different analytical approaches for quantifying AAV-based products. Information and recordings of the workshop can be found here: *https://www.fda.gov/vaccines-blood-biologics/workshops-meetings-con-*

FOR MORE INFORMATION

Further information about AAV2 and AAV8 can be found in the following references (provided links are from the ATCC website):

AAV2

Production:

Flotte, TR, Burd, P and Snyder, RO. Utility of a Recombinant Adeno-Associated Viral Vector Reference Standard. BioProcessing J. 2002;1: 75.

Potter, M., Phillipsberg, G., Phillipsberg, T., et al. Manufacture and stability study of the recombinant adeno-associated virus serotype 2 vector reference standard. Bioprocess J. 2008;7: 8-14

Characterization:

Lock M, McGorray S, Auricchio A, et al. Characterization of a recombinant adeno-associated virus type 2 Reference Standard Material. Hum Gene Ther. 2010;21(10):1273-1285. doi:10.1089/hum.2009.223

AAV8

https://pubmed.ncbi.nlm.nih.gov/18574495/

2016 Paper:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4813604/

ferences-biologics/quantitation-aav-based-gene-therapy-products-12072018-12072018

The existing AAV reference materials provide useful tools for developers to use in benchmarking equipment, methods, and analyst performance. Although the PCR targets used for these materials are different from those used in product-specific methods, they are still extremely valuable as tools for interrogating the variability of methods. The results obtained from the studies associated with these efforts were also extremely valuable in highlighting the variability of data obtained in different laboratories, which has led to investigations into potential sources of variability. In 2016, another study was published on the use of the AAV reference materials, titled: "Practical utilization of recombinant AAV vector reference standards: Focus on vector genomes titration by free ITR qPCR." The authors used the reference materials to investigate differences in titers obtained using slightly different methods and concluded that free inverted terminal repeat (ITR) qPCR eliminated the differences.

Current Landscape of Standards in Gene Therapy

Currently, additional research is needed to assess the safety and efficacy of gene therapies for commercial use, which is becoming more widespread with the regulatory approval of several products in recent years. Furthermore, factors such as variations in manufacturing, measurement, and analytical techniques across developers of experimental gene therapy products make it difficult to evaluate product quality and safety, and to assess the impact of manufacturing changes or innovations intended to improve product safety and efficacy. A common set of standards in gene therapy will advance development of treatments beyond the realm of clinical trials to approved treatments for a number of diseases and syndromes subject to genetic control.

CURRENT SDO'S INVOLVED WITH STANDARDS IN GENE THERAPY

Table 1 describes the number of important gene therapy-related standards that have been developed by several established SDOs. Some of the more active SDOs involved currently with gene therapy-related standards are discussed in greater detail below.

Table 2-1. *Existing Standards by SDO*

6	AABB
58	ASTM International
8	American Type Culture Collection (ATCC)
3	British Standards Institutions (BSI)
7	Clinical & Laboratory Standards Institutes (CLSI)
9	European Directorate for the Quality of Medicines and Healthcare (EDQM)
8	Foundation for the Accreditation of Cellular Therapy (FACT)
6	International Conference on Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)
1	International Council for Commonality in Blood Banking Automation (ICCBBA)
55	International Organization for Standardization (ISO)
1	International Society for Advancement of Cytometry (ISAC)
3	International Society for Cellular Therapy (ISCT)
1	International Society for Stem Cell Research (ISSCR)
22	National Institute for Biological standards and Control (NIBSC)
1	ONE Study Consortium
6	Parenteral Drug Association (PDA)
6	Pharmaceuticals and Medical Devices Agency (PMDA), Japan
14	United States Pharmacopeia (USP)

ASTM International

ASTM International (formerly known as American Society for Testing and Materials) is a global developer of voluntary standards using an open and transparent process. ASTM is a broad SDO with more than 12,000 standards globally and follows the World Trade Organization's principles for standards development of transparency, openness, impartiality and consensus, effectiveness and relevance, coherence, and development dimension. ASTM develops standards for regenerative medicine primarily through Committee F04. In

particular, Subcommittees F04.41 (Classification and Terminology for TEMPs), F04.42 (Biomaterials and Biomolecules for TEMPs), F04.43 (Cells and Tissue Engineered Constructs for TEMPs), F04.44 (Assessment for TEMPs), and F04.45 (Adventitious Agents Safety) are most relevant to production of regenerative medicines.

ATCC

ATCC is an ISO accredited non-profit organization that seeks to support the advancement and application of scientific knowledge by facilitating the development of standards related to biological materials and information. With respect to supporting regenerative therapies, ATCC is responsible for distributing standard doses of recombinant adeno-associated virus 2 (ATCC VR-1616) and 8 (ATCC VR-1816) vector reference standard stock (RSS). Adeno-associated virus (AAV) -based recombinant vectors are important tools used for the manufacturing clinical gene therapy (in addition to cell therapy). ATCC-distributed RSSs establish reference points that define standard particle, vector genome, and infectious unit specifications for AAVs. In line with recommendations issued by FDA's CBER, OCTGT, and DCGT, the global availability of AAV reference standard materials aids in standardization across organizations involved in gene therapy manufacturing and distribution by allowing for the accurate calibration on internal reference materials and assays for products that make use of AAV viral gene transfer-based technologies and techniques. In turn, data from different preclinical and clinical studies can be more easily and meaningfully compared.

CLSI

Clinical and Laboratory Standards Institute (CLSI) is an international SDO comprised of more than 1,400 member organizations that seeks to promote the development and use of voluntary consensus standards and guidelines within the health care community at large. In order to develop standards, CLSI uses a unique consensus-based process that includes authorization of a project, development and open review of documents, revision of documents in response to users' comments, and finally, acceptance of a document as a consensus standard or guideline. Importantly, through its role as the ANSI-appointed Secretariat for ISO Technical Committee 212 (ISO/TC 212), CLSI provides ISO/TC 212 with both draft and approved standards related to laboratory testing and *in vitro* diagnostic test systems, with specific standards topics including quality management, pre- and post-analytical procedures, analytical performance, laboratory safety, reference systems, and quality assurance. CLSI also serves as the administrator for the ANSI-accredited US Technical Advisory Group (TAG) for ISO/TC 212. With regard to gene therapy, many of the nucleic acid quantification assays commonly used during gene therapy product manufacturing are implemented using standards established by CLSI. Developers may also reference a number of standards covering a wide range of molecular-level testing used in clinical development settings.

EDQM

The EDQM supports patient access to quality medicines and healthcare in the EU. Through its European Pharmacopoeia (Ph. Eur.) arm, the EDQM determines standards for the quality of medicines, including those for gene therapy products, and codifies these standards into pharmacopoeial texts. The EDQM engages with the field of gene therapy as part of its role as the technical secretariat for the network of Official Medicines Control Laboratories (OMCLs) of Europe. The OMCLs are a network of more than 70 laboratories spanning both the public and industry healthcare sectors that support European regulatory authorities in ensuring the quality of gene therapy products by assessing the landscape of marketing licensure applications with respect to preclinical data and data gathered from monitoring marketed medicines, as well as current legal requirements. The interaction of the OMCLs with regulators allows manufacturers of gene therapy products to take into account all relevant regulatory considerations starting from the early stages of product development. In order to organize and direct the OMCLs for their role in the surveillance of gene therapy products, a gene therapy Working Group has been established within the General European OMCL Network. The gene therapy Working Group aims specifically to define the most appropriate analytical methods for gene therapy products and relevant reference standards. The gene therapy Working

Group meets once a year to review field-wide activities in the past year, define the goals for the following year, and discuss any relevant topics as needed. The areas of greatest focus include adeno-associated viral (AAV) vectors, retroviral/lentiviral (RV/LV) vectors, and plasmids.

FACT

FACT (Foundation for the Accreditation of Cellular Therapy) at the University of Nebraska Medical Center is a non-profit organization that establishes standards for medical and laboratory practice for developers of cellular therapies. FACT aims to promote the adoption of voluntary inspection and accreditation within the field of cellular therapy, which is an emerging and evolving field intimately tied to gene therapy (particularly with respect to the use of viral vector products in the manufacturing process). The standards developed by FACT are intended to represent the fundamentals of cellular therapy that can be applied to a broad range of cell sources and therapeutic applications, and are to be used throughout product development and clinical trials.

The overarching goal of the FACT Standards is to promote improvement and progress in cellular therapy and regenerative medicine across all aspects of manufacturing and administration that are relevant to the quality of products and therapeutic care. To this end, FACT Standards are evidence-based (where possible, if published data are not available, accepted scientific theory is referenced) and decided upon within committees comprised of world-renowned clinicians, scientists, technologists, and quality experts that span the entire continuum of cell manufacturing. Additionally, input from both consumers and regulatory bodies are sought wherever possible. Before final approval by the FACT Board of Directors, the standards development process undergoes both public review and legal review.

As the only set of standards that are clearly and definitively focused on promoting the use of cellular therapy products manufactured under rigorous controls, the FACT Standards form the basis of the FACT accreditation program. By gaining FACT accreditation, developers and manufacturers of cell therapy products can demonstrate a commitment to maximizing the quality of products and therapeutic care, thereby instilling confidence in patients.

ISO

The International Organization for Standardization (ISO) is a worldwide federation of national standards bodies (ISO member bodies). ISO develops standards for regenerative therapy production via ISO Technical Committee 276 Biotechnology. ISO Technical Committee 276 Biotechnology operates through four main Working Groups corresponding to the following areas:

- Terminology
- Biobanks and bioresources
- Analytical methods
- Bioprocessing

Additional topics covered in due measure by the Working Groups include data processing, validation, and comparability. In addition, ISO Technical Committee 276 Biotechnology works closely with both governmental and non-governmental agencies such as International Society for Cell and Gene Therapy (ISCT) to identify priorities with regard to standardization efforts worldwide. Such collaboration not only streamlines standardization into a comprehensively coordinated process, but also serves to allow organizations to avoid duplications and overlapping standardization activities.

USP

The United States Pharmacopeia (USP) was created nearly 200 years ago and is dedicated to instilling trust where it matters most: in the medicines, supplements, and foods people rely on for their health. The quality standards developed by USP help manufacturers deliver on their promises of safe products, while building confidence among healthcare practitioners, patients, and consumers. In the field of regenerative medicine, USP currently has multiple documentary and physical standards to aid developers in bringing safe and effective novel therapies to patients. Under the guidance of the scientific experts on the recently established Advanced Therapies Expert Committee, USP is active in developing standards for AAV and lentiviral therapies, as well as materials such as plasmid DNA used in manufacturing.

STANDARDS COORDINATING BODY FOR REGENERATIVE MEDICINE, CELL AND GENE THERAPY (SCB)

SCB is an organization that was created in 2017 to help accelerate the standards process. SDOs play a critical role in the publishing of consensus standards that are universally recognized. It was recognized that an organization was needed to help focus on the standards development process in order to accelerate this process by facilitating the use and development of standards in response to demonstrated needs expressed by a range of stakeholders. SCB functions as a 501(3)c organization with no vested interest in a particular scientific, commercial, clinical, or policy approach. This is critical to its function and success in addressing the complex challenges related to scientific protocols, product testing, and product quality and performance specifications. The field of regenerative medicine faces challenges common to emerging industries, including fragmentation of knowledge, insufficient communication and coordination, and rapid advancement of innovation.

For the field to thrive, it is recognized that development and implementation of standards and best practices will help accelerate regulatory reviews of therapeutic developers' CMC documentation. SCB's processes for development of standards and best practices address the diverse needs of stakeholder groups, including government and regulatory agencies, researchers, providers of raw materials, product developers, equipment manufacturers, and clinicians and healthcare professionals. Since 2017 SCB has been involved in 23 standards projects with four advanced to SDOs, greatly shortening the historical times it has taken (up to 10 years in the past) for standards to progress.

CURRENT DOCUMENTARY STANDARDS IN GENE THERAPY

Gene therapy involves modifying the expression of a patient's genes and/or repairing abnormal genes using recombinant DNA technology. Gene therapy products are delivered using viral or nonviral vectors that administer specific nucleic acids (DNA or RNA) into the cell for expression and replication. This sector's key challenge is to better understand and control how products interact with the human body. This can be achieved by defining variables related to delivery mechanisms, and dosing. Delivery of appropriate doses of vectors to patients is a foundational challenge that could be addressed through vector quantification standards. Additionally, factors that cause adverse reactions in gene therapy patients (e.g., immune response or complications from replication-competent retroviruses [RCRs]) must be better understood.

Specific gene therapy areas to standardize include:

- Vector genome titration
- Protocols for patient monitoring after infusion
- Factors for selection of biodistribution methods
- Methodology for screening patients for immunity to adeno-associated virus (AAV) vectors

Most current standards that are applicable to gene therapy have been compiled in SCB's The Regenerative Medicine Standards Landscape: *https://www.standardscoordinatingbody.org/landscape*

Of note, a number of documentary standards related to quantification and sequencing have been published which may be referenced by developers of gene therapy products. The main areas of focus relate to DNA diagnostic sequencing and molecular diagnostic testing, DNA extraction methodology, general best practices for manufacturing, testing, and administration of gene therapy products, reference materials (e.g., genomic DNA, vector plasmids, cell lines, reference panels and reagents) virology standards, and testing for acceptable levels of residual host-cell proteins in gene therapy products.

Some current standards of note include:

International scope

- ISO 20395:2019 Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR
- ISO 20688-1:2020 Nucleic acid synthesis — Part 1: Requirements for the production and quality control of synthesized oligonucleotides
- ISO 20391-1:2018 Biotechnology — Cell counting — Part 1: General guidance on cell counting methods
- ISO 20391-2:2019 Biotechnology — Cell counting — Part 2: Experimental design and statistical analysis to quantify counting method performance

Europe

- EP 5.14 EDQM: Gene Transfer Medicinal Products for human use
- EP 2.6.35 EDQM: Quantification and Characterization of residual host-cell DNA
- EP 2.6.34 EDQM: Host-cell protein assays

United States

- USP<1043>Ancillary Materials for Cell, Gene and Tissue-Engineered Products
- USP <1046> Cell and Gene Therapy Products
- USP <1047> Gene Therapy Products

Documentary standards intended for preclinical settings

Pre-clinical studies are intended to test a drug, procedure, or other medical treatment in animals, and are required to take place before clinical trials in humans can be started. Because the preclinical phase of research is critical for optimal decision-making about a possible future therapy, experiments done at this stage should be conducted using best practice-based methods (e.g., choosing the most appropriate animal model and ensuring that experiments are comparable and reproducible across different labs).

Currently, there are no known gene therapy standards related to preclinical studies.

Documentary standards intended for clinical settings

Clinical trials are research studies intended to determine whether a treatment or device is safe and efficacious for human use. These studies must follow strict scientific research standards (e.g., indication-specific endpoints, data collection, analytics) to ensure patients are protected and results are reliable.

In 2018, FACT published four standards (two in collaboration with JAICE) that gene therapy developers can reference in the context of clinical trials. These standards are: FACT Standards for Immune Effector Cells (First Edition, Version 1.1); FACT Immune Effector Cells Accreditation Manual (First Edition, Version 1.1); FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration (Seventh Edition); and FACT-JACIE Hematopoietic Cellular Therapy Accreditation Manual (Seventh Edition). These documentary standards are regularly updated with new editions.

FIVE IMPORTANT GENE THERAPY STANDARDS CURRENTLY IN DEVELOPMENT

1. NIST Gene Editing Consortium

A prominent standards development effort is being led by NIST through the NIST Gene Editing Consortium (https://www.nist.gov/programs-projects/nist-genome-editing-consortium). The goal of this effort is to advance the rapidly developing field of genome editing by involving experts in an ongoing consortium to develop standards for terminology, specificity measurements, and data and metadata. Though much of the current effort is only indirectly related to the gene therapy field, one directly related project is the "Feasibility study for standards for pre-existing immunity to AAV vectors."

2. NIBSC/WHO Lentiviral Vector Copy Number Standard

Gene therapy is a rapidly evolving field. A prerequisite for producing gene therapy products is ensuring their quality and safety. This requires appropriately controlled and standardized production and testing procedures that result in consistent safety and efficacy. Assuring the quality and safety of lentiviral-based gene therapy products in particular presents a substantial challenge because they are cell-based multi-gene products that include viral and therapeutic proteins as well as modified cells. In addition to the continuous refinement of a product, changes in production sites and manufacturing processes have become increasingly common, posing challenges to developers regarding reproducibility and comparability of results. The paper describing the NIBSC/WHO Lentiviral Vector Copy Number Standard discusses the concept of developing a first World Health Organization International Standard, suitable for the standardization of assays and enabling comparison of cross-trial and cross-manufacturing results for this important vector platform. The standard will be expected to optimize the development of gene therapy medicinal products, which is especially important given the usually orphan nature of the diseases to be treated, which naturally hamper reproducibility and comparability of results.

Links to further information:

NIBSC Gene Therapy page:

https://www.nibsc.org/science_and_research/advanced_therapies/gene_therapy.aspx

Publication on development of the standard:

https://www.liebertpub.com/doi/full/10.1089/hgtb.2017.078

Collaborative Study report: *https://www.who.int/biologicals/expert_committee/BS.2019.2373_Lentiviral_vector_IS_Study_Report_final.pdf*

3. ISBio Lentiviral Vector Reference Material Project

The International Society for BioProcess Technology (ISBio) has organized a consortium of stakeholders with the goal of producing a reference material for use in analytical methods used to characterize lentiviral products. This effort is currently underway and information relating to the effort can be found here: *https://isbiotech.org/ReferenceMaterials/lentivirus-home.html*

4. USP Standard Development for Gene Therapy

Over the last few years, USP has engaged with stakeholders, regulatory authorities, and government scientists to explore the development of new standards for the growing field of gene therapy. In early 2019, USP held a roundtable with representatives from industry and regulatory agencies to explore opportunities for the development of standards to support advancement of AAV-based gene therapies. The goal of the meeting was to facilitate a robust discussion on possible ways that documentary and performance standards could help standardize methods for assessing the quality of both raw materials and AAV drug substance. The roundtable participants shared challenges and discussed the need for best practices and methods, in addition to physical reference standards that could be made available through USP. The outcome of this and other more recent interactions is a list of AAV standards that USP is looking to develop, either internally or with collaborators, to aid developers of AAV products in harmonizing and standardizing their practices, methods, and materials. The list, as of early 2020, is as follows:

- Existing reference materials—new collaborative study in kit form with primers/probes, standards and with accompanying methods for quantitation and infectious titer (supply cells)
- AAV9 as a new reference material—could be produced for a platform program
- Empty and full AAV capsid preparation
- AAV plasmid standards with multiple PCR targets
- Raw materials standards (plasmid DNA), possibly both best practices and reference standards for quality of these materials.

5. Plasmid DNA Expert Panel

USP is also forming an expert panel, under the guidance of the new Advanced Therapies Expert Committee, to develop a documentary standard for the use of plasmid DNA in the manufacturing of advanced therapies.

The landing page for information relating to USP's development activities for advanced therapies can be found here: *https://www.usp.org/biologics/cell-tissue-standards*

DEVELOPING SUPPORTIVE STANDARDS IN GENE THERAPY

Supportive standards are not developed specifically for the gene therapy sector, but are applicable to one or more of these sectors. They often occur as part of new standard development and cover the same application areas as sector-specific standards. As a result, they can be used directly in each sector or as foundations for creating sector-specific standards. As the efforts to develop standards for regenerative medicine continue, more supportive standards will be identified and categorized in the aforementioned The Regenerative Medicine Standards Landscape.

NON-DOCUMENTARY STANDARDS IN GENE THERAPY

In addition to documentary standards, multiple non-documentary reference standards have been, or are being, developed that can be useful for developers of gene therapy. Groups such as ATCC are working to identify and distribute the most impactful and critical reference standards needed by the gene therapy community. Examples include ATCC Recombinant Adeno-Associated Virus 2 reference standard material (AAV2-RSM) and ATCC Recombinant Adeno-Associated Virus 8 reference standard material (AAV8-RSM).

In addition, the reference material development process is being supported by a number of organizations. It is critical to bring the community of stakeholders together to identify and discuss needed reference material for industry use (e.g., vectors, genomic DNA, cell cultures, serums) and then to determine if a given material meets the criteria for development. Key questions often asked include:

- Do existing standards or reference materials address the need at hand?
- Is the proposed material market-relevant?
- Are stakeholders committed to advancing the reference material? The community introduces the concept to a reference material development organization or group for consideration (which varies in terms of formality required).

NEEDS AND GAPS FOR STANDARDS IN GENE THERAPY BY FUNCTIONAL AREA

Variations in manufacturing, measurement, and analytical techniques across developers of experimental gene therapy products cause difficulties for evaluating product quality and safety and addressing the impact of manufacturing changes or innovations. A common set of standards in gene therapy will advance development of treatments beyond the realm of clinical trials, to safe approved treatments for genetic diseases and syndromes. The gene therapy sector's overall needs for standards development are summarized below by functional area:

- Reference materials for analytical testing
- Analytical and testing methodologies needs — identify more consistent methods for cell counting in gene therapy to reduce disparities in evaluation, standardized methods for evaluating endogenous chimeric antigen receptor T-cells (CAR-T), and consistent reporting methods and requirements to use for vector genome quantification
- Product quality and characterization needs — revisit existing standards for replication competent retrovirus/lentivirus testing, standardize methods to assess product activity or comparability of gene therapy products, and refine and clarify release criteria to ensure that products are effective and reliable
- Preclinical study needs—identify and standardize the methodologies used for collecting and evaluating biodistribution data and identify ways for studies to consistently validate their methodology to speed up approvals.
- Clinical trial standards needs

An overview of standards that are needed is provided for each of the regenerative medicine sectors in the Needed Standards Report, which is a component of the Regenerative Medicine Landscape Report. This report is a useful reference for SDOs, researchers, industry members, and other stakeholders as they work to bring forward therapies from bench to bedside. Regular updates to the report will help to ensure that the report remains comprehensive and will enable its use in identifying emerging standards needs in the field.

Implementing Standards in Gene Therapy Manufacturing

STANDARDS BASED ON INTENDED USE

Ways to implement standards based on intended use include:

- Defining the standards landscape
- Allowing stakeholders to more easily identify gaps and ways to move the field forward Coordinate and support standard development
- Driving efficiency and allow stakeholders from across the regenerative medicine community to make their voices heard
- Educating and build awareness of standard
- Encouraging adoption of the standard and help stakeholders understand the benefits the standard can bring their organization
- Prioritizing needed standards
- Allowing energy to be focused on the standards that will have the greatest impact
- Conducting feasibility assessment
- Ensuring that the standards selected are scientifically ready for development and likely to be adopted by the regenerative medicine community
- Outlining Development Process, Post-Development Process, Pre-Development Process
- Distributing educational materials that convey benefits of a specific standard, stakeholders impacted, relevant regenerative medicine sectors, and product development processes, meetings and conferences.

Table 2-2. *Gene Therapy Standards Addressing Bioprocessing and Production*

Standard Number	SDO*	Standard Name	Publication Status
EP 5.2.12*	EDQM	Raw materials of biological origin for the production of cell-based and gene therapy medicinal products	Published 2017
EP 5.14*	EDQM	Gene transfer medicinal products for human use	Published 2008
N/A*	FACT	FACT Standards for Immune Effector Cells (First Edition, Version 1.1)	Published 2018
N/A	FACT	FACT Immune Effector Cells Accreditation Manual (First Edition, Version 1.1)	Published 2018
N/A*	FACT	FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration (Seventh Edition)	Published 2018
N/A*	FACT	FACT-JACIE Hematopoietic Cellular Therapy Accreditation Manual (Seventh Edition)	Published 2018
ISO/PWI 20389*	ISO	Collection, processing, conserving, and transportation technology criteria for human genetic resources	In development
ISO/DI 20688-1*	ISO	Biotechnology—Nucleic acid synthesis—Part 1: General definitions and requirements for the production and quality control of synthesized oligonucleotides	In development
	ISO	Biotechnology—Nucleic acid synthesis—Part 1: General definitions and requirements for the production and quality control of synthesized gene fragments, genes, and genomes	In development
USP <1047>**	USP	Gene Therapy Products	Published 2011, revised 2020

*Standards Development Organization **Indications standards that apply to multiple standards application areas or sectors

GENE THERAPY BIOPROCESSING AND PRODUCTION STANDARDS FOR MANUFACTURING

Bioprocessing involves the design and development of processes, materials, and equipment for manufacturing products from raw/ancillary biological materials (with appropriate procedures for characterization or starting materials such as cells, gene therapy vectors, and biomaterials).

Table 2 lists gene therapy standards related to bioprocessing and production.

The 21st Century Cures Act—signed into law in December 2016—directs the U.S. Food and Drug Administration (FDA), in consultation with the National Institute of Standards and Technology (NIST) and industry stakeholders, to "coordinate and prioritize the development of standards and consensus definition of terms... [that] support, through regulatory predictability, the development, evaluation, and review of regenerative medicine therapies and regenerative advanced therapies." Gene therapies present complex challenges related to product testing, scientific protocols, product quality and specifications, performance characteristics, and compliance criteria. The FDA assigned Nexight and SCB the role of developing the landscape of existing standards relevant to regenerative medicine, a task not previously undertaken for this field because of its novelty and the relative lack standards specific to regenerative medicine therapies.

Standards in Gene Therapy and Regulatory Approval

FDA'S INFORMAL RECOGNITION OF VOLUNTARY CONSENSUS STANDARDS

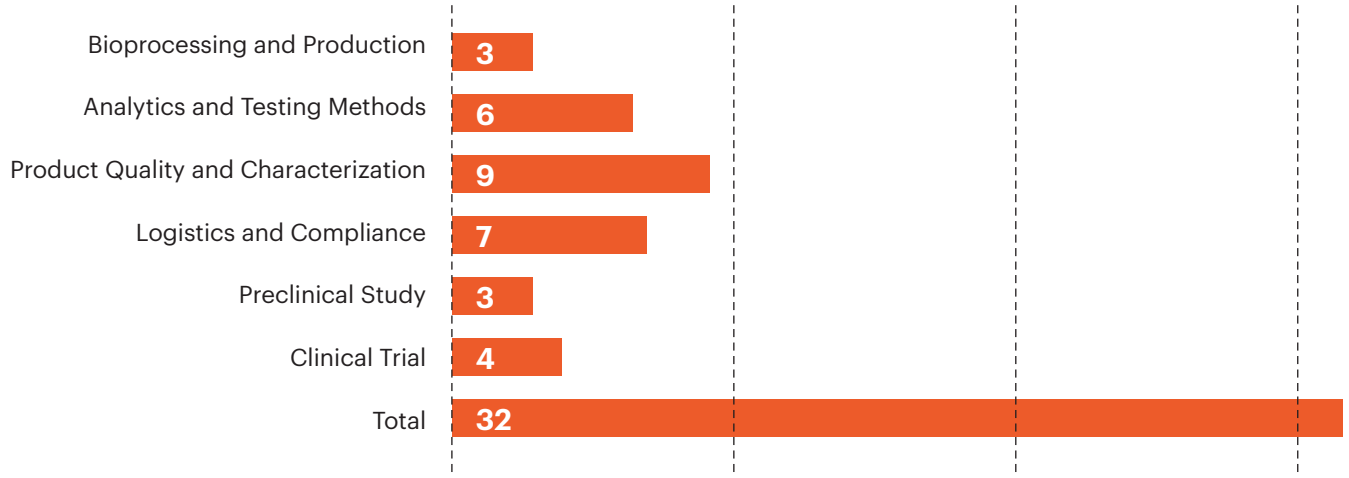
Voluntary consensus standards can be defined as standards developed by voluntary consensus standards bodies. The Food and Drug Administration Modernization Act of 1997 (FDAMA) (Pub. L. No. 105-115) and the 21st Century Cures Act of 2016 (Pub. L. No. 114-255) amended section 514(c) of the Federal Food, Drug, and Cosmetic Act (FDC Act) to require FDA recognition of voluntary consensus standards. FDA has used such standards to develop and/or evaluate performance characteristics of dosage forms, testing methodologies, manufacturing practices, product standards, scientific protocols, compliance criteria, ingredient specifications, labeling of drug products, and other technical or policy criteria.

In addition, FDA's Center for Drug Evaluation and Research (CDER) has drafted a document titled CDER's Program for the Recognition of Voluntary Consensus Standards Related to Pharmaceutical Quality Guidance for Industry. This guidance describes a proposed program at CDER to make public a comprehensive listing of informally recognized voluntary consensus standards related to pharmaceutical quality. The program, once established, will facilitate submissions by external stakeholders and CDER staff proposing voluntary consensus standards related to pharmaceutical quality for informal recognition. CDER believes that this informal program, which is different than the formal recognition standards program in FDA's Center for Devices and Radiological Health, will help promote innovation in pharmaceutical development and manufacturing and streamline the compilation and assessment of marketing applications for products regulated by CDER. It should be noted, however, that even if an applicant decides to use one of CDER's informally recognized voluntary standards, CDER may request that the applicant provide additional information to support an Investigational New Drug (IND) application or a marketing application. In addition, the applicant's use of an informally recognized consensus standard will be strictly voluntary. CDER has issued this draft guidance to obtain public comments on the proposed program. After CDER considers submitted comments, CDER will establish this program and describe it by publishing a final guidance. Thus, though not yet formalized, this draft guidance, when finalized, will comprehensively represent the current thinking of the Food and Drug Administration.

FDA'S FORMAL RECOGNITION OF STANDARDS

In addition to informal recognition, FDA also issues formal recognition of standards. FDA recognizes consensus standards are standards that FDA has vetted

Figure 2-2. *Gene Therapy Standards Needs by Functional Area*



source: Standards Coordinating Body; Needed Standards Report; December 2020

and determined are appropriate to support clearance or approval of a device. The purpose of FDA's formal recognition of consensus standards is to streamline the premarket review process. This formal recognition allows companies to submit a declaration of conformity with a recognized standard in a premarket application, rather than submit complete data and test reports demonstrating conformity with a standard.

FDA maintains a formal database of recognized consensus standards. This database consists of national and international standards recognized by FDA to which manufacturers can declare conformity and is a component of the information that regulators can use to make an appropriate decision regarding the clearance or approval of a submission.

In the Jan 2020 FDA Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)—Guidance for Industry, FDA recognizes 3 types of reference standards: 1) Certified reference standards (e.g., USP compendial standards); 2) Commercially supplied reference standards obtained from a reputable commercial source; and/or 3) Other materials of documented purity, custom-synthesized by an analytical laboratory or other noncommercial establishment.

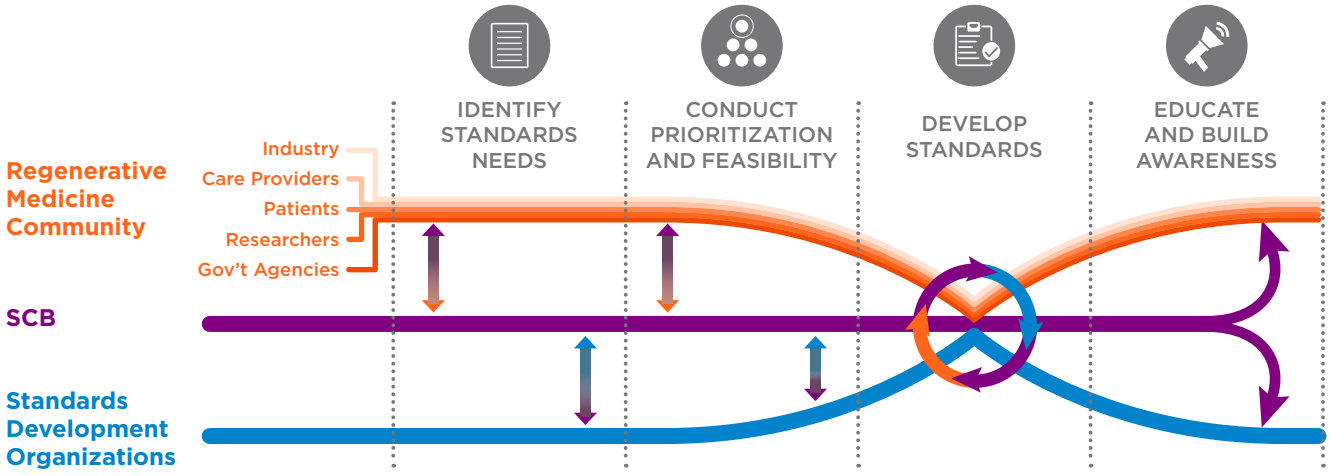
Standards Coordinating Body—Coordination, Community Engagement, and Education

The rapid development of gene therapy products – 373 products in clinical trials, and 536 developers worldwide (ARM Sector data Feb 2021)—presents challenges and opportunities for standards development. The goal of SCB was to establish processes that create an effective network of regenerative medicine community stakeholders to coordinate and complement current standards development processes. It is widely accepted that broad stakeholder involvement is necessary to ensure that the establishment of any new standards and/or reference materials provide the greatest benefits to the gene therapy community. SCB approaches these challenges by focusing on Coordination, Engagement, and Education.

Coordination: A critical first step was to identify and document the standards needed in regenerative medicine. SCB has accomplished documenting the needs in its Needed Standards Report updated in December of 2020.

In that report, 32 areas were identified as important for Gene Therapy (Figure 2).

Figure 2-4. *Standards Coordinating Body's Approach to Accelerating Standards Development*



source: Standards Coordinating Body

Figure 2-3. *Community Prioritization of Standards Needs*

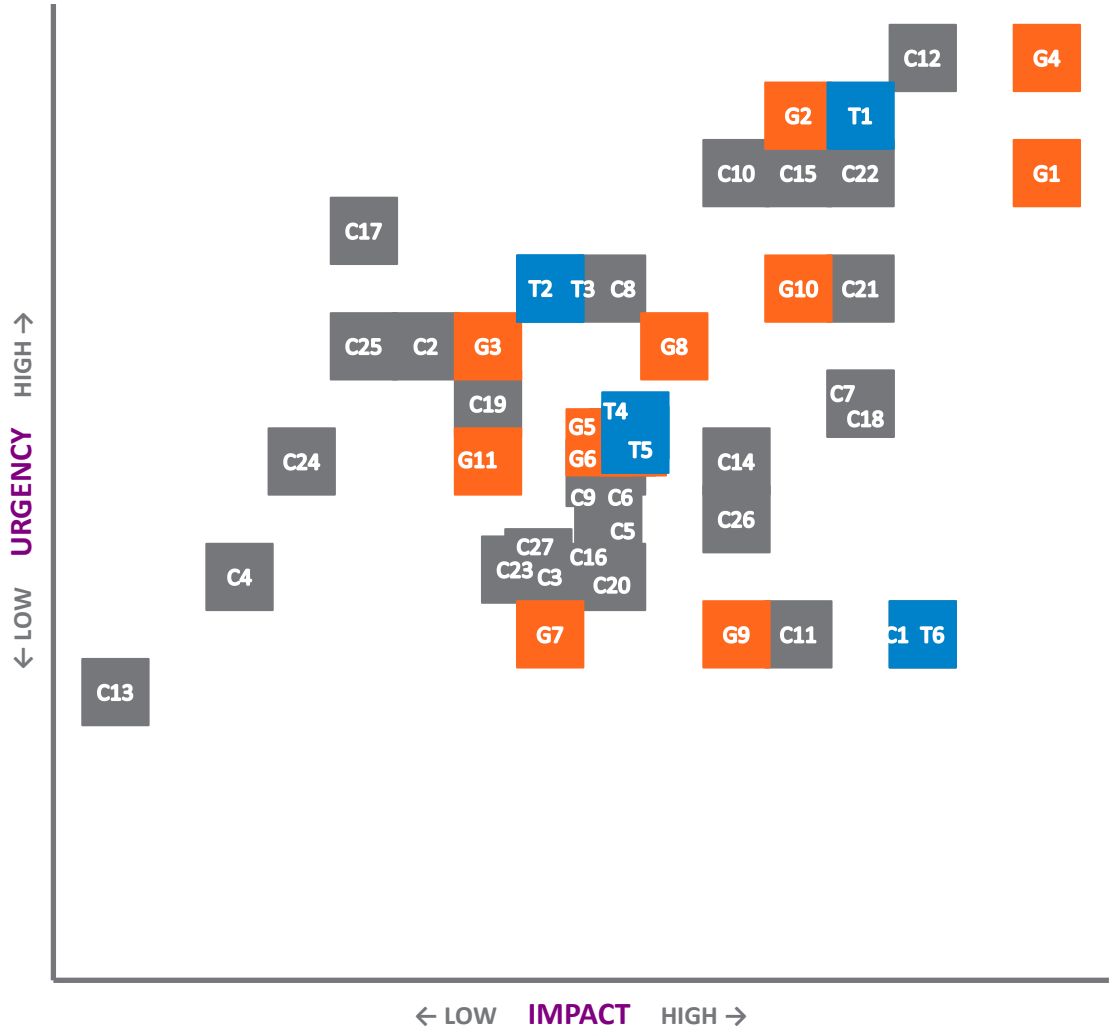


Figure from The Standards Coordinating Body Needed Standards Report; December 2020.

The areas for standards development are identified and then ranked for both urgency and impact. (G = Gene Therapy Specific, C = Cell Therapy Specific, T = Tissue Engineering Specific).

The identified areas for standards development are identified and then ranked for both urgency and impact. (Figure 3).

Engagement: SCB uses a process (Figure 3) to then both prioritize and perform a Feasibility Assessment to determine if a standard is ready to progress to formal development.

In general, operational and technical feasibilities are key to standards development. These can be defined as:

Operational Feasibility:

- Are there sufficient interested parties willing to commit individuals with appropriate training and resources to provide scientific and experiential knowledge to the project?
- Does SCB have sufficient staffing to perform the program management function for the project?
- Are sufficient funds available immediately (or likely to be obtainable) to adequately support the standards development?
- Does an existing SDO(s) have a committee project that is a good fit for the standards development?

Technical Feasibility:

- Documentary Standard
- Are there applicable scientific/engineering knowledge in the field to provide sufficient information to inform development of the standard(s) (i.e., the concept is not completely science fiction)?
- Does the documentary standard require round-robin testing?
- Standards Reference Material
- Do potential materials that could easily be adapted for a standards reference material (SRM) exist, or is discovery science needed to get there?
- Do manufacturing methods exist to create sufficient quantities of the SRM to generate a stockpile?
- Do testing methods that could be directly applied to the SRM already exist?
- Does a documentary standard for testing the SRM need to be developed concurrent with development of the SRM?

After the feasibility assessment concludes that a standard is ready for development, formal working groups are established to address that standard's needs. As standards development can take months to years to occur depending on the complexity and nature of the project, SCB's facilitated process ensures that development occurs as efficiently and quickly as possible.

Education and Implementation—SCB recognizes that access to information and help with implementation of standards are important for the industry. To help the industry, SCB has developed an online portal for searching for standards and uses its website for timely and up to date information.

It is through the processes of: Coordination—community and stakeholder engagement (identification of standards), Engagement—facilitated Working Groups (feasibility and development), and Education (outreach and implementation) that SCB works to accelerate standards development.

Chapter 3

Generation of a Quality Target Product Profile

Alliance *for* Regenerative Medicine

NIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 3 | Contents

Introduction

Cell and gene therapies cover a broad spectrum of therapies, from stem cell transplants and lymphocyte transplants to *ex vivo* and *in vivo* gene therapies and gene editing. Many of these therapies are moving from academic settings to biopharmaceutical-focused companies, and as these therapies enter late-phase clinical trials and commercialization, their manufacturing processes are being improved to increase reproducibility and robustness and to reduce the cost of manufacturing. These technologies have the capacity to revolutionize how we treat and potentially cure disease. However, because they are complex biologics, much of the development of gene therapy products will follow CMC (Chemistry, Manufacturing, and Control) aspects already established for therapeutic proteins.

Conventional biologics and gene therapies are similar with respect to replacing missing proteins or increasing the level of a protein for disorders where insufficient amounts of a given protein are produced. The primary difference between biologics and gene therapies is that a biologic is repeatedly given to the patient as a parenteral product. In the case of conventional biologics, a production cell line, such as Chinese Hamster Ovarian (CHO) or SP2/0 myeloma, is genetically modified to secret the protein of interest (e.g., monoclonal antibodies or therapeutic proteins). The majority of these proteins are

Key Terminology and Definitions

Quality Target Product Profile (QTPP)

A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy.[1] Because the QTPP defines essential criteria that relate to the development of a new product, it is critical that the development team clearly defines the quality attributes of the product. In the pharmaceutical industry, these product attributes are referred to as the target product profile (TPP). Ideally, the TPP describes how the end user will utilize the product, and includes the clinical delivery of the drug product. For the company, the TPP will help to identify project goals and potential risks.[2] The terms TPP and QTPP are sometimes used interchangeably. In practice, the TPP is broader in scope and typically includes some items that are absent from the QTPP, including marketing inputs (ie, desired claims) and clinical inputs. The QTPP is meant to identify, define, and justify quality characteristics so as to ensure safety and efficacy expectations established by the TPP. The focus is on the QTPP for the purposes of the A-Gene document.

Critical Quality Attribute (CQA)

The QTPP facilitates the identification of CQAs, which are physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality (ICH Q8.[1] Examples of CQAs of AAV products include physical viral titer (i.e., viral genomes or full AAV particles per unit volume), capsid content (i.e., percentage of empty capsids), potency, purity, and product stability. Preliminary CQAs are defined early in phase 1 of the drug development lifecycle using risk assessments. Preliminary CQAs are further investigated using design of experiments (DOEs)/process experience and continue to be refined during the early phase of the development lifecycle based on enhanced product knowledge and early clinical experience. CQAs serve as the basis to identify critical process parameters (CPPs) and facilitate development of the design space.[3]

continued on next page

Key Terminology and Definitions

continued from previous page

Critical Process Parameter (CPP)
A process parameter whose variability has an impact on a CQA and therefore should be monitored or controlled to ensure the process produces the desired quality (ICH Q8). Examples of CPPs include temperature, pH, cooling rate, rotation speed, etc. Because CPPs impact the CQA, they must be monitored or controlled via a well-designed process to enable early and accurate detection of deviations outside acceptable limits that will impact product quality. Not all process parameters have the same impact on CQAs; some may have a greater impact than others. As a result, it is important to prioritize CPPs over other process parameters as they will have the most impact. Of all process parameters, CPPs must be the most rigorously controlled. Critical material attributes (CMAs) are often used when determining CPPs and their impact on CQAs. A non-CPP is a process parameter whose variability has no significant impact on a CQA and therefore does not have to be controlled to ensure the process produces the desired quality.

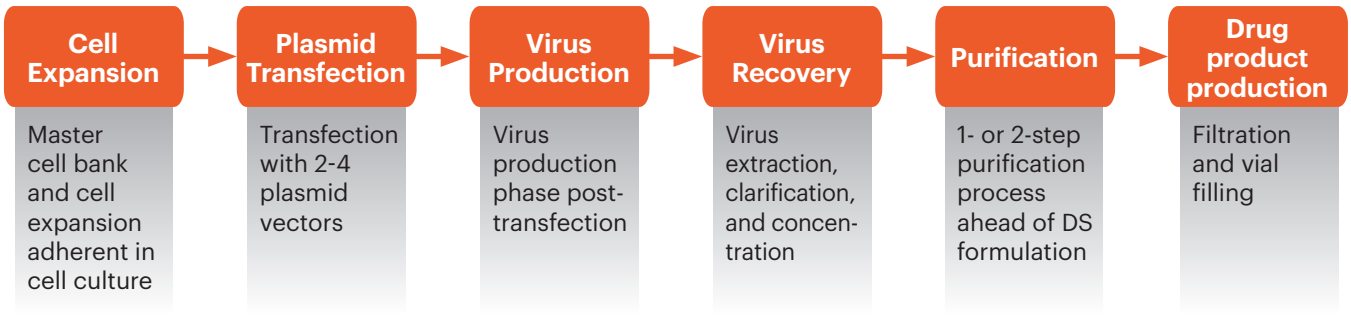
Critical Material Attribute (CMA)
A physical, chemical, biological, or microbiological property or characteristic of an input material that should be within an appropriate limit, range, or distribution to ensure the desired quality of output material.[1]

Key Process Parameters (KPPs)
Parameters of the manufacturing process that may not be directly linked to critical product quality attributes but need to be tightly controlled to ensure process consistency as it relates to product quality.[1]

Risk Assessment
Risk assessment is a valuable science-based process that can aid in identifying which material attributes and process parameters potentially have an effect on product CQAs. Risk assessment is typically performed early in the pharmaceutical development process and is repeated as more information becomes available and greater knowledge is obtained. It can be defined as "an assessment of the ability of the process to reliably produce a product of the intended quality (e.g., the performance of the manufacturing process under different operating conditions, at different scales or with different equipment). An understanding of process robustness, or process capability, can be useful in risk assessment and risk reduction and to support future manufacturing and process improvement, especially in conjunction with the use of risk management tools." Examples of common risk assessment tools include parameter risk assessments (PRA) and failure mode and effects analysis (FMEA), which are covered in detail in Chapter 4 (see also ICH Q9 Quality Risk Management).[1] Chapter 4 contains a more in-depth discussion of risk assessment approaches.

Quality by Design (QbD)
A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.[1]

Figure 3-1. *Overview of Gene Therapy Manufacturing*



For additional details on upstream and downstream manufacturing, please refer to Chapter 5.

produced, purified, and formulated with well-defined and robust processes. Many of these processes have been optimized through the application of QbD and require well-defined processes to ensure equivalent quality attributes between lots and manufacturing sites. Similarly, the FDA requires that gene therapy manufacturing processes be well characterized.[4]

The manufacturing of AAV often differs from that of conventional therapeutic proteins; additional critical manufacturing steps are required for the expression and assembly of viral vector components (Figure 1). In particular, AAV therapeutics are generally produced from a transient transfection of cells and do not derive from a stably transfected cell line. This requires the design and production of plasmids that encode the viral vector capsid, enzymes required for replication, and the transgene (sequence encoding the protein of interest). Once the appropriate plasmids have been synthesized, they are used to produce bacterial cell banks that will produce the plasmids required for producing the viral vector. The production of the plasmids can be considered as precursor starting materials required for the production of the viral vector. Viral vectors can be made using transient production by transfecting HEK293 cells with the appropriate plasmids. It is feasible that HEK293 cells could be engineered to stably express the capsid and adenovirus helper components, thus enabling a single-plasmid transfection approach. Alternative production systems include insect cells/baculovirus systems, producer cell lines in combination with adeno- or HSV-helper viruses, or upcoming helper virus–free producer cell systems.

Unique Challenges

Gene therapies are complex biologics that pose a challenge to the currently available analytic techniques for full and comprehensive characterization when compared to a monoclonal antibody. Rather than the repeated administration of a well-characterized therapeutic protein to the patient, the patient's own cells produce the missing protein or express the appropriate receptors for the desired biological function with gene therapy products. Plasmids, viral vectors, or mRNA are used to introduce the genetic material required to modify these cells. Plasmids can be introduced directly into cells using DNA nanoparticles or used as the precursor genetic material required for the production of viral vectors. In either case, QbD principles and process optimization can be applied equally to manufacturing of plasmids and viral vectors and to modified cells produced for the *ex vivo* autologous therapies (e.g., CAR-T therapies and therapies for hematological disorders). Additionally, QbD can be applied to *in vivo* or *in situ* gene therapies using lentiviral and AAV vectors, as well as nonviral vectors. The following discussion will focus on the application of QbD principles to AAV vectors.

Ensuring the process is capable and in-control is essential. Many of these complex processes are difficult to define and lack the forward planning typically deployed in the development and registration of a commercial product. Though the industry has achieved significant milestones, the initial products required additional efforts, resources, and time to retrospectively address

concerns around the quality of the material inputs, testing and characterization, quality systems, and the development of robust processes. As the gene therapy industry matures, the speed to clinical commercialization must be tempered with the appropriate development of these products.

It should be noted that though the FDA is flexible with respect to early-stage cell and gene therapies for unmet medical needs, prior to initiating pivotal clinical studies the sponsor must demonstrate that the product in development includes a solid commercial plan for meeting demands. While a product may be developed under accelerated timelines, the sponsor is expected to apply best practices in order to ensure safety and efficacy. The manufacturing process should be robust and sufficiently scaled to meet the market demand with minimal major changes during clinical development, especially during the pivotal and post-pivotal stages.

Often during DOE studies, following a process and product risk assessment, some attributes may be designated as CQAs but are adequately controlled by the process. Thus, it is not appropriate to have tests for these CQAs on the control system. However, agencies may expect characterization data to show that the applicant has evaluated these CQAs and understands the risk. An example in AAV-derived gene therapy is certain capsid post-translational modifications such as deamidation that can severely impact AAV infectivity in hepatocytes. For an in-depth discussion of risk assessment, please refer to Chapter.[4]

Table 3-1. *Comparison of Empirical vs QbD Approach*[6]

Aspect	Minimal Approaches (Empirical)	Enhanced QbD Approaches
Overall pharmaceutical development	• Mainly empirical • Developmental research often conducted with one variable at a time	• Systematic, relating mechanistic understanding of material attributes and process parameters to drug product CQAs • Multivariate experiments to understand product and process • Utilization of process analytical tools (PATs)
Manufacturing process	• Fixed • Validation primarily based on initial full-scale batches • Focus on optimization and reproducibility	• Lifecycle approach to validation and ideally continuous process verification • Focus on control strategy and robustness • Use of statistical process control methods
Process controls	• In-process tests primarily for go/no-go decisions • Off-line analysis	• PATs utilized with appropriate feedback and feed-forward controls • Process operations tracked and trended to support continual improvement efforts after approval
Product specifications	• Primary means of control • Based on batch data available at time of registration	• Part of the overall quality control strategy • Based on desired product performance with relevant supportive data
Control strategy	• Drug product quality controlled primarily by intermediates (in-process materials) and end product testing	• Drug product quality ensured by risk-based control strategy for well understood product and process controls • Quality controls shifted upstream, with the possibility of real-time release testing or reduced end-product testing
Lifecycle management	• Reactive (i.e., problem solving and corrective action)	• Preventive action • Continual improvement facilitated

MANUFACTURING AND PRODUCT QUALITY CONSIDERATIONS

The sponsor of a product that receives an expedited drug development designation may need to pursue a more rapid manufacturing development program to accommodate the accelerated pace of the clinical program. The sponsor's product quality and CMC teams should initiate early communication with the FDA to ensure that the manufacturing development programs and timing of submissions meet the Agency's expectations for licensure or marketing approval. When sponsors receive an expedited drug development designation, they should be prepared to propose a commercial manufacturing program that will ensure availability of quality product at the time of approval. The proposal should consider estimated market demand and the commercial manufacturing development plan, as well as manufacturing facilities and a lifecycle approach to process validation. Additionally, the proposal should include a timeline for development of manufacturing capabilities with goals aligned to the clinical development program.[5]

A challenge for developing gene therapies for orphan products is that the small patient population requires only a limited number of batches, impacting the ability to generate sufficient data to develop acceptance criteria for CQAs and control strategies. The small patient population impacts the justification to expend significant resources for process definition and optimization.

QTPP and QbD

QbD ensures quality by defining the critical process controls and testing that guarantee that the drug substance and drug product will meet the attribute specifications that ensure safety and efficacy. Through the application of DOE techniques, defined upper and lower control limits for CPPs can be set to ensure lot-to-lot comparability. In contrast to the empirical approach of manufacturing a product and performing release testing, the quality of the product is considered at the earliest possible stage rather than simply testing the product for quality towards to the end of the process (Table 1). Although it is evolving, most of the gene therapy industry is at the minimal approach stage due to the challenges mentioned here.

The principles of QbD are built on the foundation of defining the QTPP, which defines the product's mode of action, patient population, and dosage and product format, as well as the preliminary CQAs (pCQAs) that ensure safety and efficacy. Developers of gene therapies leverage historic processes and use QTPP, CQAs, and process risk assessments to develop the preliminary process control strategy (pPCS). The pPCS is further refined through additional studies that use the DOE approach, which permits the systematic reduction of the degree of experimentation necessary to define and optimize the process control parameters, KPPs, and non-KPPs. Through DOE, control limits can be precisely defined with respect to a parameter's impact on quality, productivity, and yield. This increases the degree of freedom or "design space" and permits continuous refinement within the product's lifecycle.[1]

A product's development lifecycle is based on various elements, including, but not limited to, the following.[1]

- In-process controls
- Process design
- Environmental controls
- Process and analytical capability
- Raw material controls
- Drug substance and drug product specifications
- Demonstrated product stability
- Process monitoring controls
- Product comparability studies
- Process validation

DEVELOPING A TARGET PRODUCT PROFILE

The TPP is the foundation for the strategy that incorporates scientific, clinical, and market information necessary for an effective development plan. The TPP is a living document that is updated continually during the drug development process.[7] The initial version of the TPP should be created at the Pre-IND stage.

A typical biopharmaceutical TPP includes the following sections:[7]

- General product information: brief description of the genetic construct, product name (or designation), general information
- Mechanism of action: mechanism by which the product produces an effect on a living organism

- Clinical pharmacology: pharmacokinetic information, distribution, and pathways for transformation and safety
- Indication for use: target disease and population, dosage, and any relevant concerns with special populations
- Target manufacturing profile: formulation, shelf life, storage conditions, and delivery system
- Primary efficacy endpoints: primary clinical outcome measures; endpoints are usually proposed as three different scenarios: minimal, base, optimal
- Secondary efficacy endpoints: additional endpoints that are not required to be met in a clinical trial
- Expected safety outcomes: primary safety outcome measures
- Contraindications: known or expected contraindications
- Commercial landscape: description of the competitive landscape at expected launch time
- Regulatory: expected BLA/approval date

DEVELOPING A QTPP

The FDA states that the QTPP should consider the safety and efficacy of the product, giving consideration to dosage strength, delivery system, dosage form, container system, purity, stability, and sterility, and more (Table 2). The QTPP describes the design criteria for the product and should therefore form the basis for development of the CQAs, CPPs, and control strategy.[8]

IDENTIFICATION OF CQAS

The second foundational step to the QbD process is defining the CQAs that impart safety and efficacy. CQAs derived from the QTPP and/or prior knowledge are used to guide product and process development. These can be modified throughout the product development as knowledge and product understanding increase through experimentation and risk assessment.[9]

CQAs are defined during development and locked for the marketing application. Using risk assessment (see Chapter 4), the QbD process is used to identify CQAs with each parameter or attribute given a rating of criticality with respect to its impact on safety and efficacy. The criticality rating of each attribute will subsequently be assessed together with other knowledge, such as information on how the process impacts the identified CQAs and the detectability of the CQA to determine a control strategy.[6] Reassessment of the criticality will continue as part of life cycle management as more data are available or as related to changes of process or methods and the ability to remove or detect an attribute. Quality risk management (QRM, as described in ICH Q9) can be used to assess the risk and criticality of variability in the identified product quality attributes during manufacture, and the resulting analyses form the basis of setting manufacturing processing parameter controls. Knowledge management (as described in ICH Q10) is key to capturing and applying prior knowledge of biological, chemical, and manufacturing principles and experience to the establishment of the QTPP and also assessing the criticality of quality attributes and the degree to which a control strategy is needed.[7] Table 2 shows an example of a QTPP for a generic AAV gene therapy product.

Analytical Methods

QbD requires the use of robust and comprehensive analytical methods to confirm product identity and measure the impact of process-related impurities. Analytical methods cover a wide array of attributes and can be tested for by a number of mechanisms. In the generation of an AAV gene therapy, a developer needs to focus on product characterization, such as strength or genomic identity, as well as process related characterization, such as empty/full capsid ratio. Safety tests may evaluate purity, toxicity, and stability, as well as the physical, chemical, and biological characteristics to the product in order to ensure that the product falls within the predetermined acceptable limits of identity, potency, quality, and purity.

Analytical procedures are similar to the method lifecycle approach to process validation in that the procedure should be developed as early as possible during the lifecycle to minimize risk. Consistent methods should be used throughout the process development and manufacturing stages. However, as process knowledge and understanding increase, analytical assays may change or be replaced. A risk-based approach must be used to determine whether manufacturing process changes necessitate revalidation of the analytical procedures that are used. In addition, methods should be evaluated to

Table 3-2. *QTPP for a Generic AAV Gene Therapy Product*

ASSUMPTIONS
• This is a quality target product profile (QTPP) for a generic AAV-based gene therapy product produced by transfection of HEK293 cells and purified via column chromatography. • This QTPP is written to be adaptable for various routes of administration such as intravenous, ocular, and via the central nervous system. • The targets for general properties and product attributes based on these assumptions are listed below. However, this approach can be adapted for other products based on dose, target tissue, or route of administration. • Targets for product quality attributes are listed below but specifications will be assessed throughout the product lifecycle based on clinical, manufacturing, and assay experience.

GENERAL PROPERTIES	
Property	**Target**
Geographic Scope	Global: test to USP, EP, JP compendia where possible
Shelf life	≥1 to 2 years at intended storage conditions
Storage conditions	Formulation stable as a liquid at 5°C or alternately frozen at -20°C or ≤-65°C
Container	Selected to support product compatibility and stability and to ensure sterility
Delivery volume	Variable based on patient weight

BULK DRUG SUBSTANCE (DS) AND FINAL DRUG PRODUCT (DP) ATTRIBUTES			
Attribute Category	**Attribute**	**Bulk DS Target**	**Final DP Target**
Safety	Bioburden	Set limit to provide confirmation of process and facility controls	NA
Safety	Sterility	NA	Sterile
Safety	Endotoxin	Set limit to provide confirmation of process and facility controls with additional consideration to USP limit specific to route of administration	Below USP body weight-based limit for the route of administration
Content/strength	Appearance and particulates	Clear to slightly cloudy, colorless solution practically free of foreign particulates	
Content/strength	pH	Appropriate for the formulation to support product stability and compatible with the route of administration	
Content/strength	Osmolality	Appropriate for the formulation to support product stability and compatible with the route of administration	
Content/strength	Vector genome titer	Stable concentration that balances volumetric impact of sampling and FDP manufacturing requirements	Dose-specific concentration for ddPCR methods [10]
Content/strength	Potency	Relative to reference standard by *in vitro* transduction (disease relevant cell line preferred)	
Content/strength	Potency/infectious genome titer	Infectivity (e.g., TCID50), cell-based assay for GOI function, animal model demonstrating clinical benefit after transduction for early phase trials	
Identity	Capsid identity	Capsid serotype confirmed	

continued on next page

Table 3-2. *QTPP for a Generic AAV Gene Therapy Product*

continued from previous page

Identity	Genome identity	Genome identity confirmed via combination of transgene-specific ddPCR method and sequence analysis	Genome identity confirmed via transgene-specific ddPCR method Complete viral genome confirmation by sequencing recommended for DS only
Process impurities	Residual host-cell protein	Base limit on amount dosed in relevant toxicology studies	NA
Process impurities	Residual host-cell free DNA	Base limit on amount dosed in relevant toxicology studies[11, 12]	NA
Process impurities	Residual host-cell packaged non-target DNA	Base limit on amount dosed in relevant toxicology studies[11, 12]	
Process impurities	Residual plasmid DNA	Base limit on amount dosed in relevant toxicology studies	NA
Process impurities	Residual cell culture media components	Base limit on supplier safety data or other available literature	NA
Process impurities	Residual transfection reagent	Base limit on supplier safety data or other available literature	NA
Process impurities	Residual chromatography ligand	Base limit on supplier safety data or other available literature	NA
Process impurities	Replication-competent AAV	≤1 replication competent AAV/10^8 genome copies	NA
Purity	Capsid protein purity	≥90%	
Purity	Capsid protein ratio	Consistent across product lifecycle	NA
Purity	% full capsids	Specific target set for general alignment with relevant toxicology studies	NA
Purity	Capsid protein modification (deamidation, oxidation)	Within set limits to ensure functional consistency in manufactured products	NA
Purity	Total capsids	When considered with % full capsids, ensure the total number of viral particles delivered does not exceed amount delivered in relevant toxicology studies	NA
Purity	Subvisible particles	NA	Meets relevant USP chapter, USP<788>, or USP<789>
Purity	Aggregates	Acceptable level so as not to affect dose or concentration	Acceptable level so as not to affect dose or concentration

For additional information on the final DP target, please refer to Chapter 6.

determine their robustness and applicability to late-phase clinical trials. Here, we discuss a number of attributes a developer must consider in their development, and have included examples of analytical methods which may be used to measure these attributes. Additional attributes and assays are discussed throughout this A-Gene document.

STRENGTH AND DOSE

Measurement of strength/dose of purified AAV vectors typically includes assays to quantify the genome concentration, infectious concentration, and functional activity of the transgene (gene of interest).[13]

Vector Genome Titration Methods

Because vector genome (VG) is the key component involved in rendering the therapeutic effect in gene therapy, focus has been on developing fast, reliable, and robust methods for its titration. ddPCR is the gold standard method to titer VG AAV. It is important to note that attention must be paid when designing the region dedicated to performing the VG titer. qPCR standards preparation and stability are key factors to consider when attempting to increase inter-assay precision.[13]

Infectious Genome Titration: Biological Activity

Methods for quantifying rAAV infectious particles that can be applied to any vector, independently of the transgene product, and rely on the detection of rAAV genome replication in the presence of AAV rep-cap genes and adenovirus. In particular, two methods are used most frequently: replication center assay (RCA) and the 50% tissue culture infective dose (TCID50) assay, which involve inoculation of serial dilutions of the rAAV vector made on HeLa rep-cap-trans-complementing cells (i.e., HeLaRC32 or C12 cells) co-infected with adenovirus type 5. Vectors carrying reporter genes such as green fluorescent protein (GFP) can be easily titrated by flow cytometry in transduction units (TU/mL). For therapeutic AAV-based transgenes, infectious genome titers (IG) are expressed as infectious units (IU)[13] The development of relative infectivity assays is on the rise leading to more precise measurements of infectivity.[14]

Table 3-3. *Impurities Encountered in AAV Vector Manufacturing* [15]

Attribute Class	Quality Attribute
Microbiological quality	Sterility
	Bacterial endotoxin
	Adventitious agents
	Bacteriostasis, fungistasis, no inhibition of growth
	Mycoplasma, mycoplasmastasis
	Replication-competent AAV
Product-related impurities	Viral capsid ratio and purity
	Empty capsid to full capsid ratio
	Aggregation
Process-related impurities	Residual host cell DNA
	Residual plasmid DNA
	Residual affinity column ligand
	Host-cell protein
	Transfection agent (PEI)
	Lysis agent (detergent)
Product quality characteristics	Appearance
	Osmolality
	pH
Biological activity: content	Gene copy number
	Viral particle number
Biological activity: strength	In vitro infectivity
Biological Activity: Potency	Transgene expression
	Transgene product functional activity
Identity	Sequence identity
	Capsid identity

Table 3-4. *Example of release testing of AAV vector product*

Attribute	Assay	Method
Strength/Dose	VG titer	Spectroscopy/fluorimetry, qPCR/ddPCR
	Infectious Genome	Relative infectivity assay, TCID50
	Total vector particles	ELISA
	Activity (expression assay)	Cell-based assay
	Potency (functional activity)	Cell-based or *in vivo* assay
Identity	Genome DNA	Sequencing
	VP proteins	Western blot, mass spectroscopy
	Host-cell DNA	qPCR/ddPCR
	Helper plasmids or virus DNA	qPCR/ddPCR
	Residual reagents and raw materials (antibiotic resistance genes, detergent, benzonase, BSA, column leachables, etc)	ELISA, HPLC
	Percentage of empty capsid	qPCR/ELISA, HPLC, electron microscopy, analytical ultracentrifugation
Safety	Sterility	EP 2.6.1 USP
	Bacterial endotoxins	EP 2.6.14, USP
	Mycoplasma	EP 2.6.7
	Adventitious viruses	EP 2.6.16
	rcAAV	Cell-based assay
	Bioburden	Direct inoculation of sample into various media; confirmed by a bacteriostasis/ fungistasis test
	Vector aggregates	Dynamic light scattering, SEC-HPLC

PRODUCT-RELATED IMPURITIES

Product-related impurities remaining after purification include, but are not limited to, nucleic acids (encapsidated DNA, such as host-cell DNA, helper plasmid, or helper virus DNA, or host-cell DNA or plasmid DNA that is not encapsidated) and vector aggregates. Particles that are empty or have encapsidated nucleic acids fragments other than the therapeutic genome cassette are considered product-related impurities. These impurities are inactive and may represent safety concerns. Therefore, reliable methods for their measurement and characterization have been suggested.[13] CQAs are derived from QTPP considering the risk assessment for each attribute due to safety/impact and uncertainty. Table 3 is an example of product-related impurities in AAV product.

Process-related impurities may include proteins and nucleic acids derived from the production cells and viruses used to manufacture the therapeutic vectors; unpackaged viral vector genomes, and empty or partially filled capsids. In addition, residual cell culture components (e.g., antibiotics, supplements, inducers) may be present, as well as residual purification buffers, chromatography media ligands, centrifugation media, detergents, enzymes, inorganic salts, etc.

PARTICLE QUANTIFICATION: PERCENTAGE OF EMPTY CAPSIDS

Empty particles are undesired AAV products that are produced at a significant level during the biosynthesis of AAV vectors. Empty particles may represent up to 90% of vector preparations. In order to determine the ratio of full to empty particles in a single experiment, techniques such as spectrometry, HPLC, and electron microscopy have been proposed. For example, one spectrometric approach uses the absorbance ratio at 260 nm/ 280 nm to quantify the number of empty and full particles of AAV2; however, this approach requires purified material and is sensitive to impurities and buffer formulation. Some laboratories have already used HPLC as an analytical tool.[13] Currently, employing analytical ultracentrifugation (AUC) has proven one of the most consistent methods for full versus empty capsid analysis.[6]

ILLEGITIMATE ENCAPSIDATED DNA

During recombinant AAV production, viral capsids are known to package not only their genomes flanked by 2 ITR but also various DNA fragments. Several types of illegitimate DNA encapsidation, helper virus sequences including rep/cap sequences, DNA fragments from plasmids and cellular genome have been identified in purified AAV vector preparations.[11, 12] However, vector design and a good manufacturing process can reduce illegitimate encapsidated DNA, and this should be considered when discussing QTTP.

Conclusion

The compilation of all analytical methods covers the end-to end of a developer's quality control program in drug substance (DS) generation and will inform the parameters for release testing of the drug product (DP). Table 4 provides an example of release testing born from the combination of a number of product- and process-related analytical methods.

Assay validation is an important consideration. Assays that measure identity, strength, quality, purity, and potency of DS and DP must be validated to demonstrate that the analytical procedures and assays are adequate and meet standards of accuracy, sensitivity, specificity, and reproducibility. Further, it is important to consider phase-appropriate development of assays and note that assays may change throughout the product lifecycle as process and product knowledge improves. Finally, as technology advances, real-time release testing and PAT will improve the monitoring of product quality, efficacy, and safety while simultaneously shortening the manufacturing time and decreasing costs.

An enhanced, carefully designed approach to gene therapy development allows for the reduction of release tests. QbD allows for testing at the appropriate control point, which may be better as a critical in-process control with appropriate rejection or acceptance limits, rather than release testing.

Endnotes

1. *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Pharmaceutical development Q8(R2): step 4 version. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use website. https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf. Published August 2009. Accessed November 15, 2020.*
2. *Lambert WJ. Considerations in developing a target product profile for parenteral pharmaceutical products. AAPS PharmSciTech. 2010;11(3):1476-1481.*
3. *Kelly M. Implementing the principles of quality by design for early stage gene therapy products. Presented at: WCBO 2012, January 23-25, 2012.*
4. *Wright JF. Manufacturing and characterization of AAV-based vectors for use in clinical studies. Gene Ther. 20008;15(11):840-848.*
5. *US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: expedited programs for serious conditions – drugs and biologics. US FDA website. https://www.fda.gov/files/drugs/published/Expedited-Programs-for-Serious-Conditions-Drugs-and-Biologics.pdf. Published May 2014. Accessed November 15, 2020.*
6. *Burnham B, Nass S, Kong E, et al. Analytical ultracentrifugation as an approach to characterize recombinant adeno-associated viral vectors. Hum Gene Ther Methods. 2015;26(6):228-242.*
7. *US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: target product profile — a strategic development process tool (draft guidance). US FDA website. https://www.fda.gov/media/72566/download. Published March 2007. Accessed November 15, 2020.*
8. *European Biopharmaceutical Enterprises. Considerations in setting specifications. European Biopharmaceutical Enterprises website. https://www.ebe-biopharma.eu/wp-content/uploads/2017/04/ebe-concept-paper-%E2%80%93-considerations-in-setting-specifications.pdf. Published March 28, 2013. Accessed November 15, 2020.*
9. *US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs): guidance for industry. https://www.fda.gov/media/113760/download. Published January 2020. Accessed November 15, 2020.*
10. *Košir AB, Divieto C, Pavšič J, et al. Droplet volume variability as a critical factor for accuracy of absolute quantification using droplet digital PCR. Anal Bioanal Chem. 2017;409(28):6689-6697.*
11. *World Health Organization. Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology. World Health Organization website. https://www.who.int/biologicals/biotherapeutics/rDNA_DB_final_19_Nov_2013.pdf. Published 2013. Accessed November 15, 2020.*
12. *CMC Biotech Working Group. A-Mab: a case study in bioprocess development (v2.1). https://cdn.ymaws.com/www.casss.org/resource/resmgr/cmc_no_amer/cmc_amab_case_study/A-Mab_Case_Study_Version_2-1.pdf. CASSS website. Published October 30, 2009. Accessed November 15, 2020.*
13. *Dorange F, Le Bec C. Analytical approaches to characterize AAV vector production and purification: Advances and challenges. Cell Gene Ther Insights. 2018;4(2):119-129.*
14. *Li Z, Wu Z, Maekawa T, et al. Analytical technology used in the latest development of gene therapy candidates. Cell Gene Ther Insights. 2019;5(4):537-547.*
15. *Fraser Wright J. Product-related impurities in clinical-grade recombinant AAV vectors: characterization and risk assessment. Biomedicines. 2014;2(1):80-97.*

Chapter 4

Process Development Using Quality by Design (QbD) Principles

Alliance *for*
Regenerative Medicine

NIIMBL The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 4 | Contents

Chapter Summary

Process development aims to establish and characterize a manufacturing process that can be scaled up to commercial-size batches while continuing to yield a consistent, quality product. Various risk-based approaches are utilized to ensure that the process stays within appropriate limits and meets all safety and quality benchmarks. In this chapter, we discuss various aspects of QbD that are used to evaluate the process and mitigate risks, quality attributes risk assessment from the quality target product profile (QTPP), and parameter risk assessment (PRA), among others. Other important pieces embedded into QbD include process development considerations, scale-down models, process characterization, parameter criticality assessments, parameter classification assessments (PCA), associated control strategy for critical process parameters, and process analytical technologies. Process analytical technologies (PAT) have been supported by agencies to gain better process control, as PAT can provide real-time monitoring for important indicators for the process that could potentially impact the quality.

Key Points

- As many gene therapy products proceed toward late-stage development and BLA-enabling activities, it is going to be increasingly important to take lessons from biologics and vaccines in applying a systematic, risk-based approach for late-stage development.
- The goal of early process development is to establish a platform-relevant process for scalable and transferable process that allows characterization and proof-of-clinical-concept to be obtained within a reasonable amount of time. Later-stage development focuses on scale, optimization (productivity and yield), and final facility.
- Establishment of process performance requirements within the manufacturing process requires knowledge of a product's critical quality attributes (CQAs) and ultimately allows the robust, reproducible production of a quality product.
- Various risk-based approaches are used throughout late-stage development to understand and summarize risks for parameters in each unit operation. The follow-up process characterization could help to define the control of process parameters and the control of raw materials. Failure mode and effects analysis (FMEA) can then identify areas that require improvement to further reduce risk. This ensures that the process delivers a quality product in a reproducible process.
- The implementation of PAT into process development and GMP manufacturing is relatively recent and aims to obtain real-time information for process control.

Introduction

The goal of process development is to optimize a high-titer process and maintain product quality at an early stage, as well as to characterize a manufacturing process to yield a consistent, quality product at late-stage development. Quality-by-design (QbD) is one approach that places emphasis on the use of the product and understanding of the process to develop a robust process control strategy that is rooted in sound science and quality risk management. For a deeper discussion of QbD, please refer to Chapter 3. Establishment of process performance requirements within the manufacturing process requires knowledge of a product's critical quality attributes (CQAs) and ultimately allows the robust, reproducible production of a quality product. In this way, quality is not maintained through product testing; instead, quality is built into the entire process though rational, intentional design. Figure 1 illustrates the overall flow of the QbD approach.

THE QBD PROCESS

Various risk-based approaches are used throughout the manufacturing process to establish processing operations and define the control of critical quality attributes, the control of raw materials, and the areas for further investigation. Such assessments ensure that the process delivers a quality product in a reproducible process. A cross-functional team of subject matter experts conducts the risk assessments using scientific understanding and product knowledge to identify areas that pose a risk of failure to deliver quality product and product that satisfies the quality target product profile (QTPP), as described in Chapter 3. Such risk-based approaches include:

- **Critical quality attribute assessment (CQAA):** A CQAA is conducted to determine which quality attributes identified in the QTTP are potentially critical to product safety and efficacy. It forms the initial list of potential analytical method requirements and the starting point for process assessment. Over time, the CQA list may be updated based on clinical history, product characterization, and increased scientific understanding.

- **Parameter risk assessment (PRA):** A PRA is conducted to determine which parameters and associated materials have potential to affect the CQAs and thus provide a defined set of parameters that may pose critical risk to quality and process performance. A PRA is carried out using a cause and effect methodology to assess each parameter for its effect on each CQA. Scientific understanding is used to score the severity of each effect. During a PRA, it is important to assess the criticality of process materials to ensure that the parameter studies consider material variability. Failure to consider material variability during process characterization may result in process characterization that is specific to material lots and not necessarily reflective of the manufacturing process.

- **Process characterization (PC):** PRA can be used as part of input for PC. Certain low-risk parameters may need to be characterized for compliance. PC defines the list of high-risk parameters and materials that should be investigated further based on high severity scores determined during the PRA. Essentially, the PC results in a prospective plan of all experiments to be conducted during process characterization.

- **Parameter criticality assessment:** The PCA is a tool that is used after process characterization studies to define which process parameters are critical, key, or noncritical based on study data and additional operational information.

SIMILARITIES AND DIFFERENCES BETWEEN AAV PRODUCTS AND BIOLOGICS/VACCINES

Some of the approaches in developing AAV products are similar to those already in place for biologics and vaccine development. For example, regulatory requirements include process characterization, process performance qualification (PPQ) for the understanding of links between process and product quality, defining acceptable/normal operating ranges, and defining critical/key parameters to enable PPQ. In addition, making sure the processes to define process and analytical control strategies to ensure product quality meets the release

Figure 4-1. *Overview of the QbD Process*

Quality Target Product Profile (QTTP)	A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product. (ICH Q8 (R2))
Critical Quality Attribute (CQA)	The physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. (ICH Q8 (R2))
Parameter Risk Assessment (PRA)	A tool used by a cross-functional team of subject matter experts to step through each process unit operation, assessing the parameters and the material used with the purpose of identifying potential risks and scoring the risk
Process Characterization	Process characterization is the step to find out the proven acceptance range (PAR) for the studied parameters. A prospective plan derived from the high severity scores determined during the PRA, as well as any specific functional studies area owners determine are needed. The high-risk parameters and materials are identified in the PC for further study to determine the effect their variability may have on the ability to control the process and produce a quality product. Qualified scale-down model is prerequsite for process characterization and could also be included in PC.
Parameter Classification Assessment (PCA)	A tool used by a cross-functional team of subject matter experts after process characterization studies are completed to define, based on study data and additional operational information, which process parameters are critical, key, or noncritical.
Process and Analytical Control Strategy (P/ACS)	A planned set of controls, derived from current product and process understanding, that assures process performance and product quality. (ICH Q10). The controls can include parameters as inputs and attributes or indicators as outputs related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, the associated methods, and frequency of monitoring and control.
Process Performance Qualification (PPQ)	A series of process runs that combines the actual facility, utilities, equipment (each now qualified), and the trained personnel with the commercial manufacturing process, control procedures, and components to produce the commercial batches. A successful PPQ will confirm the process design and demonstrate that the commercial manufacturing process performs as expected.

criteria. Throughout the development of these three types of products, it is important to maintain a vigilant eye for comparability when implementing process changes. As in biologics and other manufacturing processes, it is critical to establish controls using good manufacturing practice to ensure the quality and safety of investigational drug products.[1]

However, challenges are associated with expedited development, and abbreviated approval pathways differ from those of biologics/vaccine development. For example, multiple manufacturing platforms (e.g., HEK293, HeLa, SF9) are available for AAV products, whereas the approach is more standardized for biologics (e.g., mainly on CHO for monoclonal antibodies) and vaccines. Thus, manufacturing processes are insufficiently defined compared to other well-established methods in biologics. This variance leads to the need for different control strategies that must be implemented for AAV compared to biologics and vaccines. Further, the full structure-function relationship of AAV products is not completely characterized, and the analytical methods are complex and potentially variable. Issues associated with AAV manufacturing include low productivity/limited material, complicated production systems, low volumes and concentrations, accelerated timelines, and limited understanding of mechanisms of action.

Many gene therapies target conditions for which there are high, urgent unmet medical needs. Such conditions may allow for expedited development pathways. QbD approaches, including process development and optimization, are critical to incorporate early during process development if a product is expected to achieve breakthrough designation from the U.S. Food and Drug Administration (FDA). Without early adoption of QbD principles, the process knowledge and design information that are needed to support BLA filing may not be available to support the aggressive regulatory timelines. A breakthrough therapy, fast track, priority review, and/or accelerated approval designation may allow BLA filing based on phase 2 data or a single phase 3 trial; however, a full Chemistry, Manufacturing, and Controls (CMC) package is still required, including process development and validation. This CMC package may be needed at least 3 to 5 years prior to when it would be for a traditional filing with such designations.

Opportunities for a risk-based approach in the development of AAV products include the following:

- **Process characterization:** risk assessments must be carried out to target the most critical parameters and limit the scope of experimentation.
- **Viral clearance:** robust risk assessment must be carried out for non-helper virus processes (Ph1-Ph3).
- **Starting materials (e.g., plasmids, helper viruses, etc.):** it is important to define a streamlined path to qualifying plasmid processes. It is also critical to understand the link between plasmid quality and vector process performance.

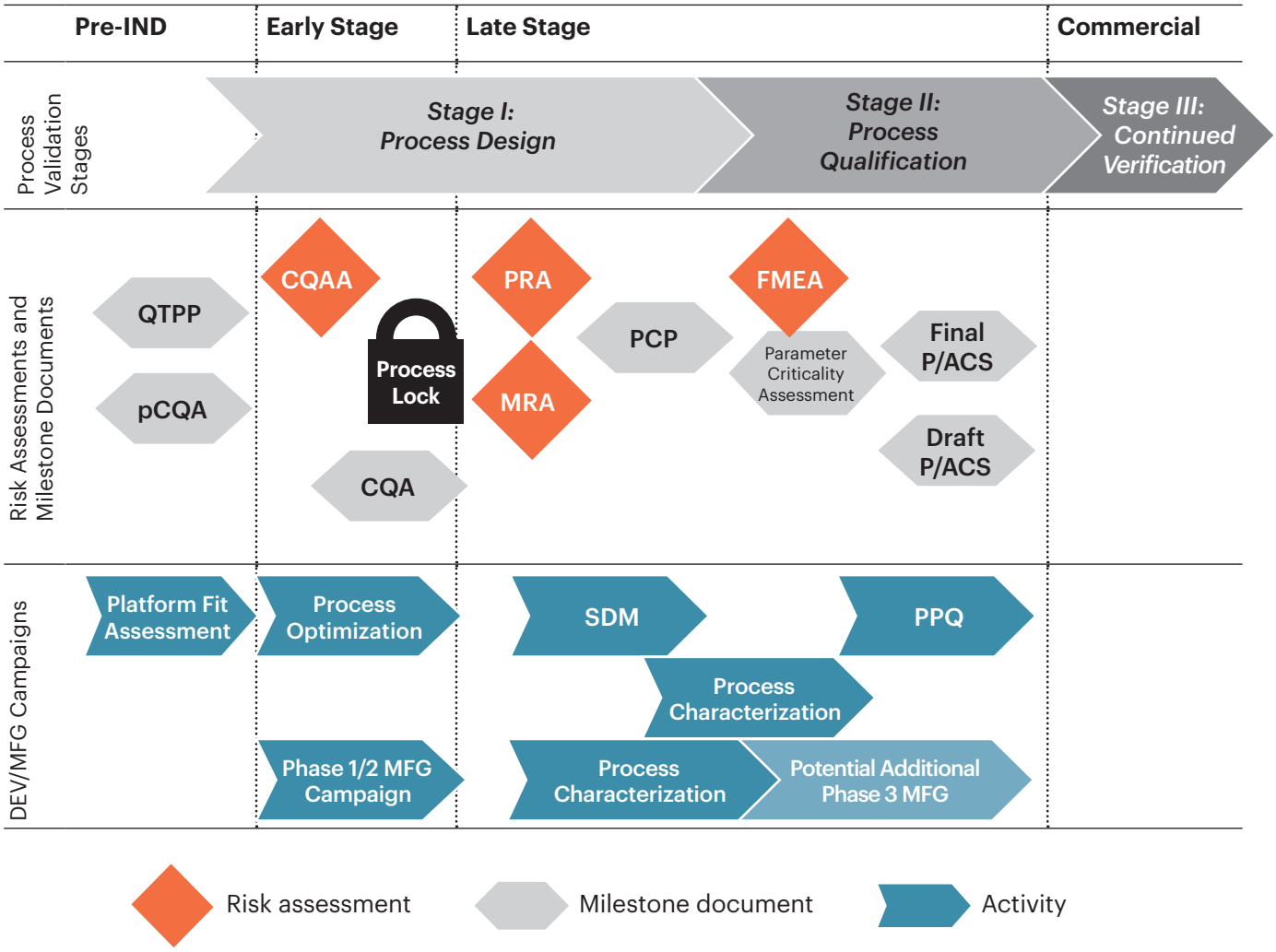
Importantly, in the development of assessment to only target most critical parameters, a greater risk approach can be used. A risk-based approach such as this could help to identify and therefore focus on the most critical parameters, while logging and monitoring uncertain risks or parameters. For example, a robust process that does not involve a helper virus may be used so that a risk assessment can be conducted in lieu of formal viral clearance studies during early stages of clinical development. While a comprehensive characterization of the plasmid processes is not necessary, it is important to streamline the path towards process characterization. It is perhaps less critical to characterize detailed operating ranges of the plasmid manufacturing process, but certainly more critical to conduct the development work to understand the link between variability in the plasmid product quality attributes and the vector process performance and product quality.

Overview of Risk-Based Strategy Timeline

Based on FDA guidance, process validation (defined as the collection and evaluation of data) involves three stages: process design, in which the commercial manufacturing process is defined based on knowledge gained through development and scale-up activities; process qualification, in which the process design is evaluated in order to determine whether the process allows reproducible commercial manufacturing; and continued process verification, in which routine production produces ongoing assurance that the process remains in a state of

Figure 4-2. *Risk-based Strategy for Late-Stage Development*[4]

IND = investigational new drug
QTTP = quality target product profile;
pCQA = potential critical quality
CQAA = critical quality attribute assessment;
CQA = critical quality attribute
PRA = parameter risk assessment;
MRA = material risk assessment;
PCP = process characterization plan; attribute;
FMEA = failure mode effect analysis
p/ACS = process and analytical control strategy
SDM = scale-down model
PPQ = process performance qualification



control. These stages allow the establishment of scientific evidence to show that a process is capable of delivering a consistent, quality product.[2]

Ultimately, a successful validation program requires information and knowledge from product and process development. In order to attain this, manufacturers must understand sources of variation, detect the presence and degree of variation, understand the impact of variation on the process and product attributes, and control the variation in a manner appropriate to the level of risk that it carries to the process and the product.[3] An overview of risk-based strategy for late-stage development for AAV products is shown in Figure 2. Note that some activities may occur in multiple stages.

STAGE I: PROCESS DESIGN

Process design defines the commercial manufacturing process that will be implemented in planned master

production and control records. The ultimate goal of this stage is to design a process that is suitable for routine commercial manufacturing and allows the consistent delivery of a product that meets its quality attributes. Steps involved in process design include building and capturing process knowledge and understanding and establishing a strategy for process control.[5]

STAGE II: PROCESS QUALIFICATION

During process qualification, the process design is evaluated in order to determine whether the process is capable of reproducible commercial manufacture. Completion of stage II is required prior to commercial distribution, and acceptable products that are manufactured during this stage may be released for distribution. This stage involves two elements: design of a facility and qualification of utilities and equipment and process performance qualification (PPQ).[6]

Qualification of utilities and equipment may include selecting utilities and equipment construction materials, operating principles, and performance characteristics; verifying that utility systems and equipment are built/installed in compliance with design specifications; and verifying that utility systems and equipment operate within the anticipated operating ranges.[7]

The PPQ combines the now qualified facility, utilities, equipment, and trained personnel with the commercial manufacturing process, control procedures, and components in order to produce commercial batches. The goal of PPQ is to confirm the process design and demonstrate that the commercial manufacturing process performs as it is expected. Successful PPQ is required prior to commercial distribution, and data from commercial-scale batches should be used as support to begin commercial distribution, supported by data from laboratory and pilot studies. During the PPQ process, previous credible experience with sufficiently similar products and processes may be helpful, and meaningful objective measures, such as statistical metrics, should be used whenever feasible.[8]

While PPQ will often have a higher level of sampling, additional testing, and greater scrutiny than would be expected in routine commercial processes, it should be sufficient to confirm uniform product quality throughout the batch.[9]

A written PPQ protocol to specify manufacturing conditions, controls, testing, and expected outcomes is critical during this stage of process validation. The protocol may include:[10]

- Manufacturing conditions (e.g., operating parameters, processing limits, and raw material inputs)
- Data to be collected and when/how it will be evaluated
- Tests to be performed and acceptance criteria for each step
- The sampling plan (including sampling points, number of samples, and frequency of sampling for each unit operation/attribute)
- Criteria and process performance indicators that allow a science- and risk-based view of whether the process is capable of consistently producing quality products
- Design of facilities and qualifications of utilities, equipment, and personnel training and qualification, and verification of material sources (such as components and container/closure)
- Status of validation of analytical methods that were used during the process, in-process materials, and the product
- Review and approval of the protocol by appropriate boards

The output of process characterization is the establishment of acceptable operating ranges for those parameters that have been fully characterized. Process performance qualification begins by the drafting of a protocol that defines the process by unit operations, set points, and acceptable parameter ranges. The protocol prospectively specifies a number of full scale manufacturing lots that will be run in the commercial manufacturing facility, and product released for commercial disposition, in order to demonstrate that the process operates as expected within predefined ranges and thereby confirms that it is operating under a satisfactory state of control.

STAGE III: CONTINUED PROCESS VERIFICATION

The control strategy after stage II (or PPQ) is maintained and updated throughout stage III based on scientific understanding, product knowledge, and gained information. In continued process verification, the goal is continued assurance that the process remains in a validated state of control during commercial manufacture. In this

stage, it is critical to have one or more systems to detect unplanned departures from the designed process.[11]

For this step, an ongoing program must collect and analyze product and process data related to product quality, including relevant process trends and the quality of incoming materials or components, in-process material, and finished products. The data should be statistically evaluated and reviewed by appropriately trained personnel. Information gathered during this stage should verify that the quality attributes are being controlled appropriately throughout the process.

Although a thorough process design and development should anticipate significant sources of variability and establish appropriate detection/control/mitigation strategies, it is likely that a process will encounter variations that were not previously detected or to which the process was not exposed. Data gathered during this stage allows the determination of ways to improve or optimize the process by altering certain aspects (e.g., operating conditions, process controls, component, in-process material characteristics). However, well-justified rationale for the change, an implementation plan, and quality unit approval should be documented prior to implementing such changes.

DEVELOPMENT AND MANUFACTURING ACTIVITIES

Development and manufacturing activities are normally designed to be aligned with FDA-guided process validation. Early-stage development involves testing on the platform if there is one or optimization to have a high titer while maintaining acceptable product quality. Scale-down model development/qualification and process characterization occur around the time for phase 3 clinical campaigns, and the output of the late-stage development contributes to the PPQ.

RISK ASSESSMENT

For risk assessment prior to IND filing, the QTPP should be defined, including dosage, administration, and other product profile information. Potential CQA can also be developed based on QTPP so that the CQA assessment could be done before entering early stage development.

Starting during late-stage development, it is important to determine the CQAs for characterization. During the early-stage process, it may be possible to create an abbreviated version of the process for evaluation (e.g., bioreactor). It could help to evaluate potential technology transfer problems, understand the scales and ranges against which the product should be compared, and determine which attributes demand focus.

After the process is locked and when the project moves to late-stage development, PRA and MRA commence. The outputs of PRA and MRA are included in the PCP, which guides process characterization. The criticality assessment is performed based on the results of characterization and the updated PRA/MRA. Manufacturing, MSAT, and/or development teams carry out FMEA.

Target Product Profile and QTPP

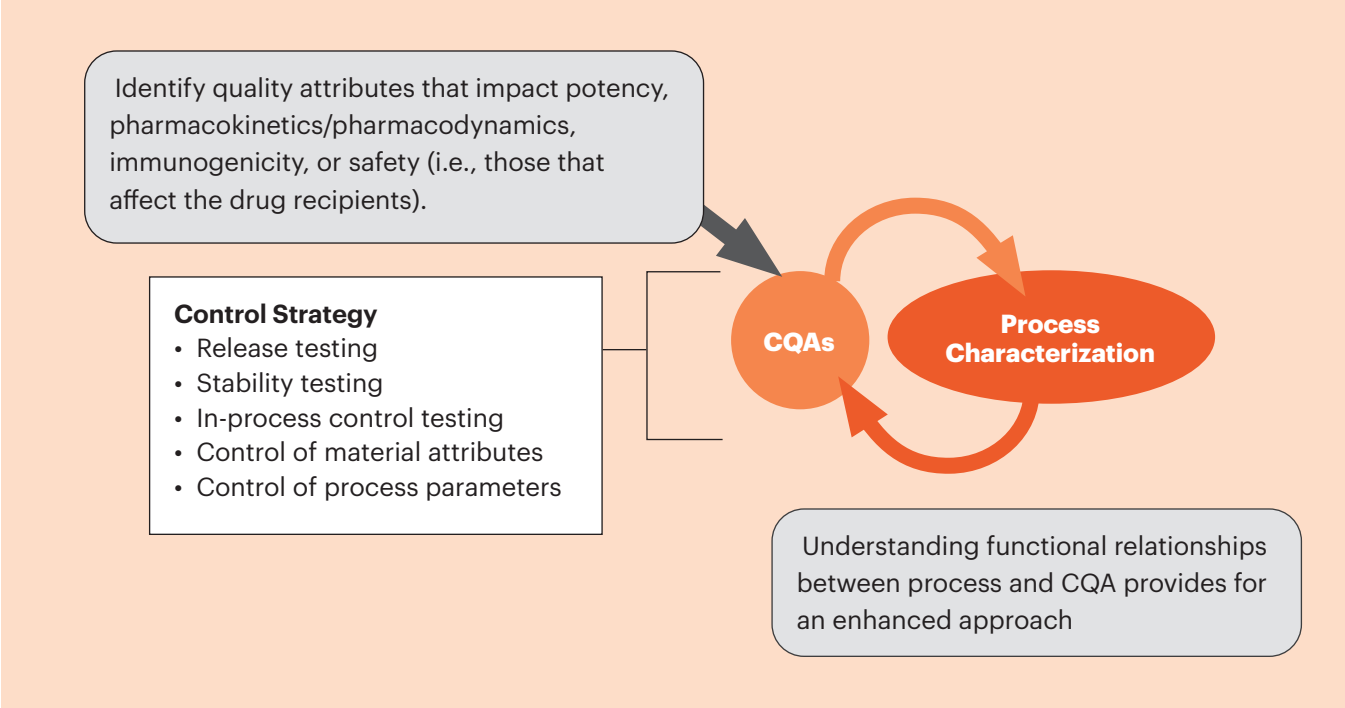
The target product profile (TPP) is a potential set of product label concepts that is refined during clinical development. The quality target product profile (QTPP) is developed from the TPP in order to provide a set of requirements for the product quality to ensure safety/efficacy. These concepts define the product design criteria and form the basis for the development of CQAs, identification of CPPs, and the overall control strategy. Importantly, early definition of QTPP forms boundaries around quality product attributes. Once the QTPP is defined, it is possible to move between manufacturing platforms so long as the QTPP is maintained and comparability is demonstrated in the respective drug substance and drug product quality attributes. For more information on QTPP, please refer to Chapter 3.

It is important to characterize vectors for clinical studies and verify that the purified clinical vectors maintain purity, potency, safety, and stability over the course of their potential use in investigational studies. Safety concerns may include sterility, mycoplasma, endotoxin, general safety, and adventitious viral agents. Potency may be measured using assays to measure the concentration and functional activity of purified AAV vectors. Of note, stability studies can be run concurrent with clinical use during early-phase studies.

OVERVIEW OF CQAS

Manufacturers of gene therapy products must take appropriate measures to ensure that products being manufactured meet the required standards of quality from the

Figure 4-3. *CQA Considerations During Development*



perspective of both patients and regulatory authorities. In the context of biopharmaceutical manufacturing and as stipulated in ICH Q6A, quality refers to the suitability of a drug substance or drug product for the intended eventual use, and specifications that are associated with this suitability are known as quality attributes. Product quality attributes are selected for their ability to help indicate the suitability of the product for its intended use. They are important to demonstrate lot-to-lot consistency, generate solid clinical data, determine relationships between product quality attributes and safety and efficacy, support establishing meaningful specifications, and show comparability after manufacturing changes.

All quality attributes must be assessed for consistently measurable and quantifiable impacts on the safety, efficacy, or other aspects of quality of the product. In turn, developers can define a profile of CQAs and CPPs. For further, more detailed discussion of CPPs and their relationship to CQAs, please refer to Chapter 5.

Understanding product CQAs is perhaps the most critical aspect of establishing a suitable manufacturing process, as well as establishing controls for assuring product quality and consistency. Because variations in CQAs indicate the importance and impact of process parameters and monitoring, CQAs serve as the benchmarks that enable the properly informed selection of operational ranges. Most importantly, CQAs help ensure that the final product will provide patients with the safest and most efficacious therapy possible. Figure 3 summarizes the purpose and involvement of considerations related to CQA considerations with respect to the overall development process.

CQAS AS RELATED TO REGULATORY TIMING AND EXPEDITED PATHWAYS

As gene therapy products provide patients with treatment options for conditions with few or no alternative therapies, the timing for securing regulatory approval is of utmost importance in allowing patients to benefit from the advancements that gene therapy products can offer. For this reason, developers often aim to achieve expedited pathway designation(s) for gene therapy products. As an example of expedited regulatory pathway development, the FDA is putting an effort into various expedited pathways to accelerate the process product approval while still ensuring safety of products. For specific information about expedited regulatory pathways with respect to gene therapy products, please refer to Chapter 1.

CMC readiness, of which identification of CQAs is a primary component, remains one of the major challenges in the expedited development of gene therapy products. Moreover, unlike traditional small molecule pharmaceuticals and well-characterized proteins, manufacturing processes for many gene therapy products must consider commercial scalability and viability at a very early stage. As a result, insufficient readiness could potentially lead to unnecessary delay and may further complicate and convolute interpretation of very costly clinical trial studies. This underscores the importance of CMC-focused communications with regulatory agencies early and often. Manufacturers should lay out a sound development and facility plan covering the product's lifecycle, including post-approval considerations. Furthermore, manufacturers should conduct comprehensive and detailed CMC readiness exercises prior to the initiation of pivotal or licensing trials or before asking agencies for one of many expedited designations. Sponsors should make sure that the treatment is adequately well understood, safe, and effective for marketing approval.

CMC Readiness

Developers, manufacturers, and sponsors should be aware of the following regarding CMC readiness and regulatory timing:

- Safety is the main focus allowing IND to proceed.
- Pivotal or innovative trials cannot be initiated without sufficient phase-based appropriate product manufacturing control.
- Expedited programs are designed to accelerate clinical development.
- An accelerated clinical development program will allow less time for CMC-related activities.
- CMC readiness for an expedited program requires additional evidence of manufacturing control.

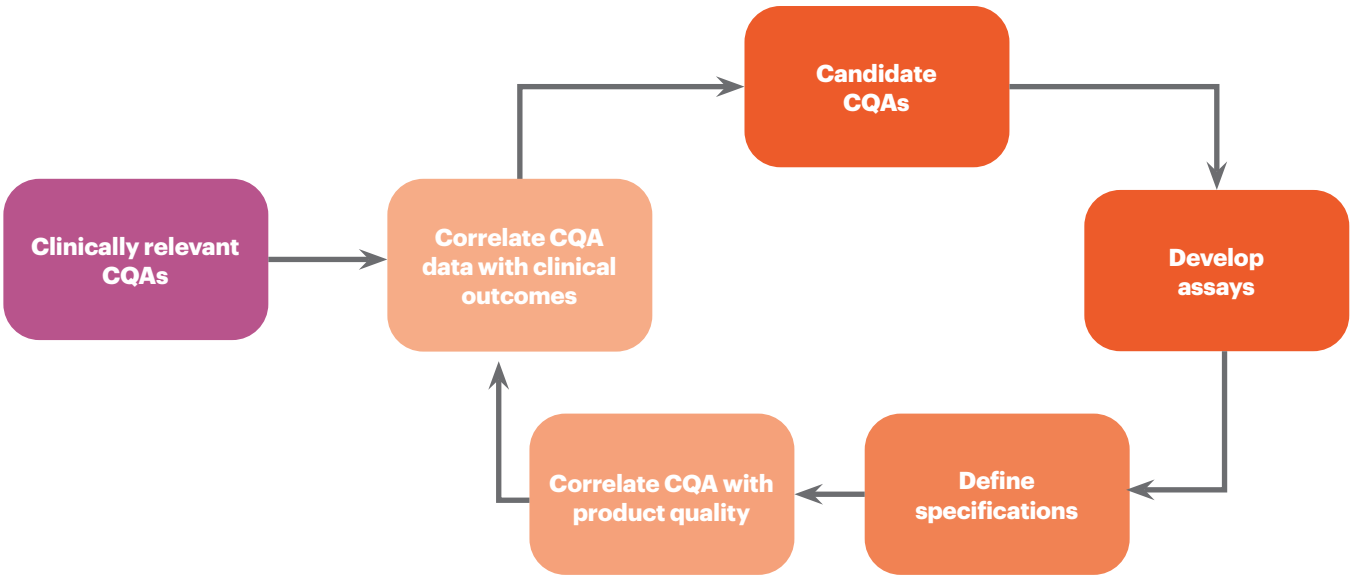
The identification of CQAs must be appropriate with respect to the context of the timing required for the regulatory pathway(s) being pursued. Changes to a process/product late in development (such as during clinical trials) could potentially change the product's critical characteristics as they relate to safety, efficacy, and other aspects of quality, which in turn could have great influence on decisions made by regulatory reviewers. However, for a variety of reasons, manufacturers are often compelled to introduce major manufacturing changes very late in the product's development life cycle. Thus, failure to detect the potential impact of these changes at critical times during the development process may detrimentally affect the commercial success of the product, leading to negative outcomes for patient access to needed therapies. To address these potential pitfalls, manufacturers are strongly encouraged by the FDA and other health authorities to devise a plan of action to understand the CQAs that could potentially correlate with product quality and the clinical outcomes, and to implement this plan at all stages of the development process at which CQAs could potentially be identified. Accomplishing this goal requires not only an in-depth understanding of the product and its associated analytics, but also a systematic approach to correlate key product attributes to various clinical outcomes.

Besides the changes intentionally introduced by the manufacturer, it is often challenging to establish and maintain consistency of the product throughout the development stages to the final commercial process. The ability to produce a consistent product will depend on controlling CPPs, which are established through the monitoring of CQAs, along with other factors that define the overall quality of the product. Collection of robust characterization data early in development can help better define CQAs and demonstrate comparability for major changes later in the development process.

At a minimum, regulators expect that manufacturing development should include "identification of potential CQAs associated with the drug substance so that those characteristics having an impact on drug product quality can be studied and controlled" (ICH Q11). Additional information is expected to be identified after commencement of clinical trials, with ongoing refinement of knowledge throughout the entirety of the development process.

Assay development should start at the preclinical phase in order to promote better decision-making throughout later phases of the development process

Figure 4-4. *Systematic and Iterative Approach to Identifying Clinically Relevant CQAs*



and provide a high level of confidence that observations during clinical phases are explainable and addressable. Assessments based on assays should consider what is known about the impact (or lack thereof) of a particular attribute on the quality attributes listed in the QTPP (refer to tables in Chapter 3). These assessments can be designed based on factors such as prior knowledge and experience (e.g., platform knowledge, published information), non-clinical data, and clinical data. In some cases, additional studies can help confirm attribute criticality or address gaps in knowledge regarding CQA impact on attributes such as potency or safety.

CQA CONSIDERATIONS RELEVANT TO GENE THERAPY

The process of acquiring in-depth product knowledge for well-characterized biologics and small molecules is well established and includes a development approach yielding in-depth understanding of the CQAs of the product and the need to better define critical manufacturing steps and CPPs.

However, this approach has, for a variety of reasons, yet to be fully adopted by the gene therapy industry, partially due to challenging technical issues and inherent biological limitations: biological products are complex, often heterogeneous mixtures, with complex mechanism(s) of action (MOA).

The FDA recognizes that identifying product-specific and clinically relevant CQAs is extremely complicated. Accordingly, the agency encourages a systematic approach involving several steps:

- Start with the identification of several candidate CQAs for each product and the development of qualified assays to measure such candidate CQAs. The knowledge gathered during the product development cycle forms a scientific basis for establishing meaningful specifications.
- In addition, there should be a systematic approach to correlate CQAs with product quality, and clinical outcomes form the basis for establishing the biologic and clinical relevance of each candidate CQA.

Through a highly systematic and iterative process, it is possible to identify potential clinically relevant CQAs (Figure 4).

Quality Attributes Risk Assessment

Risk can be defined as a metric for the amount of danger posed by a given situation or variable. With respect to quality-related risk in cell therapy product manufacturing, as is the case for other biopharmaceutical manufacturing contexts, risk should always be assessed with the potential for diminishment of safety to the patient as the

primary concern. However, it should be noted that as far as business- and operations-related matters are concerned, the attribution of risk to any given case may not be straightforward, given that a multitude of stakeholders are often involved in materializing the operations of the manufacturing program, and subjectivity and variation in the exact levels of factors such as uncertainty and/or severity are possible. If risk is inaccurately or disproportionately estimated, patient access to efficacious therapies may be reduced if risk is assessed too conservatively; similarly, patients may be exposed unnecessarily to side effects if risk is not assessed conservatively enough. Therefore, the goal of risk assessment is to minimize the potential for harm to patients while maximizing therapeutic benefit.

Quality risk management is a systematic process to assess and control risks to quality across the product's lifecycle. The level of formal documentation associated with the quality risk management process should be commensurate with the level of risk as determined through risk assessment. Risk assessments—a systematic approach to support risk decisions using accurate, analyzable, and well-organized information—serves as the basis for establishing control measures and making informed decisions when managing risk. Risk assessment may be part of a formalized, integrated risk management strategy, but can also benefit an organization when used less formally to increase the scope and accuracy of an organization's institutional knowledge of the risks and hazards intrinsic to its current processes, systems, and operational business models.

The first step of risk assessment is the identification of possible sources of harm that are present in and/or inherent to the manufacturing process. Identification can be based on factors such as historical data, theoretical analysis, and the informed opinions of experts. The next step of risk assessment is risk analysis, in which the risks associated with the identified sources of harm are estimated. Through qualitative and quantitative means, associations are established between the likelihood of a harmful event occurring, the severity of harm should the event occur, and in many cases, the ease of detecting the harmful event.

The last step of risk assessment is risk evaluation. During risk evaluation, the results of the identification and analysis steps are compared against set criteria in order to place risks in proper context with respect to the manufacturing program as a whole. Risk assessment should be considered to be an evolving process that starts at the bench-based research phase, develops through the clinical stages, and continues up through product approval and commercial manufacturing. In order to properly guide this evolution, as well as to ensure that risk assessment results in the best possible outcomes for patients, ongoing communication involving industry, regulators, and where possible, patients, is vital. Communication may relate to the existence, probability, severity, acceptability, and/or detectability, of risks, as well as other aspects.

SOURCES FOR GUIDANCE

The following sources provide guidance related to ensuring the quality of products and implementing an effective risk management system:

- FDA, *Guidance for Industry: Quality Systems Approach to Pharmaceutical cGMP Regulations* (September 2006)
- ICH Q8(R2), *Pharmaceutical Development* (August 2009)
- ICH Q9, *Quality Risk Management*
- ICH Q11, *Development and Manufacture of Drug Substances,* November 2012
- Parenteral Drug Association, *Technical Report No. 54-4. Implementation of Quality Risk Management for Pharmaceutical and Biotechnology Manufacturing Operations,* 2014

SEVERITY SCORING AND FILTERING

Severity scoring is an approach in which multiple factors associated with each quality attribute identified to be a potential source of risk are evaluated regarding the severity (also referred to as "impact") of each factor with respect to potential effects on the safety (including immunogenicity), efficacy (determined from either clinical experience or potency assays), and/or pharmacokinetics

Table 4-1. *Overview of Severity Scoring*

Value	Severity	Severity of Effect
1	**Low**	Variability in attribute has minor or negligible potential for decreased safety/efficacy. Negligible or minor transient adverse effects are expected based on historical experience.
3	**Medium**	Variability in attribute may have moderate potential for decreased safety or efficacy within the clinical history of the product. Attribute may result in manageable adverse effect seen historically but no new adverse effects.
10	**High**	Variability in attribute may have potential for severe effect on patient. Potential significant change in safety/efficacy or risk/benefit profiles. May result in a serious (reversible or irreversible) adverse effect.

and pharmacodynamics of the product, along with the uncertainty about the information used to assess the severity. In this way, scoring matrices for each factor are developed, after which the individual scores for each factor are multiplied together to generate a composite risk score. The risk score can then be compared with respect to the range of scores established by the scale generated by calculating the lowest and highest risk scores possible according to the systems of measurement being employed. Finally, "filters," in the form of weighting factors or cut-offs for risk scores, can be used to scale or fit the severity scoring to management or policy objectives.

Severity scoring is particularly helpful in situations in which the range of risks and the potential consequences to be managed are diverse and difficult to compare using other methods, and has the advantage of allowing for both quantitative and qualitative assessment of risks within the same organizational framework. If severity scoring is properly applied at key points throughout the product lifecycle, starting at the pre-IND phase and through to licensure and post-approval, developers will be able to identify the attributes that pose the highest levels of risk, and therefore will be able to implement effective precautionary measures and mitigation strategies.

Table 4-2. *Overview of Uncertainty Scoring*

Value	Severity	Prior Knowledge	Pre-Clinical Studies	Clinical Studies
1	**Low**	Well characterized effect based on extensive data (*in vitro, in vivo,* or clinical). Large body of knowledge in the literature.	Demonstrated relevance of animal model results. Extensive *in vitro* and *in vivo* studies for this product.	Significant clinical experience with this product.
2	**Medium**	External published literature available. Well characterized effect known. Internal data (*in vitro, in vivo,* or clinical) from this or similar class products.	Only moderate *in vitro* and/or *in vivo* data available for this product.	Only limited clinical experience with this product.
3	**High**	Limited or no published external scientific literature and no internal data from this or similar class products.	No data available for this product.	No data available for this product.

Table 4-3. *Critical Quality Attribute Classification Outcome*

		Uncertainty		
		1 (Low)	**2 (Medium)**	**3 (High)**
Severity	**10 (High)**	10 (Critical)	20 (Critical)	30 (Critical)
	3 (Medium)	3 (Potential)	6 (Potential)	9 (Potential)
	1 (Low)	1 (Non-critical)	2 (Non-critical)	3 (Potential)

Product- and process-related impurities, as well as microbiological quality/safety, biological activity, and product identity can be ranked using the system described in Table 1 and Table 2. By multiplying the severity score and uncertainty score, it is possible to rank and classify the critical quality attributes, as shown in Table 3.[12]

For example, bioburden is a critical quality attribute. Because the impact/severity of bioburden is high (10) and uncertainty is low (1), the overall ranking will be critical (10 x 1 = 10). Another example might be AAV impurities by SDS PAGE. The severity could be medium (3) because protein impurities may have moderate or potential immunogenic response. The uncertainty is low (1) due to the potential for preclinical or clinical data. Thus, the overall ranking of AAV impurities would be potential (1 x 3 = 3).

PRELIMINARY HAZARD ANALYSIS (PHA)

Preliminary hazards assessment (PHA) is a risk assessment approach based on applying prior experience or knowledge of a hazard or failure to identify future hazards, hazardous situations, and events that might cause harm, as well as to estimate their probability of occurrence for a given activity, facility, product, or system. The tool consists of: 1) identifying the possibility that the risk event will happen, 2) the qualitative evaluation of the extent of possible injury or damage to health, 3) a relative ranking of the hazard using a combination of severity and likelihood of occurrence, and 4) the identification of possible remedial measures.

Similar to risk ranking, PHA is based in part on severity, but unlike risk ranking, uses likelihood as the other parameter instead of uncertainty. Likelihood refers to the probability that, should a quality attribute stray outside of accepted ranges based on the most recent understanding and knowledge about the attribute (drawn from literature, clinical, and non-clinical studies relevant to the product in question or similar products), the occurrence will affect the safety and/or efficacy of the product. When limited clinical data are available for a particular quality attribute, likelihood is to be assessed conservatively. In a similar way to how risk ranking scores are determined, the risk priority number (RPN) of PHA is calculated by multiplying the severity score and the likelihood score. The direness of the risk posed by the attribute in question can then be judged based on its relative placement along the priority scale compared to the other attributes being assessed. Because the assessment of likelihood depends on prior knowledge, PHA is particularly useful when performing risk assessment in existing systems.

PHA is most commonly used early in the development of a project, at a time when there is little information on design details or operating procedures. Thus, results of PHA can inform process and facility design, as well as serve as a pointer for further study of quality attributes using other risk management tools.

CASE STUDY

In this section, a risk assessment is performed to determine which quality attributes are critical to guide process validation and process characterization experiments. The purpose of this risk assessment is to identify and summarize the CQAs for the generic AAV-based gene therapy product introduced in Chapter 3. CQAs are physical, chemical, biological, or microbiological properties or

characteristics that should be within an appropriate limit, range, or distribution in order to ensure the desired product quality. International Conference on Harmonization (ICH) Guideline Q8(R2): Pharmaceutical Development requires the following:

> *Identification of potential CQAs, including those related to drug substance, drug product, and excipients, so that any characteristics that may have an impact on the desired product quality can be studied and controlled.*

A risk-based approach has been adopted for identification and assignment of CQAs, similar to the principles outlined in the A-Mab and A-Vax case studies published by CASSS, ISPE, and the PDA for applying Quality by Design (QbD) principles to process development. The CQAs are derived from the Quality Target Product Profile (Chapter 3), which forms the basis of design for the development of the product as well as any knowledge gained during the product and process development activities, in order to assign a risk ranking score for each quality attribute. Each quality attribute is evaluated for

Table 4-4. *Quality Attribute Risk Assessment*

Quality Attribute Category	Quality Attribute	Severity Score	Uncertainty Score	Overall Ranking	Criticality
Safety	Bioburden	10	1	10	CQA
	Endotoxin	10	1	10	CQA
	Sterility	10	1	10	CQA
Content/ strength	Appearance/particulates	10	1	10	CQA
	pH	10	1	10	CQA
	Osmolality	10	1	10	CQA
	Vector genome titer	10	2	20	CQA
	Potency (protein expression)	10	2	20	CQA
	Potency/infectious genome titer	10	3	30	CQA
Identity	Capsid identity	10	1	10	CQA
	Genome identity	10	1	10	CQA
Process impurities	Residual cell culture media components	3	1	3	Potential CQA
	Residual host cell protein	1	1	1	Non-critical
	Residual plasmid DNA	3	1	3	Potential CQA
	Residual host cell DNA	3	1	3	Potential CQA
	Residual transfection reagent	3	1	1	Potential CQA
	Residual chromatography ligand	3	1	3	Potential CQA
	Replication-competent AAV	10	1	10	CQA
Purity	Capsid protein purity	10	2	20	CQA
	Capsid protein ratio	10	1	10	CQA
	% full capsids	3	3	9	Potential CQA
	Total capsids	3	1	3	Potential CQA
	Aggregates/subvisible particles	10	1	10	CQA

criticality by assessing its potential impact and uncertainty as it relates to the efficacy and safety of the product.

During stage I activities during process validation, the CQAs will be used to identify the CPPs for the proposed commercial manufacturing process via a risk assessment, observations from historical experience, and findings from process characterization experiments.

The identification and justification of CQAs are being performed in accordance with the QbD principles and recommendations made in ICH Q8(R2), Pharmaceutical Development and ICH Q9, Quality Risk Management, as well as the generally accepted approach described in guidance documents related to process validation.

Process Development Considerations for Product Quality

An overview of various process development considerations can be found in Wright JF. Biomedicines 2014;2:80-97, including vector-related impurities (e.g., empty AAV particles), residual host nucleic acids, residual helper DNA sequences, replication-competent AAV species, and noninfectious AAV vector particles.[13] Highlights and additional considerations are provided below. For additional information on processes used to remove impurities, please refer to Chapter 5 of this document.

Unencapsidated DNA

Foreign DNA can be introduced through a few ways: plasmid DNA, host cell DNA and intermediate species of the gene of interest. The capsidated foreign DNA is considered to be a product-related impurity. To minimize the amount of foreign DNA in the process, nucleases, such as benzonase, could be introduced. In addition, depth filtration and other downstream processes can remove unencapsidated DNA.

Cell Culture–Related Impurities

Depending on the media used in cell culture, potential impurities can be introduced to the system. To mitigate the risks, nonanimal-derived or even chemical-defined media can be used for production. For the working cell bank (WCB), extensive tests and growth characterization are required before release for GMP production.

Downstream-Related Impurities

During product purification, downstream processes can introduce buffer, resin, and other impurities. In order to mitigate these risks, understanding and evaluation of the tests are needed. For example, a leachable and extractable study might be needed to demonstrate the effectiveness of impurity removal during the downstream process.

Helper Viruses

Helper viruses may be used in the upstream production of AAV, and the viruses must be absent from the drug product. Additional viral inactivation steps following affinity purification may be needed, such as heat or acid inactivation after affinity step. A viral filtration step is recommended for AAV products going into late-phase production, even if viruses are not used in the system. Health authorities will require a clearance study when approaching the registration/late phase stage.

Parameter and Material Risk Assessment

The PRA determines which parameters and associated materials may affect CQAs using cause and effect methodology to assess each parameter for its effect on each CQA. The purpose of PRA is to define a set of parameters that may pose critical risk to quality and process performance. Such an assessment of the criticality of process materials is needed in order to ensure that material variability is considered during parameter studies.

Gene therapy involves complicated analytics that would consume a considerable amount of time if each parameter were evaluated. A PRA allows a company to reduce the number of parameters to a manageable number of high- or medium-risk parameters to be evaluated within a reasonable amount of time. For example, hundreds of parameters may be identified, which following PRA may be reduced to ~30, allowing laboratory work to be done within 6 months and analytics and reports within an additional 3 months. Prioritizing parameters that require further study help to reserve resources; analytics may require several samples of drug product, which is challenging in gene therapy development when product is extremely valuable and limited.

It is important to analyze all parameters involved

Table 4-5. *PRA Template*

Line no.	Unit Operation	Process Step	Parameter	Development Range	Target	PI	PI	PI	CQA	CQA	CQA	CQA	CQA	CQA	Max of Rating	Sum of Rating	Justification/ Rationale	Comments
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		

during the upstream-to-downstream process. Table 5 shows a PRA template to rank the impact of CQAs, which is then incorporated in future studies to allow faster product development. While process performance indicators are mainly business-related, critical quality attributes pertain to efficacy and safety and thus carry more weight in the risk assessment (Table 6).

The output of PRA is a process characterization plan (PCP), which defines the list of high-risk parameters and materials for further investigation based on high severity scores in the PRA. Ultimately, the PCP is a prospective plan including experiments that should be performed during process characterization. The PCP should also define aspects of the scale-down model that need to be considered.

The output of PCP is a detailed characterization protocol for each parameter study that will be conducted. Such characterization protocols should include the following:• Study purpose

- Materials and methods to be used
- Study design
- Study acceptance criteria

UPSTREAM EXAMPLES

To provide more direction on how to perform the risk assessments, three assessment examples for upstream process parameters and one raw material assessment are presented (see Table 7). The ratings and development ranges in the examples are only for demonstration and are not intended to represent the rating or ranges for any processes.

The examples illustrate how to rate, use ranges, and interpret the sum of the ratings and the maximum rating. If the rating is ≥10, further investigation is needed to show understanding and control of the process and product quality. Depending on the program, the sum of ratings requires team input to reflect both the impact and uncertainty for the evaluated raw material or parameter.

Example 1: Temperature for Thaw Stage

Table 4-6. *Risk Ranking Criteria for Operating Parameters and Material Attributes*

Parameter Risk Score	Process Performance Indicator	Critical Quality Attributes
1	No effect	No effect
4	Minimal to moderate effect	Minimal effect
7	Moderate to severe effect	NA
10	NA	Moderate to severe effect

Table 4-7. *Upstream PRA Examples.*

Line Number	Unit Operation	Process Step	Parameter	Development Range	Target	Units	VCD	Viability	Titer	E:F Ratio	Potency	Max of Rating	Sum of Rating
1	Thaw	Set-up	Incubator temp	36-38	37	°C	4	4	1	1	1	4	11
2	Production (N)	Set-up	Temp set point	35-59	37	°C	7	7	7	4	4	7	29
3	Transfection	Mixing	DNA ratio[a]	N/A	1.0:1.0:0.2	N/A	4	4	7	10	4	10	29
4	Transfection	Transfection	PEI quality	N/A	N/A	N/A	4	4	7	4	4	7	23

[a](Ad Helper:Trans:Cis(GOI))

The set point for the temperature at this stage is 37°C. Due to equipment capacity, 36-38°C was selected to cover the normal operating range. As the temperature is controlled within a relatively narrow range and it is expected that the effect on cell growth will be minimal, the rating for viable cell density (VCD) and viability for that stage are both 4. This is the first stage of the passage, and cells could resume normal growth at a later stage. Therefore, the effect for the production stage for titer and the CQAs is negligible, and the titer and CQAs were all rated as 1. This results in the maximum rating of 4, and the sum of the ratings is 11. Both the maximum and the sum indicate that this parameter has a low risk to affect the process performance or product quality.

Example 2: Temperature for N Stage

The set point for the temperature at this stage is 37°C. The developmental range was selected to cover both the normal operating range, as well as the potential characterization range. Understanding the effect of temperature variation on the N-production stage could help to better control the final productivity. For cell growth, previous experience with higher temperature (i.e., 40°C) resulted in slower growth and low titer. Therefore, all process indicators (titer, VCD, and viability) were rated at 7. There was limited knowledge on the effect of temperature variation on the quality of the product, so the CQAs were each rated a 4. Finally, the maximum rating for temperature for N stage was 7. The sum of the ratings was 29 due to the high effect on cell growth and titer, as well as the uncertainty of the effect on the CQAs.

Example 3: Plasmid Ratio for Transfection

The set point for the plasmid ratio was 1:1:0.2, and the developmental range has not been defined due to the complexity of three variables. However, from previous experiments, it was known that plasmid ratio had minimal effect on cell growth and a moderate effect (20% to 30%) on titer. Therefore, VCD and viability were rated at 4, and titer was rated at 7. Variation in the plasmid ratio affects the empty-to-full ratio (E:F) with no observed effect on potency. Therefore, E:F was rated at 10, and potency and the remaining CQAs were rated at 4. The maximum rating was 10, indicating significant effect on

Table 4-8. *Downstream PRA Examples*

Line Number	Unit Operation	Process Step	Parameter	Development Range	Target	Units	Yield	Aggregation	SDS Page Purity	E:F Ratio	Max of Rating	Sum of Rating
1	Affinity	Load	Residence time	4-10	7	min	4	1	1	1	4	7
2	CIM Q	Column load	Capacity	5-11	10	CV	4	1	4	10	10	19
3	Buffer	Formulation	Pluronic F68	N/A	10	ppm	1	4	1	1	4	7

at least 1 CQA or performance indicator. This will lead to further characterization of the parameter to understand the effect on the CQA (i.e., E:F). The sum of the ratings was 29. A high sum of the rating could reflect both high impact on some CQAs, as well as an indication of requiring more process knowledge around this parameter to sufficiently determine a control strategy to minimize risk to the process and product.

Example 4: Raw Material – Polyethyleneimine (PEI)

PEI quality is known from past experience to influence titer. Depending on the amount used, as well as lot-to-lot variation, there could be minimal to moderate effect on cell growth. Quantity of PEI will be evaluated separately as a process parameter. For the purposes of this example, only the lot-to-lot variation will be considered. The effect on cell growth was rated as 4. The CQAs were each rated 4 due to uncertainty of effect of PEI quality on each attribute. The maximum rating for PEI quality effect was 7, indicating influence on productivity or uncertainty of significant effect on CQAs. The sum of the rating was 23, reflecting uncertainty of effect on CQAs.

DOWNSTREAM EXAMPLES

To provide more direction on how to perform the risk assessments, two assessment examples for downstream process parameters and one raw material assessment are presented (see Table 8). The ratings and development ranges in the examples are only for demonstration and are not intended to represent the rating or ranges for any processes.

Example 1: Residence Time on the AAV8 Column

The proposed residence time (4 to 10 min) for the AAV8 chromatography was expected to moderately affect the performance indicator (PI) step yield. Poros resins are specifically engineered for the purification of large biomolecules, and the rated pore size of the AAV8 resin is ~0.2 µm. Therefore, the effect of residence time at the lower end of the proposed range was rated as 4 because it is expected to only moderately affect the step yield. Because AAV8 is operated in a bind-and-elute mode where the impurities will be flowing through and only the product of interest will bind, the lower residence times are unlikely to affect impurity clearance. Therefore, the effect on CQAs was rated at 1 (no impact).

Example 2: Total Load on CIM Q Column

The CIM Q step is designed to enrich the fraction of full particles. The exact mechanism of separation is unclear. However, the empty particles are relatively weakly bound and elute at a slightly lower ionic strength. In general, the chromatographic resolution is strongly dependent on the capacity of the column. Therefore, the effect of column load was rated at 10. Given the limited capacity of the column, the higher end of the proposed range is also

Figure 4-5. *Example of Outcome Using Pareto Chart*

	Max	Sum	Parameters	Materials	Total
High Risk	*10*	*NA*	*4*	*0*	*13*
	Not 10	*>40*	*5*	*4*	
Medium Risk	*Not 10*	*24-40*	*11*	*1*	*12*
Low Risk	*Not 10*	*<24*	*229*	*7*	*233*

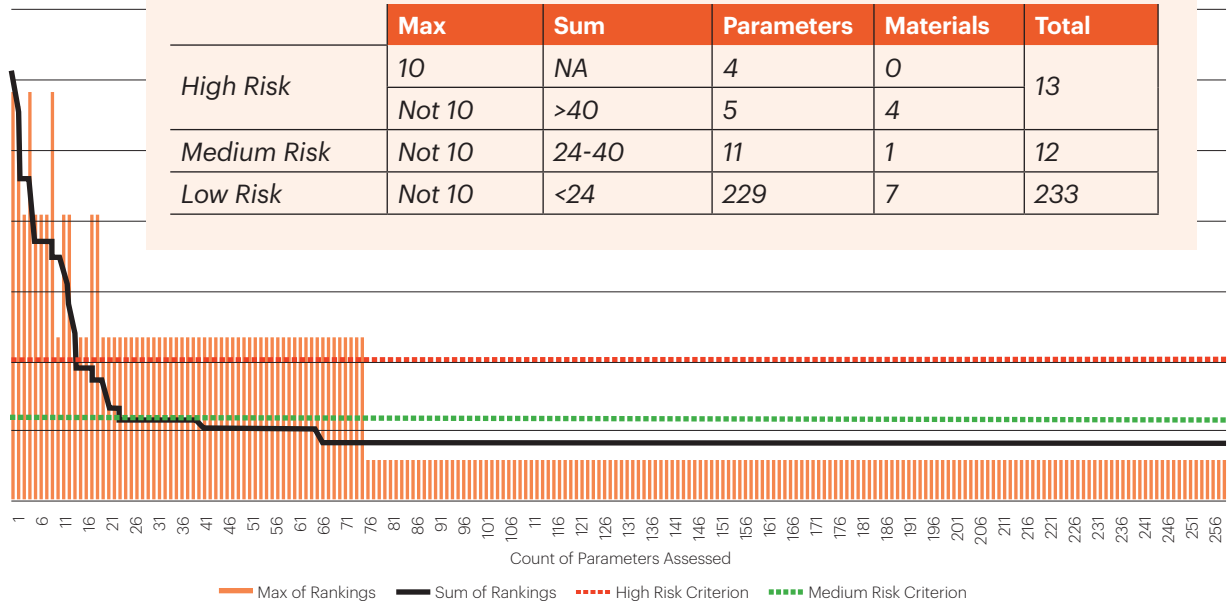


Figure 5 demonstrates an example of the risk analysis outcome. The X-axis contains each parameter that is assessed. Any parameter with a maximum of rankings of 10 is automatically considered a high-risk parameter and may require further study. Parameters with a sum of rankings >40, but no maximum ranking of 10, are also considered high-risk. Medium-risk parameters are those with a sum of rankings of 24 to 40, whereas low-risk parameters are those with a sum of rankings <24. As shown in the example, such an analysis allows the ranking of ~260 parameters into high-, medium-, and low-risk categories. Rather than performing analytics on 260 parameters, focus can be applied to the 13 high-risk and 12 medium-risk parameters, thus conserving time and resources while still producing a consistent, high-quality product.

expected to lead to yield loss. Consequently, the effect on yield was rated at 4.

Example 3: Raw Material – Pluronic F68

Pluronic F68 is used in the formulation buffer at a concentration of 10 ppm. Pluronic is a known stabilizing agent. There is conflicting literature on the use of Pluronic F68 to prevent aggregation. Therefore, a rating of 4 is assigned. Because this is a raw material risk assessment and not a PRA, the yield rating is left at 1.

Scale-down Models

In order to conduct complex process characterization studies, key areas can be scaled down. However, such a scale-down model (SDM) must represent the proposed commercial processes. Developing SDMs requires consideration of scale-dependent effects.

SDMs are associated with several challenges. For example, CQAs that are affected by upstream process parameters cannot be measured directly with upstream materials due to the high level of impurities found within the product. Products often require sample treatment with downstream purification steps, which introduces variability to the data analysis. Further, the downstream process is tied to the upstream process for a consistent supply of materials. Small-scale unit operations may not represent the actual manufacturing process scale.

Therefore, SDMs require rational design in order to produce data that are sufficiently predictive of and relevant to full-scale manufacturing (i.e., size of bioreactor or column). Sometimes, there is no perfect scale-down

Table 4-9. *Design of SDMQ Examples.*

Unit operation	Purpose	SDM	At-scale	PI or CQA selection for comparison
Seed train/Shake flasks	Biomass accumulation	NA	125 mL to 2 L	• NA • The same or modified seed train • Widely accepted that shake flask sizes can be interchangeable
Production stage	Product	3 L	500 L	• Cell growth • Titer • Product-related impurities • Identity
Affinity	Yield	1-cm ID	20-cm ID	• HCP • GC titer • Other upstream related impurities that can be cleared out at affinity step

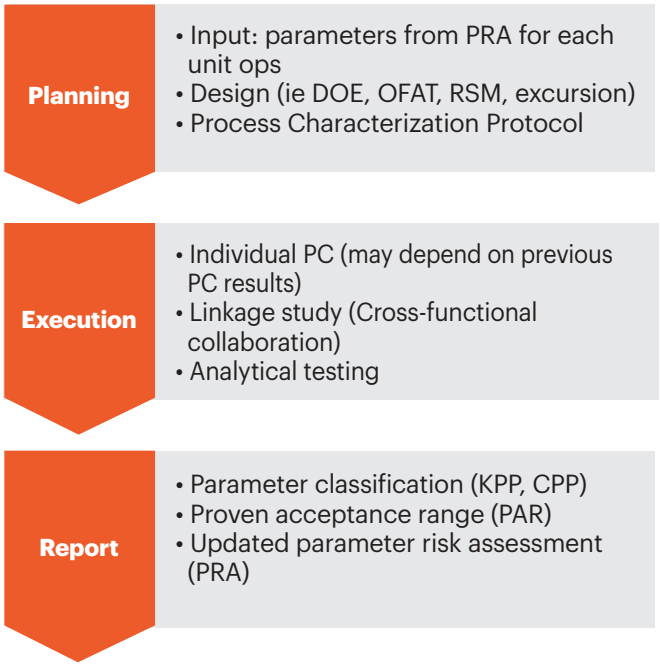
model by design (i.e., continuous centrifugation for biologics at manufacturing stage vs bench-scale filter), but a study can be designed to determine the equivalence of material quality after that step. Furthermore, it is important to understand the degree of differences between the model and the commercial process because this can impact the relevance of information derived from scale-down models. Examples of SDM at each unit operation are provided in Table 9.

In general, it is recommended to have a minimum of three runs at manufacturing scale, although more is better to enable a stronger analysis. If the model requires in-process samples to assay specific criteria such as titer, full capsid, or other specific CQAs, then an in-process sampling plan should be provided to manufacturing prior to running the process in order to maximize the collection of information from each batch.

In some cases, there may not be enough manufacturing-scale batches to provide statistically significant data for SDM generation. A risk-based decision may be made to move forward with an SDM at risk to allow process characterization studies to be conducted. The justification of the decisions based on characterization studies using a model that has not been fully qualified will need to be documented. In some instances, studies may need to be repeated with a modified model if further manufacturing scale batch data are collected that demonstrate that the SDM did not suitably represent the manufacturing-scale process.

An SDM is qualified by demonstrating its equivalent performance with that of the large-scale process. This is generally done using a statistical approach that is selected based on the availability of the data and the number of variables being evaluated in the model. The goal of the qualification is to demonstrate equivalency of the SDM to the manufacturing scale. Each unit operation SDM

Figure 4-6. *Overview of Planning, Execution, and Report*



will have a plan predefining the criteria for qualifying the SDM.

When nonequivalency is observed for a particular data point, justification for the nonequivalency is investigated first to identify potential root cause(s) before establishing that the data point truly is not comparable (i.e., too small of a sample size, too large/small analytical variation). If the incomparability is confirmed, the understanding of the offset either from theory or experiment is needed to interpret the correlation between input parameters versus output (process performance indicators and CQAs). Unjustifiable offset or nonequivalence will lead to failure of SDMQ. Failure of a qualification of an SDM will require investigation as to the cause of the failure to determine whether it is due to execution issues or that the model truly does not provide sufficient relevance to the manufacturing scale. The appropriate process development leadership will develop a strategy for how to manage the situation.

Process Characterization

Process characterization is a key part of the QbD process, as well as a key portion of the regulatory file. Process characterization seeks to not only document but also understand the impact of predetermined, deliberate variations in the process parameters and raw material attributes as a first step. Process characterization results in the identification of potential sources of variability in product quality. In turn, this allows the determination of how to control such sources of variability. When performing process characterization, the classification and level of control over process parameters should consider the corresponding risk to product quality. Essentially, process characterization provides insight into which parameters are critical to both attain and maintain product quality and process performance. Process characterization is repeated and modified as the results of process knowledge from multiple iterations, preclinical data, and clinical data become available. In gene therapy

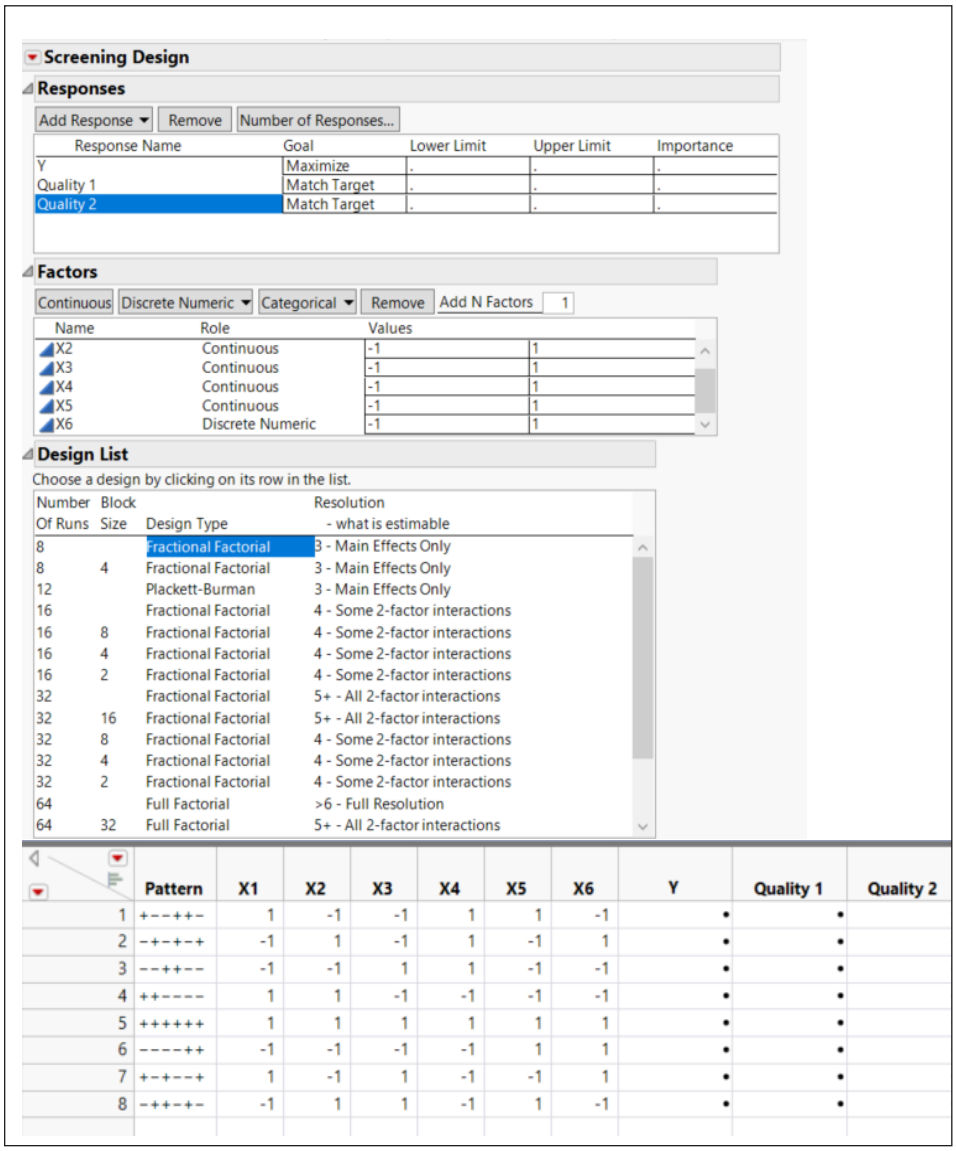
Table 4-10. *Design of Studies*

Design	Description	Example
OFAT	Main effect only	• Upstream: Harvest timing • Downstream: Affinity wash buffer volume
Screening	Main effect with some interaction Low resolution	• Upstream: pH, Temp, DO, Seeding Density • Downstream: 6 factors in AEX step (i.e., loading CV, loading pH, conductivity, elution pH, elution pH and elution CV)
RSM (Response Surface Model)	Main effect, interactions, and quadratic terms High resolution	• Upstream: ≤3 factors from above row • Downstream: 3 factors in AEX step
DSD (Definitive Screening Design)	Main effect and interactions; note that DSD is not appropriate in all situations	• Upstream: initial optimization with 6 factors
Excursion	Short time excursion	• Upstream: DO or temperature excursion
Linkage Study	Between unit operations	• Upstream: N-1 and N stage • Downstream/Upstream: Harvest

approaches, characterization approaches for plasmid products should take a minimalistic approach.

Starting materials, vector, and plasmid process characterization attempt to understand how risk profiles differ through a series of experiments that allow the definition of the protocol by which the process is qualified. Some parameters are critical by default due to compliance requirements. Experiments may include one-factor-at-a-time (OFAT) and design of experiments (DOE) approaches, with DOE moving from screen DOE approaches to response surface DOEs (Table 10).

Figure 4-7. *Example of Settings and Output for Screening Studies*



Example 1: OFAT Study

Sometimes, OFAT can be used to define the study range, especially when there is a wide range to study. For example, instead of having many levels per factor prior to DOE, OFAT can be used to define a narrow range. This helps to reduce levels in the subsequent DOE. For example pH ± 0.4 could potentially have up to five levels: -0.4, -0.2, set point, +0.2, and +0.4. These five levels in later DOE would either lead to lower resolution (i.e., screening) or too many runs to study for the main effect and interactions. Confirming the ranges to have only two levels (within a range that would not impact titer or quality) and a center point is more effective for later studies.

Another scenario could be one with no potential interactions with other factors and likely no need to study interactions by DOE. An example of an OFAT study would be investigating the effect on viability and viable cell density by varying the media hold/media storage time for a thawed cell bank vial passage step prior to transferring to shake flask production. In addition, harvest time is typically tested at control conditions so that no extra runs are needed for different harvest timing.

Example 2: Screening Study

Screening studies are typically used when multiple factors must be screened for main effects and partial interactions. The example shows six factors with two levels each for testing. The outcome from the screening study is usually whether there is a main effect from the factor tested. Using DOE, minimal runs could be tested (i.e., 2 x 6

=12 runs for each OFAT, but only eight runs are needed in the Figure 7 for the main effects only). Another option is two-factor interaction in the design if there is interest and resources are not limited.

Example 3: Response Surface Methodology (RSM)

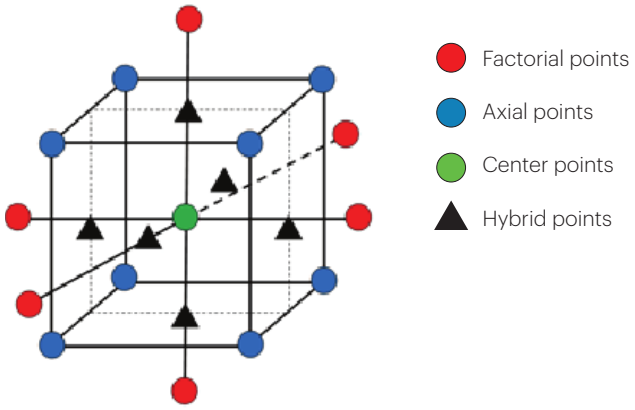
RSM provides high resolution of both main effects and interactions between factors. Figure 8 shows three factors, each with two levels. Central composite design (CCD) was used without hybrid points. CCD in a DOE design allows the determination of the settings of factors that would result in the optimum response. This experimental design builds a second-order model for the response variable without needing to complete a three-level factorial experiment. In the example here, a rotatable axial point (with 1.68 by default) was selected to test the extreme condition for 1 factor while keeping the rest of factors at target level. CCD can test main effects and interactions and also reflect any quadratic effect for factors. In addition, CCD can be used to test more levels (i.e., axial points for extreme conditions) or to build a model for prediction. However, a drawback for three factors, as in this example, is that more runs are required (i.e., 16). Therefore, CCD would be recommended after first confirming the impact for quality or titer from factors.

Example 4: Definitive Screening Design (DSD)

At times, it is necessary to do both screening and optimization simultaneously. One way to do this is through definitive screening design (DSD). DSD is a DOE methodology first published in 2011 that uses correlation-optimized designs to screen several factors for both main effects and interactions. DSD requires fewer runs than similar fractional-factorial designs and allows for the unambiguous identification of the main effects and interactions. Thus, DSD is an efficient, one-step approach to process characterization.

DSD is most appropriately used during the earliest stages of experimentation when there is a large number of factors that are potentially important and which may affect a response of interest. In particular, DSD is best used when the goal is to identify a much smaller number of factors that are highly influential. DSDs are best suited for situations in which most of the factors are continuous

Figure 4-8. *Response Surface Methodology*



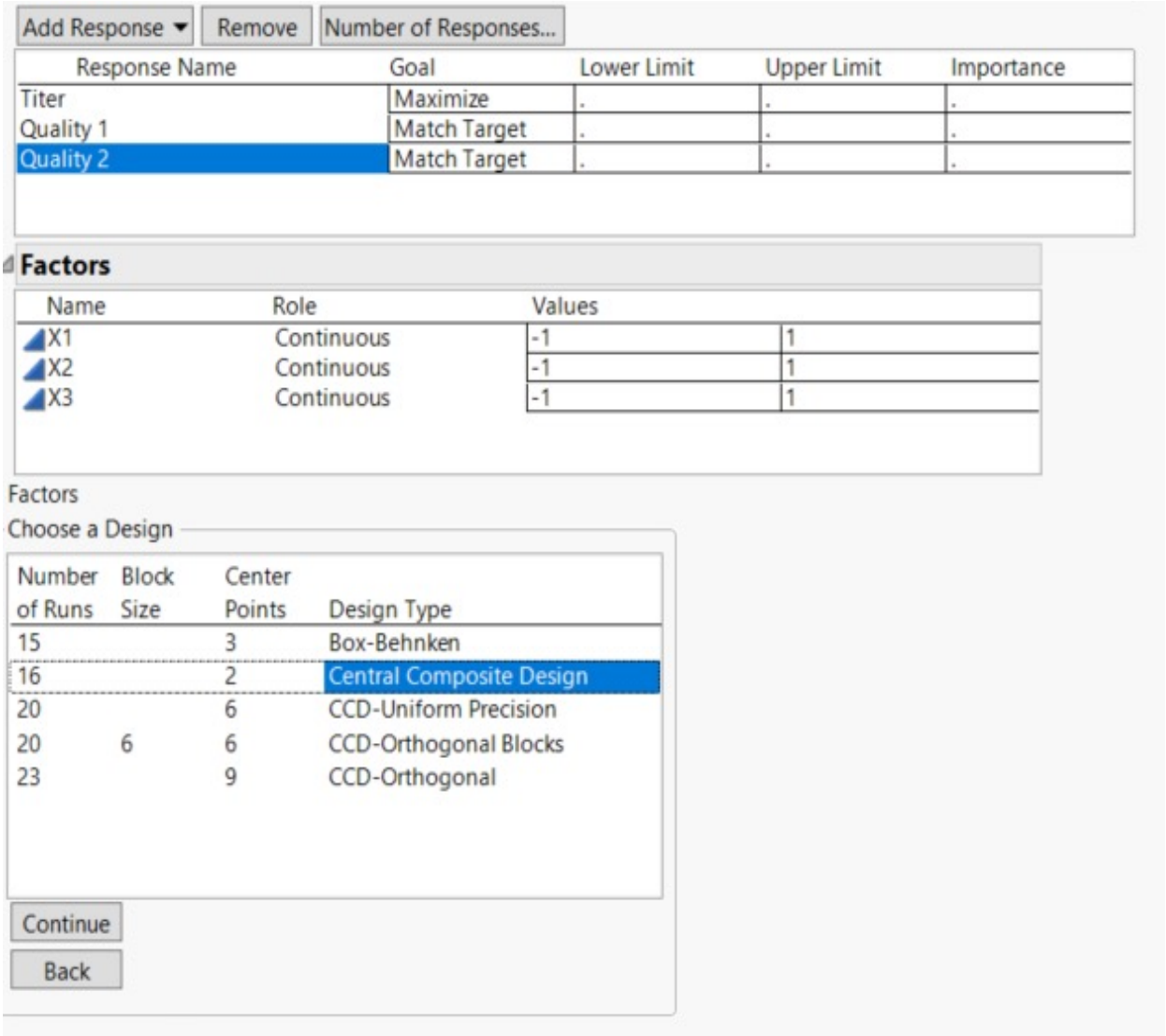
(and thus have three levels), which allows a curve rather than a straight line for each continuous factor.[14,15]

DSDs are not appropriate in some circumstances. Although DSDs are efficient, in systems with many significant factors and interactions DSDs can only screen for main effects. Further, DSDs should not be used when there are constraints on the design region because an implicit assumption behind the use of DSDs is that it is possible to set up levels of a factor independently of the level of any other factor; when this is not the case, certain factor combinations are not feasible and thus cannot be evaluated in a DSD. In addition, DSD should not be used when some of the factors are ingredients within a mixture. For example, if the percentage of one ingredient (e.g., media) is increased, the percentage of another ingredient (e.g., additives) must decrease, so these factors cannot vary independently. Another scenario in which to avoid the use of DSD is when there are categorical factors with ≥2 levels. Although DSDs can run with a few categorical factors at two levels, a DSD with too many categorical factors with ≥2 levels is inefficient. DSDs run as split-plot designs also should be avoided. Lastly, DSDs should not be used when the a priori model of interest has higher order effects because cubic terms are confounded in DSDs.[16,17]

Output for PC: Proven Acceptance Ranges and Normal Operating Ranges

After completion of the PC study and analysis of data, the results are utilized to establish process control limits and

Figure 4-9. *Response Surface Methodology Responses*



ranges and to understand criticality. Normal operating range (NOR), in which the process is allowed to vary around a set point target value with no negative effect on the process, occurs for all parameters in a process. NORs should be wider than the ability to control the parameter at target. For example, the bioreactor agitation NOR may be 180 rpm to 220 rpm, but the system can control this parameter within 10% of the target. Thus, NORs are considered ranges that are practically achievable. Most two-sided NORs have the target point within one or two equipment capacities. Once additional statistical analysis is available following characterization, statistical modeling may be used to determine the NOR.

Some NORs are carried through from the beginning of development with no changes, whereas others are adjusted and honed to improve productivity or quality during process development. NORs are not used to define design space but can be adjusted based on characterization studies. NORs can be widened if there is a need to provide more range around the ability of the equipment to control the parameter at the set point and there is no negative effect on the process. Alternatively, NORs can be narrowed if there is need to more tightly control the parameter to prevent failure, for example if the characterization study identified a NOR was too close to the failure point. In this case, the range may be

Figure 4-10. *Example of Response Surface Methodology Output*

	Pattern	Pattern Description	X1	X2	X3	Titer	Quality 1	Quality 2
1	00A	Axial	0	0	1.6817928305	•	•	•
2	+−−	Corner	1	-1	-1	•	•	•
3	−−−	Corner	-1	-1	-1	•	•	•
4	0A0	Axial	0	1.6817928305	0	•	•	•
5	+++	Corner	1	1	1	•	•	•
6	0a0	Axial	0	-1.681792831	0	•	•	•
7	−++	Corner	-1	1	1	•	•	•
8	−−+	Corner	-1	-1	1	•	•	•
9	++−	Corner	1	1	-1	•	•	•
10	000	Center	0	0	0	•	•	•
11	A00	Axial	1.6817928305	0	0	•	•	•
12	+−+	Corner	1	-1	1	•	•	•
13	00a	Axial	0	0	-1.681792831	•	•	•
14	a00	Axial	-1.681792831	0	0	•	•	•
15	000	Center	0	0	0	•	•	•
16	−+−	Corner	-1	1	-1	•	•	•

narrowed if it can be practically controlled. Alternatively, the entire range and target may shift away from the failure point if there is no risk of moving close to another failure point. For example, the affinity column residence time characterization study using a range of 1.5 to 5.5 min demonstrated that 1.5 min was too short and 5.5 had no negative effect on the process and CQAs. Operating with a NOR of 2 to 4 min with a target of 3 min may be considered too close to the failure point. It could be decided to narrow the range to 2.5 to 4 min or shift the target to 3.5 and shift the range to 2.5 to 4.5 min. For each parameter, the NOR will need to be determined following the PC.

A PAR allows deliberate change in one parameter without changing the others outside their NOR/target. PARs may be presented in the description of the manufacturing process of the drug substance as ranges. PARs for single parameters are proposed in the licensing application and are subject to regulatory assessment and approval. The PAR should be adequately justified regardless of whether the process parameter is considered a critical process parameter or not (ICH Q8 R2).

Where interaction effects between different parameters exist and the acceptable range for one process parameter depends on the setting of another parameter, the parameters should be included in a Design Space. Alternatively, a PAR can be defined for only one of the parameters in the process description, and other process parameters will be limited to target operating condition or NOR.

For some parameters, the characterization range and/or knowledge range may be outside of the PAR. This can be due to findings in development or if the characterization studies identified failure points in the range. Not all parameters will have a knowledge range and not all will have a PAR. Figure 12 shows the nesting that may occur if a parameter has all three levels.

Parameter Criticality Assessment

Parameter criticality assessment is done to assess the overall criticality of parameters (e.g., as key or non-key). Following PC, every process parameter and material is classified based on the effect it has on the CQAs and the process performance (PIs). This is done quantitatively for parameters studied in PC and qualitatively for the remaining parameters. There are 3 levels of classification used in a common process. The classification of the parameters is used to build the P/ACS (process/analytical control strategy).

Figure 4-11. *Example of DSD output*

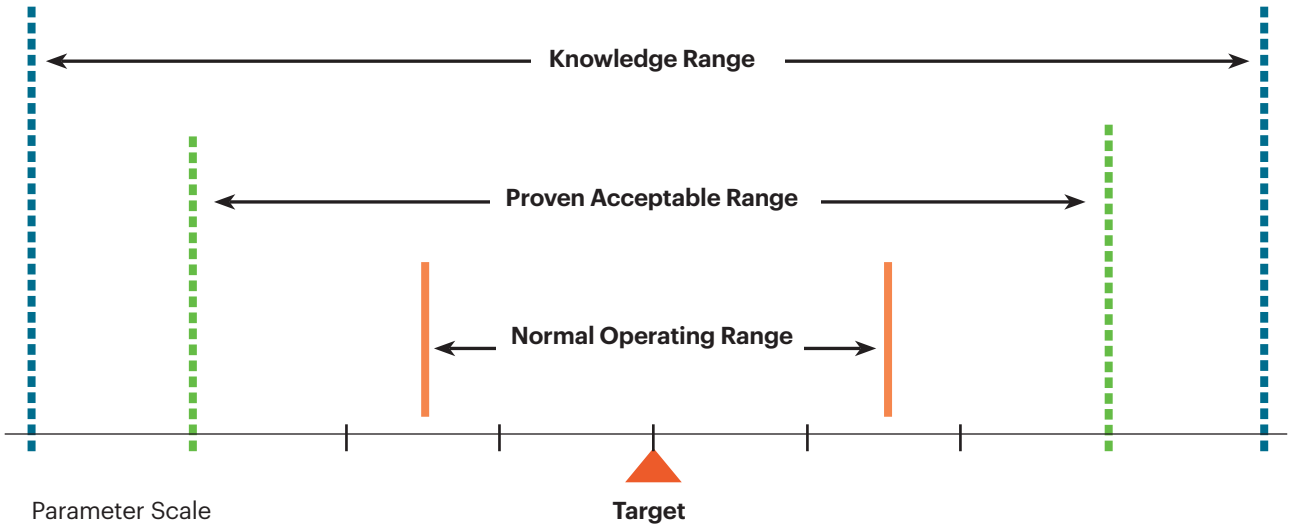
		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10
1	**13**	0	1	1	1	1	1	1	1	1	1
2	**16**	0	-1	-1	-1	-1	-1	-1	-1	-1	-1
3	**21**	1	0	-1	-1	-1	-1	1	1	1	1
4	**17**	-1	0	1	1	1	1	-1	-1	-1	-1
5	**8**	1	-1	0	-1	1	1	-1	-1	1	1
6	**5**	-1	1	0	1	-1	-1	1	1	-1	-1
7	**7**	1	-1	-1	0	1	1	1	1	-1	-1
8	**4**	-1	1	1	0	-1	-1	-1	-1	1	1
9	**15**	1	-1	1	1	0	-1	-1	1	-1	1
10	**10**	-1	1	-1	-1	0	1	1	-1	1	-1
11	**6**	1	-1	1	1	-1	0	1	-1	1	-1
12	**14**	-1	1	-1	-1	1	0	-1	1	-1	1
13	**18**	1	1	-1	1	-1	1	0	-1	-1	1
14	**2**	-1	-1	1	-1	1	-1	0	1	1	-1
15	**9**	1	1	-1	1	1	-1	-1	0	1	-1
16	**12**	-1	-1	1	-1	-1	1	1	0	-1	1
17	**20**	1	1	1	-1	-1	1	-1	1	0	-1
18	**11**	-1	-1	-1	1	1	-1	1	-1	0	1
19	**3**	1	1	1	-1	1	-1	1	-1	-1	0
20	**1**	-1	-1	-1	1	-1	1	-1	1	1	0
21	**19**	0	0	0	0	0	0	0	0	0	0
22	**22**	0	0	0	0	0	0	0	0	0	0
23	**23**	0	0	0	0	0	0	0	0	0	0
24	**24**	0	0	0	0	0	0	0	0	0	0
Best		-1	0	0	0	1	0	-1	-0.4	0	0
Worst		1	0	0	0	0	0	1	0	0	0

A process parameter is an input variable of the manufacturing process that can be directly controlled such as temperature, time, pH, flow rate, etc. Based on their impact to process performance and product quality, process parameters are divided into the following three classes (definitions of which can be found in Chapter 3): critical process parameter (CPP), key process parameter (KPP), and non-critical process parameter (non-CPP).

Two classification decision trees are provided in Figure 13 and Figure 14. Figure 13 covers parameter classification for parameters that were characterized and which have quantitative data supporting decision-making. Figure 14 covers parameters that fell below the water line and were not selected for characterization; therefore, classification is made qualitatively based on scientific knowledge, literature, or previous experience.

For a parameter that was characterized, and data reflects the potential to affect a CQA with both statistical

Figure 4-12. *Nesting of Ranges Around the Target*



and practical significance, it is deemed a CPP. Statistical significance relates to whether an effect exists. Practical significance refers to the magnitude of the effect. A CPP will have a specified range in which the operation must be maintained. Falling outside of the range will result in an investigation and will likely lead to a rejected batch due to potential effect on a CQA.

If the effect was statistically significant but is not practically significant (relating to efficacy or safety), or if there was no statistical effect on a CQA, then it will be considered further. If the parameter affected the process performance, then it will be classified as a KPP. A KPP will have a control range, and falling outside of the control range will trigger a quality action to determine the outcome of the batch. If the parameter does not affect CQAs or process performance, it is classified as a non-key parameter. Non-key parameters have control ranges and are monitored in continued process verification. For all other parameters that were not selected for PC, a science-based decision is made to classify the parameters as CPP, KPP or non-key. Each classification of a parameter will require documenting the rationale for the classification assignment, as this will be part of the licensing application. For CPPs, the CQAs that are affected will need to be identified. For KPPs, the effect on the performance and the action limits will need to be defined. The P/ACS will be used to define the controls used to reduce risk to the quality and robustness of the process.

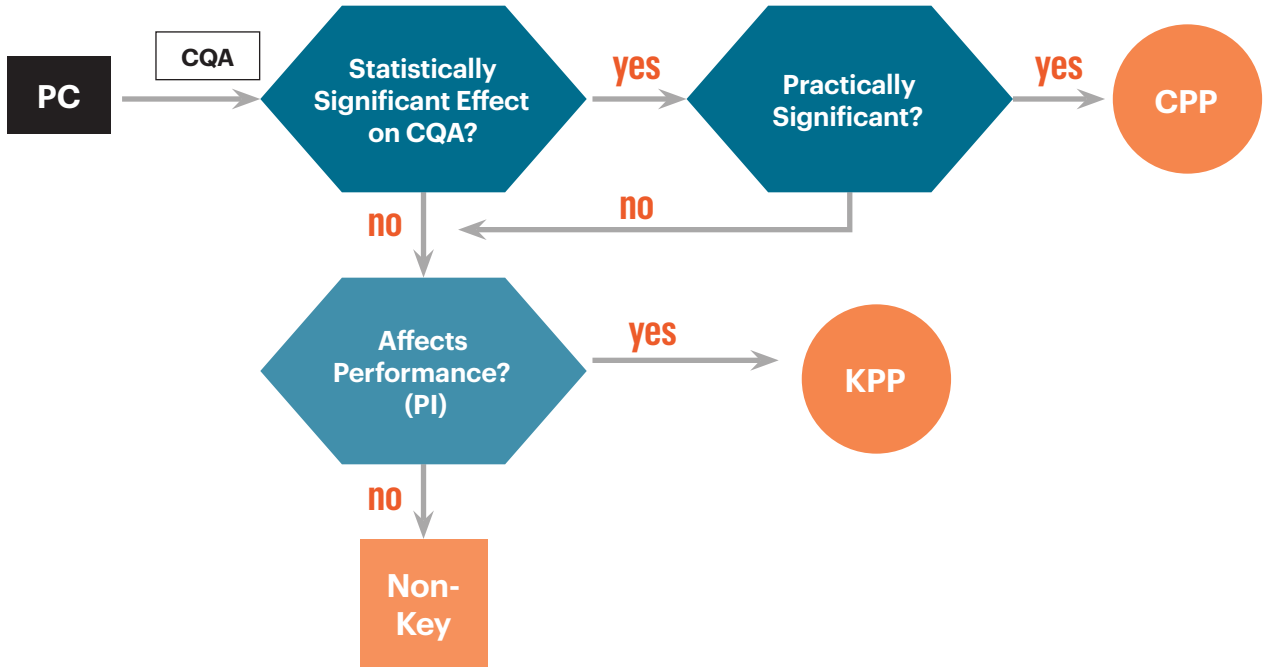
Parameter Classification Assessment (PCA) or FMEA

Failure mode and effects analysis (FMEA) is a step-by-step approach to identify all possible failures in the manufacturing process. In FMEA, failures are categorized based on how serious the consequences of the failure are, how frequently the failure occurs, and how easily they can be detected. Ultimately, the goal of FMEA is to eliminate or reduce failures starting with those that have the greatest effect on the process. The use of FMEA in QbD processes allows the documentation of current knowledge and actions about the risk of failures to be used in continuous process improvement. FMEA should begin during the earliest stages of process design and continue throughout the entire process.

For gene therapy or biologics, prior to the generation of the P/ACS (process/analytical control strategy) for PPQ, a process control FMEA will be conducted by development and manufacturing teams to ensure any failures with potential impact to product quality have a documented mitigation and control strategy.

Process parameter classification is governed by FMEA and is also assessed by results of the process parameter risk assessment and using data from completed PC studies. The purpose of process parameter classification is to use the results of these completed PC studies to classify process parameters and input material attributes based on their likelihood of having an impact on process

Figure 4-13. *Decision Tree for Parameter Classification for Characterized Parameters Based on Quantitative Data*

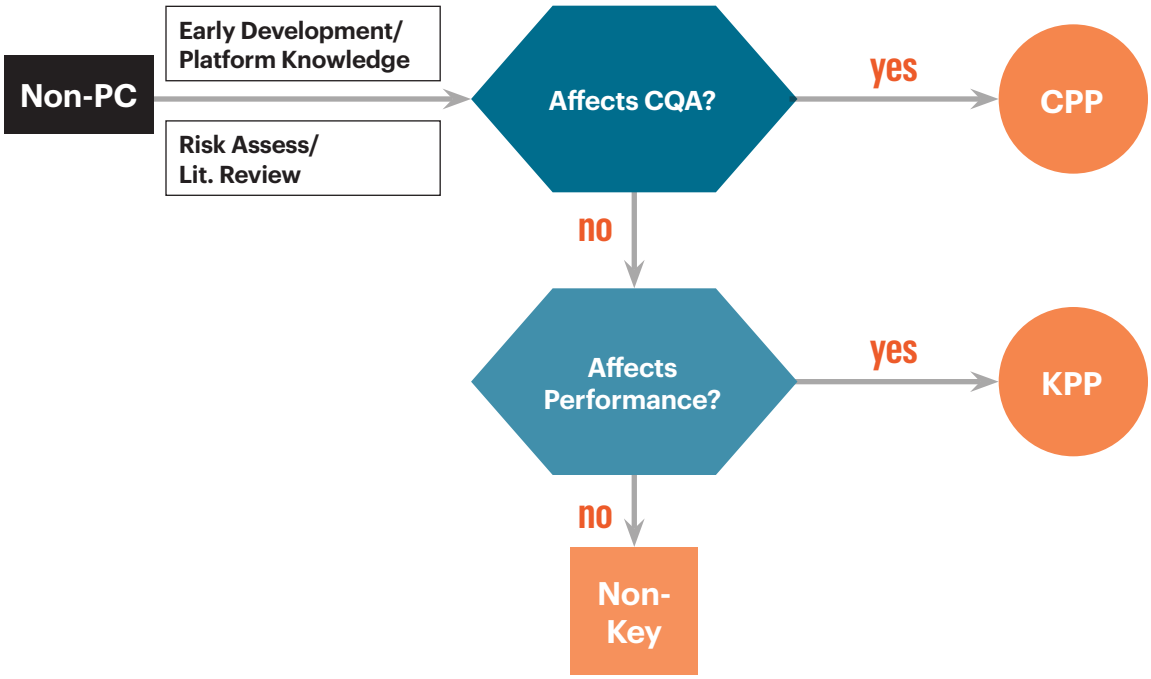


performance or product quality. Criticality is assigned based on the impact to drug substance and drug product quality, by establishing a link between CPPs and CQAs. Each process parameter is classified as either a CPP or non-CPP based on its potential impact on quality. A process parameter is classified as a KPP based on its potential impact to critical process performance attributes. This classification is used to develop a P/ACS that ensures that CPPs are adequately monitored and/or controlled. In addition, the FMEA should be used to designate certain materials as critical raw materials and to develop an appropriate strategy to ensure that raw materials, starting materials, reagents, solvents, intermediates, and process components do not have a negative impact on product quality.

The process control FMEA builds upon the risk assessment performed during the initial PRA phase and begins with a formal risk assessment of each process parameter based upon the "SOD" model of severity (potential magnitude of impact of parameter excursion to the quality of the product), occurrence (predicted likelihood or frequency of an excursion, based on historical process knowledge), and detection (ability of the manufacturing team to observe the excursion and take steps to mitigate). In the highest severity scores, excursion may have potential for severe effect on safety and efficacy, may result in a serious adverse event, or may result in loss of product and adversely affect subsequent unit operations. In the highest occurrence scores, the excursion has been documented frequently with the historical platform and potential for excursion is generally expected to be significant in a high percentage of runs. In the highest detection scores, no known controls are available to detect the particular failure mode and as such excursion may occur without real-time knowledge. Based on individual SOD scores for each process parameter, a risk priority number (RPN) is calculated, which is essentially the product of individual parameter SOD scores.

Whereas the initial SOD stage of the FMEA defines high-risk parameters, the second stage of the process control FMEA assesses multiple aspects of the manufacturing process (equipment capacity, normal operating ranges, process characterization range, parameter setpoint, potential failure modes/causes) along with various controls in place to prevent failure (engineering controls, process controls, facility controls, personnel controls, testing, PAT, etc.). The purpose of the second stage of the FMEA is to compile all potential for risk mitigation on a parameter-specific basis with the intent of

Figure 4-14. *Decision Tree for Parameter Classification for Uncharacterized Parameters Based on Science-Based Knowledge*



modifying the RPN to reflect a mitigation-adjusted RPN. As such, knowledge and experience compiled during PC and prior manufacturing runs can be applied to some of the higher-risk parameters to modify the criticality based on process control. The end result of the process control FMEA is a shorter list of parameters that are deemed critical.

Control Strategy for Critical Process Parameters

In addition to establishing parameter control ranges as the previous section discussed, certain steps within a process may require additional controls to ensure that the process is performing as expected. In these instances, in-process controls (IPC) are established. These are determined by the process development team using the combination of process development, scale-up, and characterization study data to provide expected ranges at specific steps. Some examples of IPC that may be filed in the licensing application as part of the control strategy include: viability and viable cell density of the cells following transfer of the cell bank vial contents to the shake flask, viable cell density and cultivation time prior to transfection, elution volume of the affinity column, step-yields at key unit operations, and IPC. IPC, process monitoring, material control, product control through quality testing, and stability are all parts of the control strategy, which will be discussed thoroughly in Chapter 6. In this chapter, we will focus on points that are gene therapy–specific through the next few examples.

POTENCY

The FDA published guidance for potency testing of gene therapy in 2011. Potency is defined 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." Regulations require potency testing through *in vitro* and/or *in vivo* tests that have been specifically designed for each product. Potency measurements are used to demonstrate that product lots meet the predefined specifications or acceptance criteria not only during all phases of development, but also following market approval. Often, a single biological or analytical assay will not provide sufficient measurement of potency, so multiple complementary

assays, referred to as an assay matrix, can be developed that measure potency through measures of quality, consistency, and stability. An assay matrix may include assays that provide both quantitative readouts (e.g., units of activity) and qualitative readouts (e.g., pass/fail).[18]

VIRAL CLEARANCE

Some AAV platforms require the addition of a virus, such as adenovirus. In the downstream process design, virus inactivation (i.e., heat, acid) and viral clearance need to be in place before filing. Extensive viral clearance using another model virus is also required to demonstrate the process capability. Normally, viral clearance is a product-specific practice and required for the production of every product. For additional information, please refer to Chapter 5 on Upstream/Downstream Processing.

HCDNA

AAV can package a large amount of nonvector DNA (e.g., plasmid DNA, helper virus sequences, host DNA), and it may be challenging to remove this DNA from the product to the agency-requested level to ensure safety.[19] Therefore, cell lines and helper vectors must be designed and selected carefully to reduce product risks. Quality data, risk assessments, and/or details of their process, and product control strategies should be in place to address and mitigate potential risks using the selected system.

Process Analytical Technologies (PAT)

Another way of ensuring that quality is built into the process is to mitigate some key process risks by utilizing process analytical technology (PAT). PAT is a framework used to design, analyze, and control "manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality.[20,21] Through the use of PAT, better process control is gained by identifying and managing the sources of variability throughout the process and proactive decision-making throughout the process. It also results in reduced cost due to optimized use of raw materials and minimization of product cycle times.[22] Ultimately, the main objective of PAT is to monitor in real-time the values of some process parameters, such as viable cell concentration (VCC) and nutrient and metabolite concentrations that may impact product quality attributes.[23]

PAT uses a risk assessment template for parameter criticality to mitigate some of the risk. The PAT used affects the number of critical process parameters in the process. Relative to monoclonal antibodies, viral products are notoriously uncharacterized, but some considerations may include monitoring particle size of transfection complexes (lentiviral vectors and AAV vectors), and cell size/growth and metabolites (i.e., Raman, capacitance, microscopic based instruments).

Whereas much monitoring has been "retrospective" in the past, it is important to obtain real-time information to inform the process strategy and control using PAT. For example, bioreactors typically have probes that allow for the monitoring of the culture environment, such as physicochemical factors (e.g., temperature, pH, dissolved oxygen); however, it is more difficult to measure other cell culture parameters, such as glucose/lactate concentration, cell density, or cell population characterization. It is vital that characteristics such as these be monitored to ensure efficiency and safety of the product and reduce batch-to-batch variability. Online monitoring technology is important for automatic feedback control of the culture, increasing knowledge of the process and facilitating QbD approaches. The following sections discuss online monitoring technology that can be used to improve batch consistency and efficiency.[24]

Compared to the process for monoclonal antibodies, unique considerations exist for PAT in gene therapy.

CELL GROWTH/MORPHOLOGY MONITORING:

Even for capacitance probe or Raman, which is commonly used for CHO cell and monoclonal antibody production, multiple platforms are used within the same company for gene therapy. Therefore, tools may be either process- or platform-specific (i.e., HEK vs Sf9). In addition, cells will experience either transfection or infection along the process, and morphology or growth could be impacted for that unique step, which introduces additional challenges. On the other hand, if the changes can be captured, the infection/transfection step can also be monitored.

TRANSFECTION/INFECTION MONITORING:

The majority of biologics production utilizes stable cell lines. Although some protein production processes also use transient transfection, normally only a single plasmid is involved. In contrast, for AAV production, either multiple plasmid transfections or virus infections are included in the upstream step. This is a critical step that requires careful monitoring. For transfection specifically, transfection efficiency may be monitored through product production or cell morphology changes or via the transfection complex (the size of the complex is related to the transfection efficiency).[25] Some DLS methods mentioned below could be used to monitor the kinetic change for transfection complex size.

PRODUCT MONITORING:

For downstream and formulation steps, most of the protein monitoring system can be used to track AAV. However, the product is a protein capsid with DNA inside. Tracking both protein and DNA can reflect not only the yield but also quality of the product (i.e., whether empty capsid has been removed).

CAPACITANCE PROBE

VCC is a key performance indicator during upstream technologies. VCC is often measured through offline methods (e.g., staining dead cells with Trypan Blue and counting cells microscopically). Online monitoring of biomass remains challenging due to complex calibration and integration of analytics. However, one such online technique is radio frequency impedance, which can be measured in the cell broth via capacitance probes to monitor cell concentration online. This principle is based on the polarization of the cells by applying a periodic electric field to the system. Only viable cells are polarized and thus can be evaluated to correlate VCC. In the past, capacitance sensors have been used to monitor growth and infectious status. A study published in 2020 found that capacitance sensors are also able to successfully facilitate the scale-up of bioreactor processes from 50 L up to 2000 L. Using such an approach can help to preserve resources and reduce failures by keeping the batch within the approved trajectory for VCC. In addition, capacitance probe technology allows faster process development and better mitigation of process risks compared to older technology. Thus, capacitance sensors likely represent a viable method for monitoring VCC in gene therapy applications, but it is not without unique challenges, such as limitations of measurement during the stationary growth phase and death phase due to cell diameter changes of apoptotic cells.[26,27]

DIFFERENTIAL DIGITAL HOLOGRAPHIC MICROSCOPY (DDHM)

Few methods are available to monitor viral particle production during cell culture. Existing processes use chemometrics approaches by measuring process variables related to viral production kinetics or changes in cell morphology or physiology. Ideally, these processes should be monitored via label-free methodologies to avoid the addition of compounds that may influence cellular behavior. While most label-free cell culture monitoring methods use spectroscopic techniques, image-based cell monitoring is also an option. Because cells are mostly transparent, systems must be in place to generate the needed image contrast. One such method is digital holographic microscopy. A study published in 2020 found that DDHM was successfully used to monitor cell concentration and viability and also assess AAV production kinetics in an insect cell system. While most attributes that are calculated via DDHM have no biologic meaning per se, they can be used collectively to characterize a dynamic phenotype that is indicative of cell adaptation to various biological situations. However, some attributes calculated with DDHM may be more directly relevant, such as phase correlation (e.g., time-specific characteristics that are similar to the culture viability profiles). Even more so, attributes more directly related to viable cell concentration rely on light intensity (due to light dispersion caused by suspension cells, which is analogous to turbidity-based measurements). In AAV in particular, DDHM appears to create "phase skewness" (which refers to a lack of symmetry for the phase histogram of the cell) due to the molecular density of certain organelles (e.g., nucleus, nucleolus) compared to surrounding regions, and the location of AAV capsid assembly (nucleolus). DDHM appears to be a valuable tool to support online monitoring to determine time of

Table 4-11. *Summary of Spectroscopic Techniques*[43]

Method	Measured Attributes	Application	Reference	Notes
Near-infrared	Vibrational overtones of peptide backbone	Identification of analytes	Rüdt (2017)[44]	Low sensitivity and selectivity
Raman spectroscopy	Vibrational; peptide backbone	Glucose, glutamine, glutamate, lactate, and ammonium concentrations; VCC; product concentration	Rüdt (2017)[45]	Generally low sensitivity but high selectivity
Fluorescence spectroscopy	Intracellular fluorophores	rAAV production; aggregation; amino acid concentration	Pais (2019)[46] and Pais (2020)[47]	Broad measurement ranges possible; difficult calibration; low-cost; high selectivity
Dynamic light scattering (DLS)	Diffusion behavior of macromolecules	Protein folding Particle size	Rüdt (2017)[48]	Based on time correlation
Dielectric spectroscopy	Dielectric potential of cells in an alternating electrical field	VCC, cell biovolume	Pais (2019)[49] and Pais (2020)[50]	Based on changes in cell physiology; continuous monitoring; high sensitivity

Adapted from Rüdt M, et al. J Chromatogr A. 2017;1490:2-9.

harvest and to establish controlled feeding strategies, and allows a simpler workflow with real-time monitoring compared to other methods.[28]

SPECTROSCOPY TECHNOLOGY

Glucose or lactate concentrations can be measured online in bioreactor cultures via spectroscopic analysis, such as Raman spectroscopy, near infrared (NIR) spectroscopy, or fluorescence techniques.[42] An overview of spectroscopy techniques is shown in Table 11.

Raman spectroscopy is a technique that can be used to observe molecular vibrations to identify and quantify molecules by measuring changes in the wavelength of laser light to identify which molecules are present within the cell culture media. It can be used to noninvasively measure time-dependent molecular properties of cells (without labels) during bioreactor growth by providing information about cell phenotype.[38] Markers, which may include glucose, glutamine, glutamate, lactate, ammonium, VCC, and product concentrations, among others, can be evaluated in real-time with Raman spectroscopy to inform adaptive manufacturing and decision-making by providing immediate feedback on process performance.[39] However, the success for Raman models depends on calibration techniques. Traditionally, Raman spectroscopy calibration generates highly specific models, but these models are only reliable in the exact conditions in which they are calibrated. Thus, it is expected that model performance would degrade over time due to changes in the process (recipe changes, raw material variability, process drifts). To circumvent this issue, a real-time just-in-time learning (RT-JITL) framework or other methods can be used to automatically calibrate, assess, and maintain Raman models. The RT-JITL framework

allows the calibration of generic models that can be used in cell culture experiments with various conditions. The use of generic calibration models allows the real-time prediction of cell culture performance parameters without jeopardizing the calibration component of this highly important process.[40]

NIR spectroscopy is an online, time-efficient, noninvasive technique that measures the interaction of near-infrared light with a sample to facilitate the identification of analytes, especially glucose, within the bioreactor. NIR spectroscopy can also facilitate the scale-up process by developing models. However, NIR use may be limited for glucose concentration monitoring due to deviations in accuracy during some phases of cell culture, such as when feeds are added to the culture.[41]

It is impossible for one sensor to measure all product quality attributes during production. In fact, even one attribute may require multiple sensors. Therefore, multimodal spectroscopy may be needed. Multimodal spectroscopy may include various types of spectroscopy, including UV spectroscopy and DLS. When using multiple sensors in a process stream, it is important to account for dispersion between the detectors. Therefore, accurate data analysis is necessary to extract correct conclusions.[42] DLS has been used for particle size and concentration for AAV gene product. The potential application for AAV production would be product formulation and purification as well as to monitor the transfection mix.

Fluorescence spectroscopy uses electronic and vibrational states and is based on the excitation of species from the ground electronic state to a vibrational state in its excited electronic state. It can be used to monitor cell and product formation, as well as metabolite consumption and production in various biological systems. For example, fluorescence spectroscopy can help to assess the concentrations of aromatic amino acids in the bioreactor (e.g., tryptophan) and has been proposed as an in-line PAT tool for a chromatography purification step of a fusion protein.[43] It is also possible to monitor VCC and recombinant protein titers in mammalian cell culture systems, as well as CQAs such as aggregation. A recent study found that in situ fluorescence spectroscopy with recombinant AAV can predict relevant process variables, such as viability and product titer, to enable PAT. However, complications associated with fluorescence spectroscopy in gene therapy–related applications include difficult interpretation of results due to the rAAV production profile, which increases for some time before decreasing and then plateauing throughout the remaining culture time. In addition, it may take more time for spectra acquisition than other methods.[44]

For biologics, many techniques are already included in GMP. Most of the techniques used in biologics may also be used in AAV-specific technologies, although some technologies are gene therapy–specific. For AAV, especially for an SF9 system, multiple PAT tools apply, but most techniques are still in the early stage (at bench scale).

Conclusion

In closing, as many gene therapy products proceed toward late-stage development and BLA-enabling activities, it is going to be increasingly important to take lessons from biologics and vaccines in applying a systematic, risk-based approach for late-stage development. The individual PRAs described in this chapter allow a large pool of hundreds of parameters to be whittled down to a smaller number of parameters deemed important for further characterization during late-stage development. This allows a greater amount of focus on aspects of the process that present the highest amount of risk to the overall quality of the product. Risk continues to be mitigated throughout the process by identifying critical process parameters and using a combination of risk assessments and process analytical technology to ensure that the commercial manufacturing process is robust and reproducible.

CMC Readiness Checklist/Considerations For Expedited Pathways

Because the FDA does not have guidance in this area, a helpful list of questions to be considered by manufacturers of cell and gene therapy products is found here.

- ☐ Have you performed a careful review of your manufacturing process to ensure that you are entering phase III trials with a product that is optimal?
- ☐ Have you introduced major manufacturing changes that may require conducting comparability studies and if so, what is your plan for conducting such comparability studies?
- ☐ What is the status of your analytical method development? Have you qualified or preferably validated your assays prior to initiation of your pivotal trial?
- ☐ Do you have appropriate potency assays in place for the final drug product?
- ☐ Do you have knowledge of CQAs, CPPs, and KPPs?
- ☐ Have you determined the shelf life of the final drug product by conducting stability assays using assays that are appropriate and qualified/validated?
- ☐ Do you have a well-defined plan to collect materials and reserve samples for in-process and the final drug product?
- ☐ What is your plan of action for conducting process validation to demonstrate that the final drug product can be successfully manufactured consistently?
- ☐ Have you defined standard operating procedures (SOPs), protocols, and/or instructions for use in outlining any additional manufacturing, processing, formulation, or thaw/dilution of the final drug product at clinical sites?
- ☐ Do you plan to gain a better understanding of the requirements for conducting leachable and extractable studies for materials that are in direct contact with your product?
- ☐ What is your plan for manufacturing of the final drug product? Do you anticipate needing to make a change to your existing facility? Do you plan for automation, scale-out, or scale-up post approval or prior to initiation of phase III study?
- ☐ Have you made a final determination of whether the current release specifications are adequate for ensuring safety and potency of your final drug product?
- ☐ Have you conducted shipping validation for source materials and the final drug product under worst-case scenarios or conditions of transport?
- ☐ Have you reviewed the quality of ancillary materials as well as the reliability and sustainability of your supply chain, and do you have a plan to review your quality agreements and SOPs that are in place for material qualification and vendor qualification? Have you developed an identity test for your critical ancillary materials?
- ☐ Have you finalized your choice of the final container and have a plan for how to affix the label on the final drug product?
- ☐ What is your plan for testing of the source material, in-process materials, or the final drug product? Do you plan to outsource your testing, or will it be conducted in-house?
- ☐ Do you need to develop any in-house standards (physical or performance standards) for your assays? Do you know what standards are needed for your product development and release testing?
- ☐ Have you had an End of Phase 2 (EOP2) meeting with the agency to assess your CMC readiness?

Endnotes

1. *Wright JF. Gene Therapy. 2008;15:840-848.*
2. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
3. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
4. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
5. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
6. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
7. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
8. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
9. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
10. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
11. *Food and Drug Administration. Guidance for industry. Process validation: general principles and practices. Food and Drug Administration website. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf. Published January 2011. Accessed May 4, 2020.*
12. *Parenteral Drug Association. PDA Technical Report No. 60 (TR 60): Process Validation: A Lifecycle Approach. February 2013; Bethesda, MD: Parenteral Drug Association.*
13. *Wright JF. Biomedicines 2014;2:80-97.*
14. *Jones B, Nachtsheim CJ. A class of three-level designs for definitive screening in the presence of second-order effects. J Qualt Technol. 2011;43(1):1-15.*
15. *Tai M, Ly A, Leung I, Nayar G. Efficient high-throughput biological process characterization: definitive screening design with the Ambr250 Bioreactor System. Biotechnol Prog. 2015;31(5):1388-1395.*
16. *Jones B, Nachtsheim CJ. A class of three-level designs for definitive screening in the presence of second-order effects. J Qualt Technol. 2011;43(1):1-15.*
17. *Tai M, Ly A, Leung I, Nayar G. Efficient high-throughput biological process characterization: definitive screening design with the Ambr250 Bioreactor System. Biotechnol Prog. 2015;31(5):1388-1395.*
18. *US Department of Health and Human Services, Food and Drug Administration. Guidance for industry: potency tests for cellular and gene therapy products. https://www.fda.gov/media/79856/download. Published January 2011. Accessed June 26, 2020.*
19. *US Department of Health and Human Services, Food and Drug Administration. Guidance for industry: chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs). https://www.fda.gov/media/113760/download. Published January 2020. Accessed July 8, 2020.*
20. *US Department of Health and Human Services, Food and Drug Administration. Guidance for industry: PAT – a framework for innovative pharmaceutical development, manufacturing, and quality assurance. US Food and Drug Administration website. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance. Published 2004. Accessed June 26, 2020.*
21. *Baradez M, Biziato D, Hassan E, Marshall D. Application of Raman spectroscopy and univariate modeling as a process analytical technology for cell therapy bioprocessing. Front Med. 2018;5:47.*

Endnotes, continued

22. *Marshall D, Ward S, Baradez M. Requirement for smart in-process control systems to deliver cell therapy processes fit for the 21st century. Cell and Gene Therapy Insights. http://insights.bio/cell-and-gene-therapy-insights/wp-content/uploads/2016/12/Marshall-et-al.pdf. Accessed June 26, 2020.*
23. *Zavala-Ortiz DA, Ebel B, Li M, et al. Interest of locally weighted regression to overcome nonlinear effects during in situ NIR monitoring of CHO cell culture parameters and antibody glycosylation Biotechnol Process. 2020;36:e2924.*
24. *Pezzoli D, Giupponi E, Mantovani D, Candiani G. Sci Rep. 2017;7:44134.*
25. *Joshi PRH, Cervera L, Ahmed I, et al. Achieving high-yield production of functional AAV5 gene delivery vectors via fedbatch in an insect cell-one baculovirus system. Mol Ther Methods Clin Dev. 2019;13:279-289.*
26. *Metze S, Ruhl S, Greller G, Grimm C, Scholz J. Monitoring online biomass with a capacitance sensor during scale-up of industrially relevant CHO cell culture fed-batch process in single-use bioreactors. Bioprocess Biosystems Engineering. 2020;43:193-205.*
27. *Pais DAM, Galrão PRS, Kryzhanska A, Barbau J, Isidro IA, Alves PM. Holographic imaging of insect cell cultures: online non-invasive monitoring of adeno-associated virus production and cell concentration. Processes. 2020;8:487.*
28. *Rodrigues CAV, Nogueira DES, Cabral JMS. Next-generation stem cell expansion technologies. http://insights.bio/wp-content/uploads/sites/2/2018/11/0408_Bioprocessing_Spotlight.pdf. Published October 31, 2018. Accessed July 8, 2020.*
29. *Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics – spectroscopy: an emerging process analytical technology. J Chromatogr A. 2017;1490:2-9.*
30. *Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics – spectroscopy: an emerging process analytical technology. J Chromatogr A. 2017;1490:2-9.*
31. *Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics – spectroscopy: an emerging process analytical technology. J Chromatogr A. 2017;1490:2-9.*
32. *Pais DAM, Portela RMC, Carrondo MJT, Isidro IA, Alves PM. Biotech Bioeng. 219;116:2803-2814.*
33. *Pais DAM. Development of advanced monitoring and control tools for rAAV production in the insect cell system [thesis]. https://run.unl.pt/bitstream/10362/97879/1/Daniel%20Pais%20PhD%20Thesis%20Final.pdf. Published April, 2020.*
34. *Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics – spectroscopy: an emerging process analytical technology. J Chromatogr A. 2017;1490:2-9.*
35. *Pais DAM, Portela RMC, Carrondo MJT, Isidro IA, Alves PM. Biotech Bioeng. 219;116:2803-2814.*
36. *Pais DAM. Development of advanced monitoring and control tools for rAAV production in the insect cell system [thesis]. https://run.unl.pt/bitstream/10362/97879/1/Daniel%20Pais%20PhD%20Thesis%20Final.pdf. Published April, 2020.*
37. *Rodrigues CAV, Nogueira DES, Cabral JMS. Next-generation stem cell expansion technologies. http://insights.bio/wp-content/uploads/sites/2/2018/11/0408_Bioprocessing_Spotlight.pdf. Published October 31, 2018. Accessed July 8, 2020.*
38. *Baradez MO, Biziato D, HassanE, Marshall D. Application of Raman spectroscopy and univariate modelling as a process analytical technology for cell therapy bioprocessing. Front Med. 2018;5:47.*
39. *Tulsyan A, Wang T, Schorner G, Khodabandehlou H, Coufal M, Undey C. Automatic real-time calibration, assessment, and maintenance of generic Raman models for online monitoring of cell culture processes. Biotech Bioengin. 2019;117(2):406-411.*
40. *Koxma B, Salgó A, Gergely S. On-line glucose monitoring by near infrared spectroscopy during the scale up steps of mammalian cell cultivation process development. Bioprocess Biosystems Engineer. 2019;42:921-932.*
41 *Rolinger L, Rüdt M, Hubbuch J. A critical review of recent trends, and a future perspective of optical spectroscopy as PAT in biopharmaceutical downstream processing. Analytic Bioanalytic Chem. 2020;312:2047-2064.*
42. *Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics – spectroscopy: an emerging process analytical technology. J Chromatogr A. 2017;1490:2-9.*
43. *Pais DAM, Portela RMC, Carrondo MJT, Isidro IA, Alves PM. Biotech Bioeng. 219;116:2803-2814.*
44. *Pais DAM. Development of advanced monitoring and control tools for rAAV production in the insect cell system [thesis]. https://run.unl.pt/bitstream/10362/97879/1/Daniel%20Pais%20PhD%20Thesis%20Final.pdf. Published April, 2020.*

Chapter 5

Upstream and Downstream Processing

Alliance *for* Regenerative Medicine

NIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 5 | Contents

UPSTREAM MANUFACTURING PROCESS DEVELOPMENT

continued on next page

Chapter 5 contents, continued

DOWNSTREAM PROCESS DESCRIPTION AND CHARACTERIZATION

continued on next page

Chapter 5 contents, continued

NOTE:
Due to inherent similarities in the processes for the production of therapeutic monoclonal antibodies and gene therapies, some of the overall content and flow of this chapter was primarily based, but adapted from when applicable, A-Mab: a Case Study in Bioprocess Development, a document in the public domain. The authors of this A-Gene chapter acknowledge the work of the authors and editors in constructing the A-Mab case study.

For further details on the A-Mab process, please review:
CMC Biotech Working Group. A-Mab: A Case Study in Bioprocess Development (chapter 3). CASSS website.

https://cdn.ymaws.com/www.casss.org/resource/resmgr/imported/a-mab_case_study_version_2-1.pdf. Updated October 30, 2009. Accessed February 16, 2021.

Upstream Manufacturing Process Development

Upstream Process Overview

OVERVIEW OF PROCESS STEPS

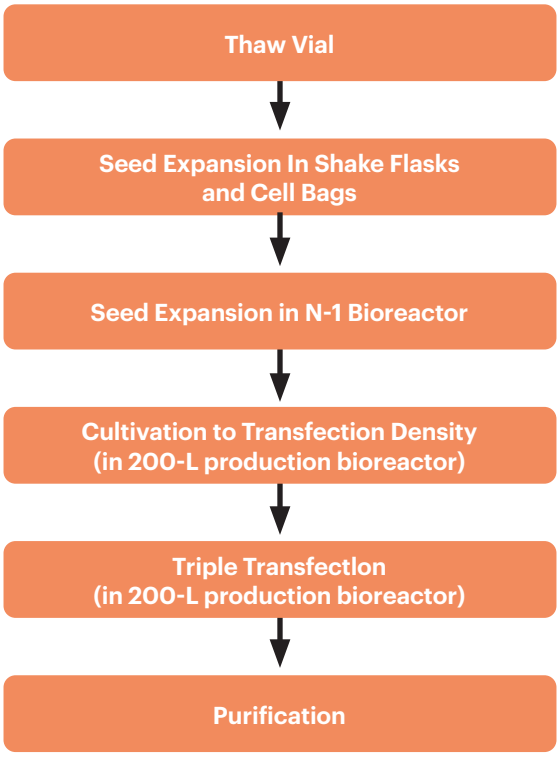
A number of different systems may be used to produce AAV, including: the HEK293 – plasmid transfection system, the baculovirus system, and the producer cell line/helper virus system that could include HSV or adenovirus. As discussed earlier in this document, for the purposes of the A-Gene case study we will describe a manufacturing process utilizing a HEK293 suspension cell line with transient plasmid transfection to produce adeno-associated virus (AAV) at a scale of 200 L (Figure 1). The upstream process is conducted entirely using disposable raw materials to maintain flexibility during manufacturing. Major steps in the upstream process include:

Step 1. Vial thaw from cell bank
Step 2. Seed expansion
Step 3. Production bioreactor
- **a.** Cultivation to transfection density
- **b.** Triple transfection with gene of interest (transgene), rep/cap, helper, plasmids

The A-Gene upstream process uses a commercially available medium for the seed train and the production bioreactor steps. During the seed expansion steps (Steps 1 and 2), one or more vials of the working cell bank (WCB) are expanded through a series of passages of increasing volume, generating sufficient biomass to inoculate the production bioreactor at the target inoculation cell density (Step 3). To reduce time between successive production batches, the seed train can be maintained as a continuous or 'rolling inoculum' so that multiple production bioreactor batches can be inoculated from the same vial thaw.

Cells are cultivated in the production bioreactor until a target cell density is reached, at which point the cells are transfected. The transfection involves addition of three plasmids (gene of interest, rep/cap, and helper plasmids) and a transfection reagent. The production bioreactor is harvested approximately 2-4 days post transfection. At the time of harvest, detergent is added to the production bioreactor to lyse cells and release AAV. A nuclease is also added to digest endogenous DNA. Following lysis, the contents of the bioreactor are clarified by filtration, and further downstream purification operations are commenced.

Figure 5-1. *A-Gene Upstream Process Overview.*



Note: for the purpose of simplicity, the risk assessments presented in the upstream section of the case study do not include extensive raw material and medium composition considerations. In a real-life scenario, upstream process risk analysis would require a thorough understanding of the impact of medium and raw material variability on process performance and product quality.

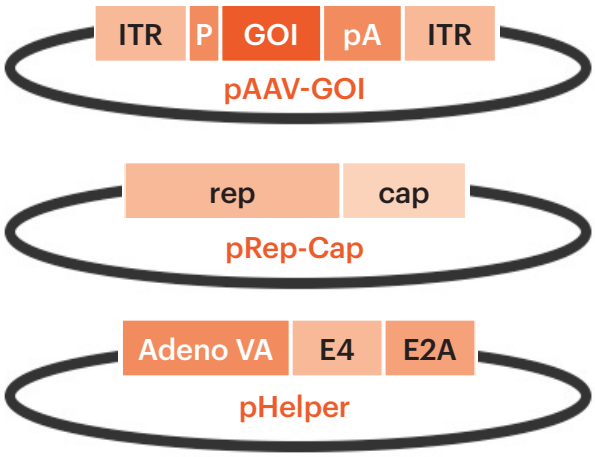
PLASMIDS

The A-Gene process uses 3 plasmids (Figure 2). One plasmid encodes the rep and cap genes of AAV (pRepCap)

using their endogenous promoters; the helper plasmid (pHelper) encodes three additional adenoviral helper genes (E2a, E4, and VA RNAs) not present in HEK293 cells; and the final plasmid (pAAV-GOI) contains an expression cassette with the gene of interest (GOI) flanked by two inverted terminal repeat (ITR) sequences.[1]

Of note, the helper plasmid may be a common component across multiple programs. X-Gene and Y-Gene (hypothetical) therapies also utilize the same helper plasmid with the same HEK293 host to manufacture AAV vectors. It should be noted that the helper plasmid can be considered a universal component across programs and be procured in greater quantities to reduce costs and provide operation efficiencies. However, the AAV serotype is chosen based on biodistribution in the target tissue; hence, the plasmid expressing the AAV capsid may also vary between programs.

Figure 5-2. *Plasmids Used to Manufacture A-Gene Using a HEK293 Cell Line*



AR, antibiotic resistance gene; ITR, inverted terminal repeat; GOI, gene of interest; P, promoter; pA, polyadenylation signal.

QUALITY ATTRIBUTES OF THE AAV VECTOR INFLUENCED BY CELL CULTURE

During development of the commercial process for A-Gene, cross functional assessments were conducted to understand the impact of each step on product quality attributes, as well as process attributes of the next step in the A-Gene manufacturing process. A summary of product quality attributes influenced by the upstream process is shown in Table 1. A detailed assessment to understand the impact of each process parameter on process performance and product quality is presented in the Process Understanding section.

PROCESS DESCRIPTION

Master Cell Bank (MCB) and Working Cell Bank (WCB) Generation, Characterization, and Testing

Cell banks used in the manufacture of A-Gene are shown in Table 2. The commercial process uses the WCB, which is established from the MCB.

The A-Gene HEK293 MCB was generated from HEK293 cells acquired from a commercial source. The cells were expanded per standard aseptic cell culture methods in a dedicated clean-room environment. Inoculation density for each passage was approximately $0.3x10^6$ cells/mL with harvest densities between $1\text{-}3x10^6$ cells/mL. The cells were cultured at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform. After the generation of sufficient cell mass, the cell bank was created by aseptically harvesting cells by centrifugation and resuspending in cryopreservative medium (90% SuperExpress Medium and 10% dimethyl sulfoxide [DMSO]). SuperExpress Medium is a fictitious, chemically defined, serum-free and animal origin-free medium containing no proteins, hydrolysates, or components of undefined composition. The cells were frozen at a final density of $1x10^7$ cells /mL using a controlled rate freezing apparatus and stored in the vapor phase of liquid nitrogen. The MCB was approximately 9 generations (doublings) from the cells acquired from ATCC. Testing for the MCB is outlined in Table 3.

In addition to the testing strategy provided in Table 3, testing for AAV serotypes, JC/BK polyoma viruses, and HSV I and II may also be applicable.

The A-Gene HEK293 WCB was generated from the A-Gene HEK293 MCB using a similar procedure as described for the MCB. Briefly, a vial of A-Gene HEK293 MCB was expanded by passaging using standard aseptic cell culture methods in a dedicated clean-room environment. Inoculation density for each passage was approximately $0.3x10^6$ cells/mL with harvest densities

Table 5-1. *Summary of Quality Attributes of the AAV Vector Influenced by Cell Culture*

Attribute	Description	Analytical Method	Notes
% Full Capsids	Capsids containing transgene	AUC, qPCR/ELISA, HPLC, electron microscopy	
Capsid protein identity	Capsid Serotype	Mass Spectrometry	
Capsid protein purity	>90%	Capillary Electrophoresis	
Viral protein ratio	Relative abundance of viral proteins VP1, VP2, and VP3 in the capsid	CE-SDS	
Vector genome sequence (Inclusive of ITRs and all other components and gene of interest itself)	Integrity of the transgene sequence; may also be controlled at the drug substance level	Sanger sequencing or NGS	
Vector genome species	DNA size distribution (GOI or any fragments)	Capillary Electrophoresis	
Potency	*In vitro* measure of activity	ELISA or RT-qPCR	RT-qPCR may be used to read out transgene mRNA expression as a measure of potency
Infectious titer	Concentration of viral particles that can transduce cells	TCID50	
Replication-competent AAV	≤1 replication competent AAV/10^8 genome copies	Cell based assay	Serotype specific positive control
PTMs	Intact mass	Liquid chromatography, MS/MS methods	
Adventitious virus	From cell line or reagents; not an issue for defined medium	EP 2.6.16	Controlled at the cell line
Mycoplasma	From cell line or reagents	EP 2.6.7	Controlled at the cell line
Aggregation	Propensity of the capsid to aggregate	Acceptable level so as not to affect loss in concentration or potency	
Residual host-cell DNA	Residual host-cell packaged and non-target DNA and free DNA	Base limit on amount dosed in relevant toxicology studies	
Residual Host Cell Protein		Base limit on amount dosed in relevant toxicology studies	
Residual plasmid DNA		Base limit on amount dosed in relevant toxicology studies	
Capsid degradation and modification	Capsid protein modification (deamidation, oxidation)	Within set limits to ensure functional consistency in manufactured products	

AUC, analytical ultracentrifugation; CE-SDS, capillary electrophoresis sodium dodecyl sulfate; ELISA, enzyme-linked immunosorbent assay; GOI, gene of interest; HPLC, high-performance liquid chromatography; ITR, internal terminal repeat; MS, mass spectrometry; NGS, next-generation sequencing; PTM, post-translational modification; TCID50, median tissue culture infectious dose (signifies concentration at which 50% of the cells are infected).

Table 5-2. *Cell Banks Used to Manufacture A-Gene*

Cell Bank	Description
A-Gene HEK MCB1	Master cell bank (MCB)
A-Gene HEK WCB1	Working cell bank (WCB)

Table 5-3. *A-Gene MCB Testing*

Test Performed	Method Description	Specification
Cell line species identity	Isoenzyme analysis to determine cell line identity	Cells confirmed to be of human origin
Cell line identity	STR DNA Profiling Analysis	≥80% match between the cell line and its original source
Mycoplasma detection	FDA "Methods to Consider" – Inoculation of Indicator Cell Line and Direct Cultivation	Free of detectable Mycoplasma contamination
***In vitro* assay to detect of adventitious viral contaminants**	Direct inoculation into MRC-5, Vero, and A549 cell lines followed by an extended incubation; cell lines are observed for changes in morphology attributable to viral agents as well as testing for hemadsorption	No CPE or hemadsorption observed
***In vivo* assay for viral contaminants**	*In-vivo* assay utilizing guinea pigs, adult mice, and suckling mice; after injection with the test substance, animals are observed for survival and good health	Free of detectable adventitious viruses
Detection of HIV-1 DNA	Detection of HIV-1 DNA by qPCR	Negative: HIV-1 DNA sequences not detected
Detection of HIV-2 DNA	Detection of HIV-2 DNA by qPCR	Negative: HIV-2 DNA sequences not detected
Detection of HTLV-1 DNA	Detection of HTLV-1 DNA by qPCR	Negative: HTLV-1 DNA sequences not detected
Detection of HTLV-2 DNA	Detection of HTLV-2 DNA by qPCR	Negative: HTLV-2 DNA sequences not detected
Detection of HCV RNA	Detection of human HCV DNA by RT-qPCR	Negative: HCV not detected
Detection of HBV DNA	Detection of HBV DNA by qPCR	Negative: HBV DNA sequences not detected
Detection of CMV DNA	Detection of CMV DNA by qPCR	Negative: CMV not detected
Detection of EBV DNA	Detection of human EBV DNA by qPCR	Negative: EBV sequences not detected
Detection of parvovirus B-19 DNA	Detection of human parvovirus B-19 DNA by qPCR	Negative: parvovirus B-19 DNA sequences not detected
Detection of HHV-6 variant A & B DNA	Detection of HHV-6 Variant A & B DNA by qPCR	Negative: HHV-6 A & B DNA sequences not detected
Detection of HHV-7 DNA	Detection of HHV-7 DNA by qPCR	Negative: HHV-7 DNA sequences not detected
Detection of HHV-8 DNA	Detection of Human Herpes Virus 8 DNA by qPCR	Negative: HHV-8 DNA sequences not detected
Detection of HAV RNA	Detection of HAV DNA by RT-qPCR	Negative: HAV RNA sequences not detected

continued on next page

Table 5-3. *A-Gene MCB Testing*

continued from previous page

Detection of human Ad5 (hexon) DNA	Detection of human Ad5 by qPCR	Negative: Ad5 DNA sequences not detected
Cell morphology and virus detection/ tabulation	Electron microscopy for retrovirus-like particles	No viral particles detected
Reverse transcription assay	Fluorescence PCR–based reverse transcriptase assay	Free of detectable reverse transcriptase activity
Detection of adventitious bovine viruses	Direct inoculation of vero and bovine turbinate cells followed by an extended incubation. The cells are stained to detect changes in morphology as well as antibody testing against the following viruses: bovine viral diarrhea virus, bovine parvovirus, bovine adenovirus, bovine respiratory syncytial virus, blue tongue virus, reovirus, and rabies virus	Free of detectable adventitious viruses
Detection of adventitious porcine viruses	Direct inoculation of vero and swine testis cells followed by an extended incubation. The cells are stained to detect changes in morphology as well as antibody testing against the following viruses: bovine viral diarrhea virus, porcine parvovirus, porcine adenovirus, reovirus, transmissible gastroenteritis virus, hemagglutinating encephalomyelitis virus, and rabies virus	Free of detectable adventitious viruses
Sterility	USP <71> Immersion	Free of viable microbial contamination
Bacteriostasis/ fungistasis	USP <71> Immersion	Free of detectable microbial inhibitors
Endotoxin	Kinetic chromogenic LAL	<5 EU/ml
Cell viability assay	Thaw cells after freezing to determine the percentage of viable of cells by trypan blue staining	Generally >70% is considered suitable

between 1-3x10^6 cells/mL. The cells were cultured at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform. After generation of sufficient cell mass, the cell bank was created by aseptically harvesting cells by centrifugation and resuspending in cryopreservative medium (90% SuperExpress Medium and 10% DMSO). The cells were frozen at a final density of 1x10^7 cells/mL using a controlled rate freezing apparatus and stored in the vapor phase of liquid nitrogen. The WCB was approximately 9 generations from the MCB. Testing for the WCB is outlined in Table 4. The information to document qualification and characterization for a WCB is generally less extensive than that for the MCB. While tests of purity and limited tests of identity should be performed once on each WCB, a risk-based approach can be adopted to establish additional WCB testing needs. Specifically testing for the presence of the adventitious agents that may have been introduced during the manufacture of the WCB from the MCB should be conducted. For example, testing for specific viruses may be executed on the WCB if not tested on the MCB.

An important consideration of cell line choice is post bank viability assessment, characterization, and tumorigenicity. Depending on the specific cell line being used, tumorigenicity may need to be evaluated through risk assessments though the data may be leveraged across projects that utilize the same host cell line. Animal studies may be conducted if deemed necessary to mitigate the risk.

Table 5-4. *A-Gene WCB Testing*

Test Performed	Method Description	Specification
Cell line species identity	Isoenzyme analysis to determine cell line identification	Cells confirmed to be of human origin
Mycoplasma detection	Ensuring lack of Mycoplasma	Free of detectable Mycoplasma contamination
***In vitro* assay for detection of adventitious viral contaminants**	Direct inoculation into MRC-5, vero, and A549 cell lines followed by an extended incubation. Cell lines are observed for changes in morphology attributable to viral agents as well as testing for hemadsorption	No CPE or hemadsorption observed
***In vivo* assay for viral contaminants**	*In vivo* assay utilizing guinea pigs, adult mice, and suckling mice. After injection with the test substance, animals are observed for survival and good health	Free of detectable adventitious viruses
Cell morphology and virus detection/ tabulation	Electron microscopy to inspect for retrovirus-like particles	No viral particles detected
Reverse transcription assay	Fluorescence PCR-based reverse transcriptase assay	Free of detectable reverse transcriptase activity
Sterility	USP <71> Immersion	Free of viable microbial contamination
Bacteriostasis/fungistasis	USP <71> Immersion	Free of detectable microbial inhibitors
Endotoxin	Kinetic chromogenic LAL	5 EU/ml
Cell viability assay	Thaw cells after freezing to determine the percentage of viable of cells by trypan blue staining	Generally >70% is considered suitable

Seed Expansion in Shake Flasks and Cell Bags

The seed culture expansion stage is performed using SuperExpress Medium supplemented with L-glutamine to a final concentration of 4 mM prior to use. The seed expansion stage involves cultivation in shake flasks of increasing volume, followed by cultivation in cell bags. The medium is supplemented with 0.2% v/v Pluronic-F68 or other shear protectant for cultivation in cell bags.

A single vial of the A-Gene HEK293 WCB was thawed in SuperExpress Medium and expanded in shake flasks. A seed density of $0.3x10^6$ was used for inoculation. For cultivation in shake flasks, one or more flasks of successively larger volume (250 mL to 2.5 L) were cultured at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 80 to 135 rpm depending on flask volume. The culture density, viability, and growth rate (doubling time) were monitored during the seed train expansion stages.

In order to generate sufficient biomass for inoculation of the N-1 bioreactor, a passage at the 25-L scale in cell bags was required. One cell bag with a working volume of 20 L each was used. The cell bag was agitated by rocking at 25 rpm with a rock angle of 10°. The cells were cultured at 37°C while maintaining a continuous supply of air through the cell bag using dissolved oxygen at 40% of equilibrium with air. The pH was kept at 7.2 using CO_2. Culture duration was generally between two and three days. Table 3 outlines seed expansion process parameters.

Seed Expansion in N-1 Bioreactor

In order to generate sufficient biomass for inoculation of the 200-L production bioreactor, a passage at the 50-L stirred

Table 5-5. *Seed Expansion Process Parameters*

Process Step	In-Process Monitoring and Control	Parameter Range
Vial thaw	Thaw duration	6 minutes
	Final thaw temperature	37°C
	Duration at final thaw temperature	5-15 minutes
	Viability after thaw	≥70%
Seed expansion (shake flasks)	Viable cell density	Inoculation target: 0.3×10^6 cell/mL At passage target: 3×10^6 cell/mL
	Viability	≥85% viability at passage
	Passage duration	3 ± 1 days
	Incubator temperature	37°C
	Incubator relative humidity	80%
	Incubator rpm/throw radius	2.5 cm
	Incubator CO_2 %	8%
	Flask nominal volume/working volume	250 mL/100 mL 500 mL/250 mL 1000 mL/500 mL 2500 mL/1000 mL
Seed expansion (cell bags)	Viable cell density	Inoculation target: 0.3×10^6 cell/mL At use target: 3×10^6 cell/mL
	Viability	≥85% final viability
	Passage duration	3±1 days
	Temperature	37°C
	pH	7.2
	Dissolved oxygen	40%
	Rock rate	25 rpm
	Rock angle	10°
	Gas flow	Air: 0.5 lpm max Oxygen: 0.25 lpm max
	Nominal volume/working volume	25 L/20 L

bag bioreactor was required (Table 6). The N-1 bioreactor operates with a 50-L working volume. Once sufficient biomass was obtained through the seed train, the N-1 bioreactor was inoculated at a seed density of 0.3 x 10^6 cells/mL. SuperExpress Medium supplemented with L-glutamine to a final concentration of 4 mM and Pluronic-F68 (0.2% v/v) or other shear protectant. An agitation rate of 70 rpm was used during cultivation. Dissolved oxygen was maintained at 40% of equilibrium with air through a continuous air sparge and was further supplemented with pure oxygen on demand. The pH was controlled at 7.2 with CO_2. The culture was maintained for two to three days until it reached the transfection density. The culture density, viability, and growth rate (doubling time) were monitored during the seed train expansion stages.

A rolling inoculum may be established as needed to support a multibatch manufacturing campaign for A-Gene. Based on development data, the seed train may extend 50 generations from the MCB to production bioreactor inoculation or 40 generations from the WCB to production bioreactor inoculation without significant loss in productivity or impact on product quality attributes.

Table 5-6. *Seed Expansion in N-1 Bioreactor Process Parameters*

Process Step	In-Process Monitoring and Control	Parameter Range
N-1 Bioreactor	Maximum working volume	50L
	Temperature	37°C
	pH	7.2
	Dissolved oxygen	40%
	Agitation	70 rpm
	Gas flow	Headspace air: 1.2 lpm Air sparge: 1 lpm max Oxygen sparge: 0.25 lpm max
	Sparger type	0.5 mm ring sparger
	Viable cell density	Inoculation target: 0.3 x 10^6 cell/mL
	Viability	≥85% final viability at passage
	Passage duration	3 ± 1 days
	Antifoam	50 ppm maximum

Production Bioreactor

The production bioreactor operates at a 200-L scale and utilizes SuperExpress Medium. To ensure that sufficient volume was available for the addition of transfection reagents, the culture was started at approximately 190 L. Once sufficient biomass was obtained through the seed train, the production bioreactor was inoculated at a seed density of 0.5x10^6 cells/mL. SuperExpress Medium was supplemented with L-glutamine to a final concentration of 4 mM and Pluronic-F68 (0.2% v/v), or another shear protectant. An agitation rate of 120 rpm was used during cultivation. Dissolved oxygen was maintained at 40% of equilibrium with air through a continuous air sparge and was further supplemented with pure oxygen on demand. The pH was controlled at 7.2 with CO_2. The culture was maintained for 2 to 3 days until it reached the transfection density. The culture density, viability, and growth rate (doubling time) were monitored during the seed train expansion stages.

Once transfection density was reached, the transfection mixture was added to the production bioreactor. The transfection mixture included sufficient plasmid DNA to ensure 2.0 µg of DNA/mL of cell culture (0.5 pg pDNA/cell). The plasmid DNA in the transfection mixture was at a ratio of 2:1:1 for pHelper:pRepCap:pAAV-GOI. The transfection reagent polyethyleneimine was added at a ratio of 2:1 relative to plasmid DNA. SuperExpress media was used to dilute the plasmid DNA and transfection reagent to a volume of 10 L. After 10 to 15 minutes of incubation at room temperature, the transfection cocktail was pumped into the production bioreactor. At 3 hours post-transfection, HEK293 media was pumped into the production bioreactor at a volume of 10% of the final volume of cell culture. Cell culture was harvested from the bioreactor bag at least 72 hours post-transfection. Table 7 outlines production bioreactor process parameters.

Batch History

To-date, eight at-scale batches have been produced in this hypothetical case study. Manufacturing history for A-Gene is shown in Table 8.

Three manufacturing processes were used for the manufacture of A-Gene. Process 1 was used to generate material to support toxicology studies to enable the phase 1 IND. This process was scaled up to meet clinical demand in the form of Process 2. This process was further optimized to support late-stage clinical studies as Process 3, which was then validated for commercial manufacture. Updates made between Process 1, Process 2, and Process 3 to accommodate facility fit requirements and increase process productivity and robustness are listed in Table 9. Pilot batches (PLT-001 and PLT-002) support toxicology studies. ENG-001 supports process development to

Table 5-7. *Production Bioreactor Process Parameters*

Process Step	In-Process Monitoring and Control	Parameter Range
Production Bioreactor	Maximum working volume	200 L
	Temperature	37°C
	pH	7.2
	Dissolved oxygen	40%
	Agitation	120 rpm
	Gas flow	Headspace air: 1.2 lpm Air sparge: 2 lpm max Oxygen sparge: 0.5 lpm max
	Sparger type	0.5 mm ring sparger
	Viable cell density	Inoculation target: $0.3x10^6$ cell/mL Transfection target: $4x10^6$ cell/mL
	Viability	≥85% final viability at transfection
	Passage duration	3±1 days
	Antifoam	50 ppm maximum

A product yield of $3x10^{10}$ vector genomes/mL is expected from the production bioreactor at harvest.

Table 5-8. *A-Gene Manufacturing History*

Process	Lot #	Bioreactor Scale	Purpose
Process 1	PLT-001	50 L	GLP toxicology study
	PLT-002	50 L	GLP toxicology study Reference Material 1
	PLT-002	50 L	Process consistency
Process 2	ENG-001	200 L	Process development and development stability Reference Material 2
	GMP-001	200 L	FIH clinical studies and stability studies
	GMP-002	200 L	FIH clinical studies
Process 3	ENG-002	200 L	Process development and development stability Reference Material 3
	GMP-003	200 L	Phase 2/3 clinical studies and stability studies
	GMP-004	200 L	Phase 2/3 clinical studies and stability studies
	EN-003	200 L	Process development and development stability Reference Material 4
	PPQ-001	200 L	Launch supplies Confirm design space and control strategy
	PPQ-002	200 L	Launch supplies Confirm design space and control strategy
	PPQ-003	200 L	Launch supplies Confirm design space and control strategy

Table 5-9. *Process Steps to Accommodate Facility Fit Requirements and Increase Process Productivity and Robustness*

Process Step	Process 1	Process 2	Process 3
Vial thaw	Controlled rate thaw using thaw device	Controlled rate thaw using thaw device	Controlled rate thaw using thaw device
Seed expansion	N-1 at 25-L scale medium 1	N-1 stage at 50-L medium 1	N-1 50-L scale medium 2
Cultivation to transfection density	50 L medium 1	200 L medium 1	200 L medium 2
Triple transfection	• PEI 40K transfection reagent • Platform transfection and harvest parameters	• PEI 40K transfection reagent • Platform transfection and harvest parameters	• PEI MAX 40K transfection reagent • Optimized feed addition at transfection • Optimized transfection density • Optimized plasmid ratios • Optimized harvest time

facilitate technology transfer. GMP-001 and GMP-002 generate materials for FIH studies. ENG-002 supports process development to facilitate technology transfer of the updated upstream process. GMP-003 and GMP-003 generate materials for phase 2/3 clinical studies. The process is continuously changed and updated to improve robustness and to ensure facility fit.

Details regarding development conducted prior to implementation of process changes are described in the Process Understanding Section.

Process Understanding

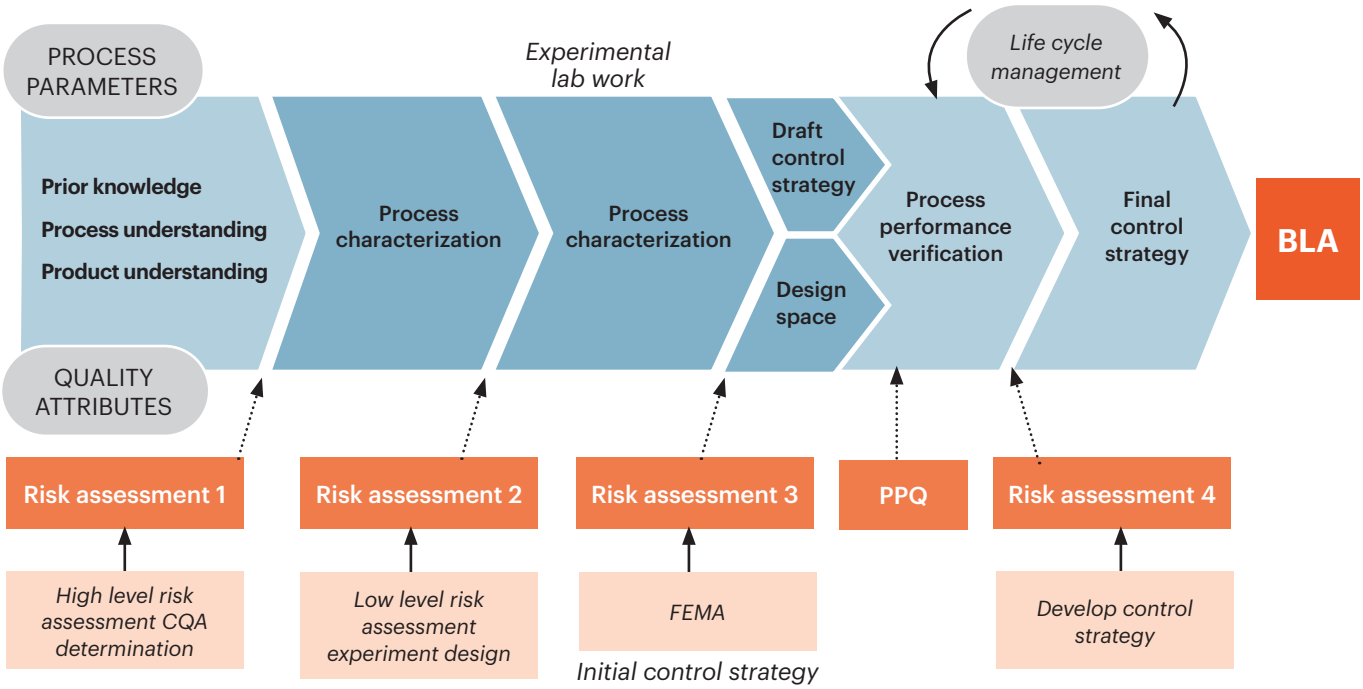
The following section describes the development history and summarizes process characterization that enabled incorporation of a QbD approach for A-Gene manufacturing. The overall strategy that guided process development for the upstream process is presented in Figure 4. This section only includes discussion through the establishment of a design space and a draft control strategy. Process performance qualification, control strategy, and life cycle management are not included.

Early-stage process development was conducted mainly to support early clinical development and used readily available raw materials to meet immediate demand. As commercial process development was initiated, a high-level assessment considered the impact of raw material and process parameters on productivity and product quality. Preliminary identification of critical quality attributes (CQAs) was important for this assessment. The crucial role of raw materials, specifically plasmids, cell culture media, and transfection reagents, was considered

Table 5-10. *Raw Material Risk Assessment*

Raw Material	Risk of Impact to Product Quality Attributes	Risk of Impact to Key Process Attributes
Plasmids	High	High
PEI	Low	High
Cell culture medium	High	High
Post-transfection medium	High	High
Antifoam	Low	Medium

Figure 5-4. *Process Characterization Strategy*



(Table 10). A template for a high-level risk assessment is shown in Table 11. Commercial process development was conducted to optimize AAV productivity while ensuring suitable product quality attributes were achieved.

Upon completion of clinical stage process development, detailed risk assessments were conducted to assess the impact of process parameters on product quality attributes. The risk assessment was based on knowledge developed during early- and late-stage process development and also identified gaps in knowledge. On the basis of this risk assessment, laboratory process qualification/characterization was conducted. Statistically designed experiments were planned and executed to enable development of models to link process parameters to productivity and product quality. Through process characterization and statistical modeling of results, process parameters having significant impact on the product quality and productivity were identified, as well as potential ranges for these parameters that would ensure desired product quality and productivity. This represented the design space for A-Gene. Finally, optimized process parameters were established at a large scale through process validation.

Implementation of this development approach for upstream process unit operations is described in this section, starting with raw materials. Please note that the raw materials section does not cover all raw materials for simplicity. A similar treatment would be applied to other key raw materials as well.

CRITICAL RAW MATERIALS

HEK293 Cell Bank

The host cell line is a critical material. Maintaining a sufficiently characterized cell bank is essential to manufacturing success. The HEK293 cell bank establishment and characterization has been described in preceding sections. The cell bank should be periodically assessed for stability. This may include basic assessment of growth and viability for three to five passages out of thaw. In certain circumstances full production of AAV may also be considered. For A-Gene, the HEK293 cell bank was established prior to phase 1 GMP manufacturing (Process 1) and stored in the vapor phase of liquid nitrogen. A vial of the cell bank was thawed and passaged three times every 5 years to assess stability. If a vial of the cell bank was used for GMP manufacturing within 5 years from

Table 5-11. *High-Level Risk Assessment Template*

Category	Process Step	Vial Thaw	Expansion in Shake Flasks/ Bags	N-1 Bioreactor	Production Bioreactor
Product Quality Attributes	Vector genome concentration				
	% full capsids				
	Capsid protein identity				
	Capsid protein purity				
	VP ratio				
	Vector genome sequence				
	Vector genome species				
	Potency				
	Infectious titer				
	Replication-competent AAV				
	Capsid PTMs (Deamidation, oxidation)				
	Adventitious virus				
	Aggregation				
	Residual host cell DNA				
	Residual HCP				
	Residual plasmid DNA				
	Residual E1A oncogene DNA				
	Capsid degradation and modification				
Process Attributes	Pre-transfection doubling time				
	Viability at transfection				
	Cell density at harvest				
	Viability at harvest				
	AAV titer at harvest				

the prior stability pull point, data from initial passaging were used to support stability.

Cell Culture Media

Cell culture media is of critical importance to the upstream process because it impacts not just cell growth but also productivity and product quality. The process for A-Gene uses SuperExpress medium supplemented with glutamine in the cell expansion and production bioreactor. Additionally, HEK293 media is used to add supplementary nutrients to the culture after transfection. Ensuring that the supply and performance of the medium can be maintained across multiple lots during late stage clinical and commercial manufacturing is essential. To this end, the hypothetical project team conducted a high-level risk assessment for A-Gene prior to initiating late-stage development to ensure that the choice of media was appropriate for late-stage and commercial manufacturing from technical and business perspectives.

A high-level risk summary template has been provided for illustrative purposes (Table 12). The level of detail in these categories as well as the classification of risks

Table 5-12. *High-Level Risk Assessment Template for Media Selection*

Risk Category	Associated risks	Justification/Mitigation
Process performance	Transfection efficiency Productivity Product quality Consistency across lots Potential for future improvement	
Media characteristics	Media stability Shipping/handling considerations Testing requirements beyond CoA	
Business and quality risk	Cost Supply continuity	
Regulatory and safety	BSE/TSE risk Animal-derived components Recombinant factors	

into categories is dependent upon the team conducting the assessment and the details of the medium itself. For example, in the case of internally developed medium with in-house clinical/commercial manufacturing capabilities, the nature of such an assessment will be different. Similarly, contract manufacturing facilities using commercially available media may require consideration of different factors. For A-Gene, the initially chosen commercially available media was changed after Process 3 development. The risk assessment was conducted and the commercial process was updated.

PEI

Polyethylenimine (PEI) is a synthetic polycation that has relatively high transfection efficiency and is commercially produced for GMP manufacturing. PEI adheres to and condenses plasmid DNA to form a complex that is endocytosed by the host cell. Upon entry into the cell, the endosome swells from osmotic pressure and lyses, releasing plasmid DNA into the cytoplasm. The plasmid DNA then migrates into the nucleus and the host cell begins replication of the transgene as well as transcription of the capsid protein genes and helper genes.

In summary, efficacy of PEI is dependent on many factors, including molecular weight, branching, cationic charge density, genetic material load, and buffer capacity.[3] PEI can be linear or branched with added functional groups to improve transfection efficiency. The key characteristic of PEI that determines its ability to transfect cells is a repeated protonatable nitrogen at every third atom. This attracts and holds onto positive charges due to low pKa values (a phenomenon commonly referred to as the proton sponge effect) and enables complexing with negatively charged pDNA molecules. Another key attribute is the length of the PEI molecule and heterogeneity of the PEI molecules with respect to the length. PEI with an optimal molecular weight (length) manages cell toxicity attributable to long fragments and has reduced complexation potential attributed to shorter fragments. Heterogeneity in the length of PEI molecules in the final product leads to heterogenous complex size formation and poor reliability in transfection efficiency. When using PEI, an addition and complexing protocol should be optimized for the specific plasmid constructs.

Several commercially available PEI products are used for transient transfection. As a critical raw material, PEI products for GMP manufacturing require a validated manufacturing process and quality control with quality attribute testing. Manufacturing of material for toxicology also requires high-quality material with appropriate quality documentation. However, it is not necessary to follow GMP standards. We used hypothetical SuperTransfect PEI transfection reagent for A-Gene. Key quality attributes used to assess quality of the PEI include identity, potency, purity, quality, and safety (Table 13).[4] Molecular weight and heterogeneity are generally measured using SEC-based methods. Free nitrogen available for complex formation may be measured using analytical techniques such as NMR, but performance assays are commonly used as a surrogate.

Table 5-13. *PEI Quality Attributes*

Attribute	Description	Assay Method
Identity	Polymer structure	Fourier-transform infrared spectroscopy or similar
	Molecular weight	Size Exclusion Chromatography
	Polydispersity index	
	Appearance	Visual inspection for color and clarity
	pH	Transfection efficiency through activity test
	Osmolality	
Potency	Performance of material	
Safety	Endotoxin	Applicable USP Methods
	Sterility	
	Mycoplasma	
Purity	Heavy metals	Applicable USP Methods

Table 5-14. *Testing for Plasmid Used in the Manufacturing Process for A-Gene*

Assay	Method
Absorbance 260/280 ratio purity	UV spectrophotometry
Appearance	Visual inspection
Concentration	UV spectrophotometry
DNA homogeneity	Densitometry analysis of EtBr-stained agarose gel electrophoresis
Endotoxin	Kinetic Chromogenic LAL
Identity	EtBr-stained agarose gel electrophoresis
Plasmid identity	Double-stranded primer walking sequencing
Residual host genomic DNA	qPCR
Residual host protein	Micro BCA
Residual host RNA	SYBR gold-stained agarose gel electrophoresis
Restriction digest	EtBr-stained agarose gel electrophoresis
Sterility	USP <71> Direct Inoculation
Sterility validation (bacteriostasis/ fungistasis)	USP <71> Direct Inoculation
Mycoplasma contamination	qPCR
Osmolality	USP <785>
pH	USP <791>
Bioburden	Testing for total aerobes, anaerobes, spore-formers, and fungi
Conductivity	Conductivity meter
Detection of Kanamycin	ELISA

ELISA, enzyme-linked immunosorbent assay; EtBr, ethidium bromide; qPCR, quantitative polymerase chain reaction; UV, ultraviolet

Table 5-15. *Testing for Cell Bank Used to Manufacture Plasmid for A-Gene Manufacturing*

Assay	Method
Final product appearance testing	Visual testing
Host cell identity	Bacterial colony morphology
Lytic phage contamination	Plate bacterial cells on media without antibiotics
Host cell identity	Gram stain analysis
Antibiotic resistance	CFU isolation on antibiotic-containing and antibiotic-free plates
DNA homogeneity	Densitometry analysis of EtBr-stained agarose gel electrophoresis
Identity	EtBr-stained agarose gel electrophoresis
Restriction digest	EtBr-stained agarose gel electrophoresis
Plasmid identity	Double-stranded primer walking sequencing
Cell bank viability	CFU/mL plate count analysis
Host cell purity	TSA and SDA
Detection of lysogenic bacteriophage	Plated in the presence of Mitomycin C
Plasmid retention	Antibiotic typing

CFU, colony-forming units; EtBr, ethidium bromide; SDA, Sabouraud dextrose agar; TSA, trypticase soy agar.

Plasmids

Plasmids are critical raw materials for the manufacture of AAV, and high-quality plasmids are required for the manufacturing process. For the A-Gene case study, it is assumed that no changes are made to the plasmid or the plasmid manufacturing process during the course of process development. However, if changes are made to a plasmid or its manufacturing process, a risk assessment should be conducted to thoroughly document the impact on the process, such as a change in transfection efficiency or impurity profile of the final product. Any gaps in knowledge to mitigate risk should be addressed through experimental work to ensure safety and comparability of the AAV material generated.

Plasmids used in the manufacture of A-Gene are manufactured under good manufacturing practice (GMP). The plasmids are produced in *Escherichia coli* (generic strain) in animal product-free medium with the appropriate antibiotic. The cultures are harvested by centrifugation, and the biomass is suspended in Tris/EDTA buffer containing RNase A (sourced from Australia or New Zealand due to lower risk of BSE and TSE) and subjected to alkaline lysis. The crude lysate is clarified by centrifugation followed by filtration. The clarified lysate is loaded onto an anion exchange column. After elution, the plasmid is diafiltered into the final buffer. The plasmid DNA concentration in the final formulation is assayed and may be further diluted to reach the desired concentration. The plasmid preparation is then sterile-filtered using a 0.22-μm membrane and dispensed into final vials in a Class 100 laminar flow hood. The final vials are labeled and visually inspected prior to frozen storage. Table 14 describes qualities that must be monitored and methods by which to do so, and Table 15 provides an overview of cell bank testing.

The generation of *E. coli* cell banks to facilitate plasmid manufacturing is highly recommended for process reproducibility.[5] A significant secondary structure of ITR sequences can result in deletion of these sequences during plasmid propagation in *E. coli*.[6,7] Plasmids that lose the ITRs have a replication advantage in transformed cells. Because intact terminal repeats must be maintained for efficient replication and packaging of the transgene, various strategies are employed to maintain ITR integrity. This includes use of specific cell lines, maintenance of selection pressure through appropriate antibiotics (after assessing safety risks), and limited propagation time after thaw of the cell bank. Stability of these banks should be

assessed periodically for cell growth, plasmid retention, and integrity. Additional characteristics of the purified plasmids (among those listed in Table 14) may be assessed as part of the stability plan.

VIAL THAW

Development History

The vial thaw is a well-established manufacturing step and no development was undertaken for Process 1, 2 or 3. The vial thaw and initial expansion have been executed similarly for X-Gene and Y-Gene for pilot scale and GMP runs with the exception of media into which the thawed vial is inoculated. Before starting Process 3 development, a risk assessment was undertaken to document the impact of the vial thaw on key process and product quality attributes. Since no product accumulates at this stage, the impact of this step on the final productivity and product quality is through impact to cell health. The risk of such impact has been demonstrated to be low based on prior experience.

This risk assessment assumes that the seed expansion process is operated following well established and successful process control strategies to ensure that seed culture performance is robust and reproducible. Batch record procedures, SOPs, process descriptions, and process controls ensure that the seed expansion steps are monitored and operated within established limits. This would include limits for parameters and attributes such as inoculation seeding density, culture duration, viability, pH, temperature, and CO_2.

This risk analysis has been simplified by not including medium and raw material considerations along with this step. It could be assumed that such sources of variability have been identified and that the appropriate raw material control strategies are in place based on platform process knowledge and prior experience with other gene therapy products. If such knowledge and controls are not available, the risk assessments would be used to guide a comprehensive evaluation of the impact of medium and raw material variability on process performance and product quality. The results of such studies would then serve as a basis to establish appropriate testing and control strategies to ensure that raw materials and media meet their respective quality acceptance criteria.

SEED EXPANSION IN SHAKE FLASKS AND CELL BAGS

Development History

The purpose of the seed stage is to build biomass while maintaining the health of the cells in suspension. The seed expansion process for A-Gene corresponds to a well-established platform process, and the same host cell line has been used across multiple toxicology and phase I and phase II manufacturing campaigns in different media. These data were compiled and reviewed as part of the initial assessment prior to Process 1. Performance of the seed train was measured by assessing growth rate (doubling time) of the culture during each passage and viability at the end of each passage. Similar and consistent performance was observed for A-Gene, X-Gene and Y-Gene despite the different cell culture medium. Considering the absence of product accumulation during seed expansion and extensive experience with routine passaging of the host cell line in shake flasks, rocking bag, and stirred bag bioreactors, the impact of this step on final product quality was deemed low. Note that the only difference for this step across these 3 processes was scale of operation. For Process 1, the production bioreactor was 50 L, so a rocking cell bag served as the N-1 stage. Process 2 and Process 3 were executed at the 200-L scale, so the rocking bag reactor served as the N-2 stage.

This initial assessment assumed that the seed expansion process is operated following well established and successful process control strategies to ensure that seed culture performance is robust and reproducible. Batch record procedures, SOPs, process descriptions, and process controls ensure that the seed expansion steps are monitored and operated within established limits. This would include limits for parameters and attributes such as inoculation seeding density, culture duration, viability, temperature, and CO_2.

Given these considerations an initial assessment of the parameters assessed during development of the seed stage included:

- **Culture media:** While a preferred media formulation was adopted for early-stage manufacturing, different commercially available media formulations were examined prior to late-stage manufacturing. One of the goals of the media screening was to enable use of the

Table 5-16. *Impact of Process Parameters and Risk Assessment for Seed Expansion in Shake Flasks*

Process Parameter	Impact to Process	Product Quality Risk
Inoculation viable cell density	May introduce lag (low density) or exceed the at-passage cell density/viability criteria (high)	Low
Final ("at passage") cell density	May introduce lag for next passage	Low
Viability at passage	May introduce lag for next passage or indicate other issues with the culture	Low
Passage duration	Manage through inoculation density	Low
Incubator temperature	Impacts cell growth rate (generally easy to control)	Low
Incubator relative humidity	Impacts evaporation rate (generally easy to control)	Low
Incubator rpm/throw radius	Impacts oxygenation (not significant at low cell densities during seed train)	Low
Incubator CO_2 %	Enables maintenance of pH during the culture	Low
Flask nominal volume/working volume	Impacts oxygenation (not significant at low cell densities during seed train)	Low

Table 5-17. *Impact of Process Parameters and Risk Assessment for Seed Expansion in Cell Bags*

Parameter	Impact to Process	Product Quality Risk
Inoculation viable cell density	May introduce lag (low density) or exceed the at passage cell density/viability criteria (high); leverage shake flask data	Low
Final ("at passage") cell density	May introduce lag for next passage	Low
Viability at passage	May introduce lag for next passage or indicate other issues with the culture	Low
Temperature	Impacts cell growth rate (generally easy to control)	Low
pH	Impacts cell growth rate (generally easy to control)	Low
Dissolved oxygen	Impacts cell growth rate (generally easy to control)	Low
Rock rate	Impacts oxygenation and hence cell growth rate (generally easy to control)	Low
Rock angle	Impacts oxygenation and hence cell growth rate (generally easy to control)	Low
Gas flow	Impacts oxygenation and hence cell growth rate (generally easy to control)	Low
Nominal volume/working volume	Impacts oxygenation (not significant at low cell densities during seed train)	Low

same medium for seed train and production bioreactor to streamline raw material sourcing. Consideration of a different medium for the production bioreactor also necessitated examination of the same medium for the seed stages. Cost, availability for GMP manufacturing, and ability to support sufficiently high cell densities were used as criteria for assessment. Productivity was considered in case the same medium was tested for the production bioreactor stage as well.

- **Inoculation density:** Inoculation density must be assessed at each passage to minimize any lag that may occur from the introduction of cells at a relatively low concentration in a nutrient-rich environment.
- **Maximum attainable cell density:** It is important to understand the maximum attainable cell density at the end of each passage that enables consistent growth for the successive passage. Maximizing the usable biomass at the end of each step can reduce the passages required to obtain the target cell number to inoculate the production bioreactor. Exceeding the maximum 'at use' density may introduce a lag (decline in growth rate) for the next expansion step due to depletion of nutrients or accumulation of toxic metabolic byproducts that adversely impact cell health. For early-stage manufacturing, media vendor recommendations regarding the maximum recommended cell density were followed, but further characterization of media capabilities was undertaken in preparation for late-stage manufacturing prior to process characterization.
- **Seed train development:** Seed train development involved assessment of the impact of cell age on productivity (i.e., the number of generations from the WCB that supported good performance in the production stage) to enable establishment of a rolling inoculum so that multiple production batches could be inoculated from the same seed train.

SEED EXPANSION IN DISPOSABLE BIOREACTORS

Development History

Seed expansion in disposable stirred bag bioreactors was implemented for Process 2 and Process 3 only, as required by the larger scale of operation. Similar to culture expansion in shake flasks and cell bags, the A-Gene seed expansion step in stirred bag reactors uses a well-established platform process where process understanding is derived from prior knowledge with other AAV products.

To establish operating parameters for Process 2, performance of the production bioreactor step for Process 1 prior to transfection was considered. These data were applicable since the goal of cultivation in the N-1 step is the same as cultivation in the production bioreactor prior to transfection: to build biomass while maintaining cell health. Process 3 development resulted in selection of a different media than that used for Process 2, but the operating parameters were unchanged. Appropriate performance, as measured by culture growth rate and viability, was observed using these parameters even with the new cell culture media. This information has demonstrated that the N-1 expansion step is robust and reproducible.

PRODUCTION BIOREACTOR (TRANSFECTION/INFECTION)

Development History

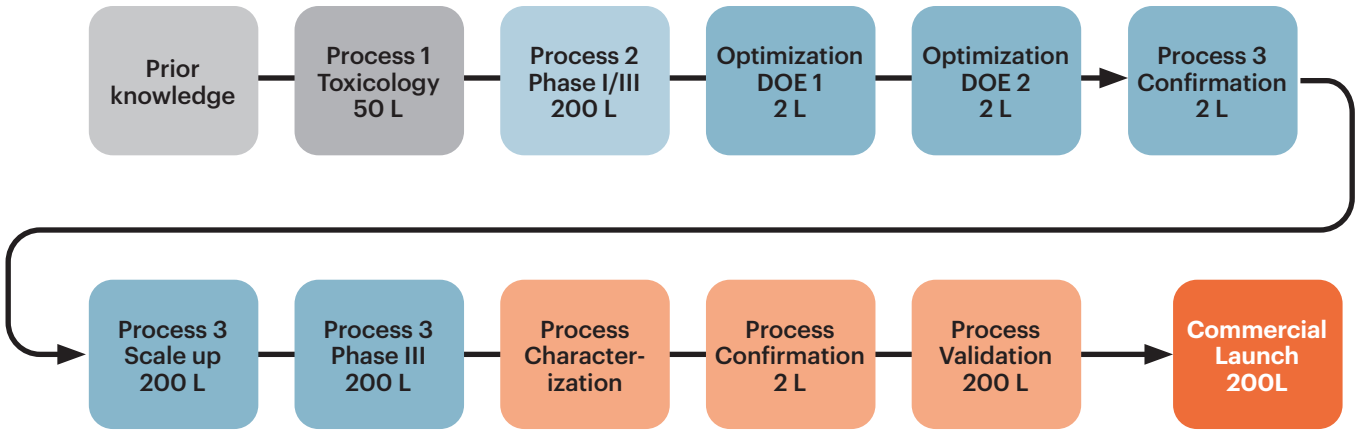
The development history through implementation of the commercial process is presented in Figure 5. This section does not cover process validation.

Process 1: Process history for A-Gene is shown in Figure 5. Early-stage process development focused on ensuring adequate clinical supply within program timelines with appropriate quality, and late-stage optimization focused on robustness and productivity (with productivity being mainly a business target). The initial process developed for A-Gene (Process 1) was used for manufacture of regulatory toxicology supplies.

The Process 1 development strategy involved the following considerations:

- Material demand for toxicology and clinical material was combined with downstream yield projections to assess the range of productivity appropriate for the upstream process and whether the demand could be met with the available bioreactor (50 L for toxicology production and 200 L for GMP manufacturing).
- Commercially available cell culture medium that had been used previously to generate material for nonclinical and clinical studies was used for Process 1. Extensive screenings of medium and feeding were not conducted.

Figure 5-5. *Development History. DOE, Design of Experiments*



- The transfection process used at the laboratory scale and for prior programs was tested without significant development to assess.
- Suitability of the process to scale up to 200 L was considered early to ensure that the process could quickly be transferred to GMP manufacturing as needed after toxicology manufacturing at 50-L scale.

Process 2: In order to manufacture GMP supplies, Process 1 was modified to manufacture A-Gene at the 200-L scale instead of the 50-L scale used for Process 1. Process 2 was largely the same as Process 1 except for the seed train and N-1 stage, which was conducted in slightly larger volumes to ensure sufficient biomass to operate at the larger scale. No additional process development was undertaken. Similarity of the material produced for toxicology and clinical studies was assessed on the basis of product quality attributes used for release and characterization of the material.

Process 3: As the A-Gene program approached late-stage, a more comprehensive assessment of the process was conducted to determine how the process may be optimized to improve the productivity of the process. It was intended that the resulting process could then be characterized and ideally commercialized, so a thorough consideration of all process parameters was needed. At this time, a preliminary list of CQAs was also available for the drug substance, representing a conservative assessment of quality attributes that may be critical to safety and efficacy of A-Gene (Table 18). Additionally, important process attributes that are linked to CQA's had been characterized(outputs).

Process knowledge accumulated across programs was considered when determining the development plans (Table 19).

Statistically designed experiments were executed, with the initial goal of selecting the medium and feed added post transfection. The statistical design allowed combinations of the factors to be tested while also detecting interactions (Table 20). Vendor recommendations were followed for use of the media and reagents as applicable, with some variability introduced to understand response of the process. Experiments assessed cell growth, ability to produce AAV, and product quality. A standard transfection protocol used for Process 1 and 2 was employed. All transfection related factors were held constant for this evaluation.

The primary criteria to evaluate performance was productivity (vg/mL). The proportion of empty capsids and potency were evaluated, but these product quality attributes can be sensitive to the sample preparation method. Thus, the experiment was conducted in bioreactors (2-L working volume) to ensure that sufficient material was available for in-depth, multi-step sample preparations that reduce yield but are more representative of the larger scale purification process for A-Gene.

Medium 2 (SuperExpress Medium) was chosen as the basal medium and dilution medium for the transfection mixture. The SuperTransfect transfection reagent was chosen with Nutrient Mix 1 post transfection to boost productivity.

Following the selection of medium other aspects of the process were assessed, including:

Table 5-18. *Process Attribute Impact and Mitigation Strategy Template*

Attribute	Severity	Probability	Detectability	Risk Score	Mitigation
Productivity					
Proportion of full capsids					
Capsid protein purity					
VP ratio					
Vector genome sequence (inclusive of ITRs and all other components and the GOI itself)					
Vector genome species					
Potency					
Infectious titer					
Residual plasmid DNA/fragments					
Residual E1A oncogene DNA					
Capsid degradation and modification					
Capsid PTMs					
Pre-transfection doubling time					
Viability at transfection					
Cell density at harvest					
Viability at harvest					
AAV titer at harvest					

GOI, gene of interest; ITR, inverted terminal repeat; PTM, post-translational modification; VP, viral protein.

- Total plasmid DNA per cell
- Ratio of the Rep/Cap to transgene to helper plasmid
- Plasmid to PEI ratio
- Transfection cell density
- Pre-transfection additives
- Complexation volume
- Time of harvest

The pH, temperature, and dissolved oxygen level of the culture were not included in this study because previous experience has shown little benefit in varying these relative to established values. A statistically designed experiment was executed to understand the impact of factors as well as interactions (Table 21).

Following the completion of the experiment, the most productive conditions were repeated alongside Process 1 to generate more data and confirm the performance of Process 2. Performance of the optimized process at varying generations from vial thaw were also assessed at this time (Table 22).

Culture productivity, reported as vg/mL at the time of harvest, was a key performance parameter. While batch yield is an important business objective, this was considered in the context of scaling up or scaling out. Process development assessed whether scaling up from the 200-L scale to a larger scale was warranted post approval as demand increases or whether extending manufacturing campaigns at the 200-L scale in a given year would meet projected peak demand.

Scaling up presents challenges associated with the engineering aspects of the process (mass and energy transfer) as well as cell biology (ensure transfection efficiency does not decline at larger volume). Facility-related operational challenges associated with handling

Table 5-19. *Parameters Optimized to Define the Commercial Production Bioreactor Step*

Parameter	Process impact	Product quality impact and risk
Media	Basal medium and media additives impact productivity (e.g., compensate for any noted nutrient deficiencies or facilitate cell maintenance)	
Pre transfection temperature	Temperature can impact cell metabolism and growth rate	
Pre transfection pH	pH impacts metabolism and growth rate	
Dissolved Oxygen	While maintaining a minimal level of dissolved oxygen in the bioreactor is essential for cell growth, the level of excess dissolved oxygen maintained has been shown to affect the productivity of the culture	
Agitation	Agitation impacts the mass transfer of oxygen to meet cellular demand and can also impact cell clumping. At lower cell densities, mass transfer is not a significant issue, but the shear stress to which the cells are exposed needs to be considered. The level of shear protectant (e.g., Pluronic® F-68) may be addressed to mitigate stress due to mechanical shear	
Gas flow	Gas flows impacts oxygen mass transfer and CO_2 stripping. Excessive sparging can also lead to bubble-related shear stress in the cell	
Post transfection pH	pH impacts growth rate andmay impact productivity after transfection	
Post transfection temperature	Temperature impacts growth rate and may impact productivity after transfection	
Transfection reagents	Selection of transfection reagents to maximize transfection efficiency is a key aspect of maximizing AAV productivity. Commonly used transfection agents include calcium phosphate, PEI, and commercial reagents (e.g., Lipofectamine, ViaFect). Rather than selecting a platform transfection agent, a platform approach to selecting the transfection reagent may be used. In addition to the transfection reagent used, the ratio of this reagent to the total plasmid present as well as the bioreactor conditions during transfection should be considered	
Plasmid ratios	The ratio of the three plasmids utilized (pAAV-GOI, pHelper and pRepCap) were optimized. Plasmid ratio may impact the percentage of full capsids	
Total plasmid DNA	Total plasmid DNA may impact the percentage of full capsids	
Inoculation density	Inoculation density can impact cell metabolism and growth rate	
Transfection cell density	Cell density and cell health at transfection play important roles in productivity optimization. Allowing cells to achieve transfection cell density or to grow past the target transfection density and diluting down to the target density with fresh media were considered	
Dilution to transfection density	Allowing cells to grow past transfection cell density in order to then dilute to transfection density allows the provision of additional nutrients at the time of transfection	
Cell age/ passage number	Generations from vial thaw	
Cell thaw time	Cell thaw time may affect viability	
Anti-clumping agent	Anti-clumping agents were considered to improve transfection efficiency. These agents may be incompatible with the transfection reagent	
Anti-foam addition	Anti-foam agents were considered to improve transfection efficiency. These agents may be incompatible with the transfection reagent	
Harvest time	The time of harvest was selected to maximize productivity of the culture by maximizing the synthesis of fully formed capsids with packaged GOI, while also considering the potential degradation of capsids post transfection	

Table 5-20. *Factors and Conditions for Experiments*

Factor	Conditions
Medium	Medium 1 Medium 2 Medium 3
Transfection reagent	SuperTransfect
Complexation medium	Medium 1 Medium 2 Medium 3
Post transfection medium addition	Nutrient Mix 1 Nutrient Mix 2

Table 5-21. *Experimental Parameters*

Parameter	Low	Mid	High
Transfection density (x10^6 cells/mL)	2	3	4
Plasmid ratio		1:1:1	
Total plasmid per cell pg/cell	0.5	1	2
PEI to plasmid ratio (mg:mg)	2:1	3:1	4:1
Additive	**Sodium butyrate**		
Feed addition post transfection (v/v)	1%	2.5%	5%
Time of harvest post transfection (h)	48	72	96

larger volumes while minimizing hold time may also be a consideration. However, experience from large-scale recombinant protein manufacturing can be applied.

Scaling out presents different operational challenges related to maintaining increased batch frequency through the year. Additionally, if a high dose is required, multiple bioreactor batches may need to be pooled to generate a single batch with an appreciable number of doses. This is desirable because it enables purification of a batch with multiple doses as opposed to a single dose through a single downstream train. Under these circumstances, understanding the consistency of batches that are pooled, establishing the point in the process at which batches are pooled, and determining pooling criteria for lots are important. It is important to understand the definition of a batch from a quality perspective and regulatory considerations.

Process Characterization

The goal of process characterization for the production bioreactor were:

- Identify process parameters that impact onto product quality and yield;
- Justify manufacturing operating ranges and acceptance criteria;

Table 5-22. *Process Parameter Conditions*

Parameter	Level 1	Level 2	Level 3
Process Conditions	**Process 2 (Ctrl)**	**Optimum 1**	**Optimum 2**
Generations from MCB thaw	10	20	40

Table 5-23. *Process Attribute Template During the Production Bioreactor Unit Operation*

Unit Operation	Attribute	Severity	Probability	Detectability	Risk Score	Mitigation
Production bioreactor	**PRODUCT QUALITY ATTRIBUTES**					
	Proportion of full capsids					
	Capsid protein purity					
	Vector genome sequence (inclusive of ITRs and all other components and the GOI itself)					
	Vector genome species					
	Potency					
	Infectious titer					
	Residual plasmid DNA					
	Residual E1A oncogene DNA					
	Capsid degradation and modification					
	Capsid PTMs					
	PROCESS ATTRIBUTES					
	Pre-transfection doubling time					
	Viability at transfection					
	Productivity Viability at transfection					
	Cell density at harvest					
	Viability at harvest					
	AAV titer at harvest					

- Identify interactions between process parameters and critical quality attributes; and
- Ensure that the process delivers a product with reproducible yields and purity.

Risk Assessment 1: Prior upstream knowledge, process understanding, and quality attributes that may be impacted by the upstream process have been described in earlier sections of this chapter. These led to the initial risk assessment conducted prior to late-stage process development, as discussed during the description of Process 3 development. Critical quality attribute assessment was also conducted at this time (as addressed in Chapter 4 and Chapter 5). Hence, the appropriate subset of critical attributes was selected for assessment during process development for Process 3. Each potential critical attribute was risk ranked based on severity, probability, and detectability. The outcome of the first risk assessment included the unit operations to be evaluated for the process as presented earlier and that resulted in the process used for manufacturing material for phase 3 clinical studies. Table 23 lists the process attributes that can be influenced during the production bioreactor unit operation.

Risk Assessment 2: A second risk assessment was completed following late-stage process development activities for Process 3. Initial assessment of process parameters that may influence quality attributes completed during process development of Process 3 was used as input. The risk assessment is focused on identifying bioreactor equipment design, control parameters,

Table 5-24. *Process Parameter Template*

Process Parameter	Risk Ranking				Baseline Parameter	Experiment Setpoints		
	AAV Titer	Full/ Empty (%)	Potency	Totals		Low	Mid	High
Temperature setpoint								
DO setpoint								
VCD at infection								
Inoculation VCD								
Agitation (RPM)								
Hours post transfection								
Air sparge setpoint								
Air overlay setpoint								
Media warming duration								
Antifoam – daily (mL)								
Media lot								
Transfection ratio								

processing conditions, and starting materials that may have a significant influence over quality attributes of the product (Table 24). A risk ranking score is assigned to each process parameter with respect to their potential to affect a particular process attribute.

Risk mitigation activities are designed to include process parameters with a high risk assessment score. These activities include some or all the following:

- DOE: multivariate studies to establish relationships between parameters and CQAs
- DOE indirect: parameters that were indirectly varied during DOE studies
- EOPC: end of production cell studies to establish limits of *in vitro* cell age
- Medium hold studies: studies performed to justify medium and feed hold times
- Not required: indicates that no special risk mitigation was performed; parameters were controlled and recorded, and data were retrospectively analyzed for correlations.

Process Characterization Studies: Operation design space and process control strategy were defined based on process characterization studies conducted using a qualified scale-down model of the production bioreactor. Multivariate and univariate studies (DOE) designed as an output of the second risk assessment were executed as part of process characterization experiments. These studies helped quantify the relationship between process parameters and critical quality attributes. Process parameters ranked either high or medium in the above risk analysis were defined as factors in D-optimal study design. The ranges for each factor were low and high experimental setpoints recorded in the risk analysis above. Critical quality attributes and measures of productivity influenced by the production bioreactor step are defined as responses for the study. The study design was sufficiently powered to resolve any main effects, effects from interaction between factors, and quadratic effects to assess curvature in the responses.

For each response, model fitting is performed using data collected from study execution. Outliers are identified using studentized residuals or adjusted jackknife distances. Data points that score >1.96 absolute value are identified as potential outliers with 95% confidence and are removed from model fitting. Figure 6 contains a matrix of plots indicating each of the effects found in the

Table 5-25. *Parameter Estimates for Fitted Models to Responses*

	CQA 1		CQA 2		CQA 3	
Term	Estimate	P value	Estimate	P value	Estimate	P value
Factor 1	305.24	<0.001	-0.5	<0.001		
Factor 2 * Factor 4	67.17	<0.001			2.34	<0.001
Factor 1 * Factor 1	15.32	0.0104	0.045	<0.001		
Factor 3	7.63	<0.001	0.11	<0.001	-1.98	0.0116
Factor 3 * Factor 4	5.0	<0.001	-0.09	0.0190	1.50	<0.001

DOE. This matrix plot is made by combining statistical models generated for individual responses. Table 25 summarizes the process parameters and factor interactions found to significantly affect CQAs (i.e., P≤0.05). Scaled estimates provide a measure of how much a given response changes as a function of an input parameter, or combination in the case of interactions. These estimates form the coefficients of the transfer function for each response. The models are suitable for predicting mean levels of the CQAs over the ranges of the process parameters that were included in the experiments.

The design space for the production bioreactor unit operation was defined by combining models for each studied response and the levels of CQAs. Acceptable CQA levels were decided during critical quality attribute assessment as described in the CQA section above. The ranges for CQA values are based on safety and efficacy data and account for the analytical method used. For example, cell-based assays are often more variable than capillary- or PCR-based quantification methods. The design space defines a multivariate space within the defined ranges of process parameters that provides high

Figure 5-6. *Combined Profiler*

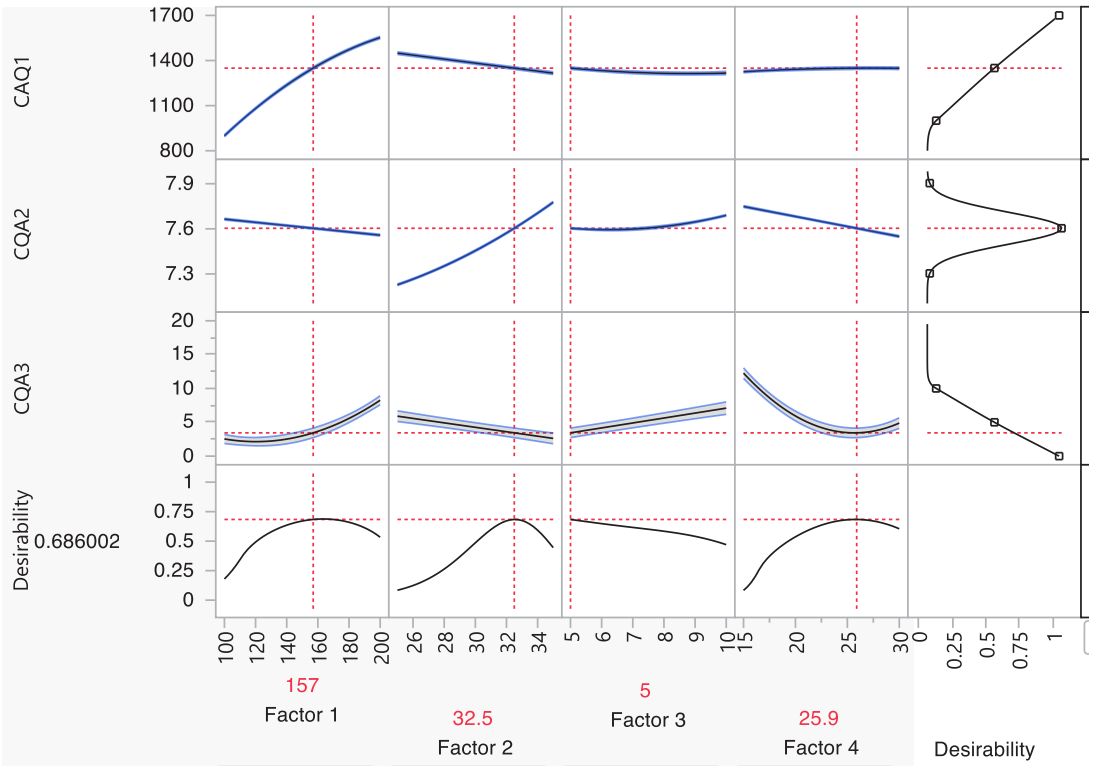


Table 5-26. *Levels of CQAs Used to Define Design Space*

Critical quality attribute	Lower limit	Higher limit
CQA 1	70	140
CQA 2	50	100
CQA 3	90	100

confidence that all CQAs will be within acceptable levels as indicated in Table 26.

Figure 7 shows the graphical representation of the design space for the production bioreactor unit operation. The shaded region in these 2D graphs represent process parameter values that would lead to mean levels of CQAs that will be outside the acceptable limits or specifications. The colors represent different CQAs that may fall outside acceptable limits.

Once a design space is defined for the production bioreactor unit operation, edge of failure analysis can be performed by using the combined model and stimulating CQA values based off variation in process parameter inputs. This analysis uses the fitted model, not the experimental data to stimulate response values. It uses a Gaussian process statistical model with the failure rate

Figure 5-7. *Design Space*

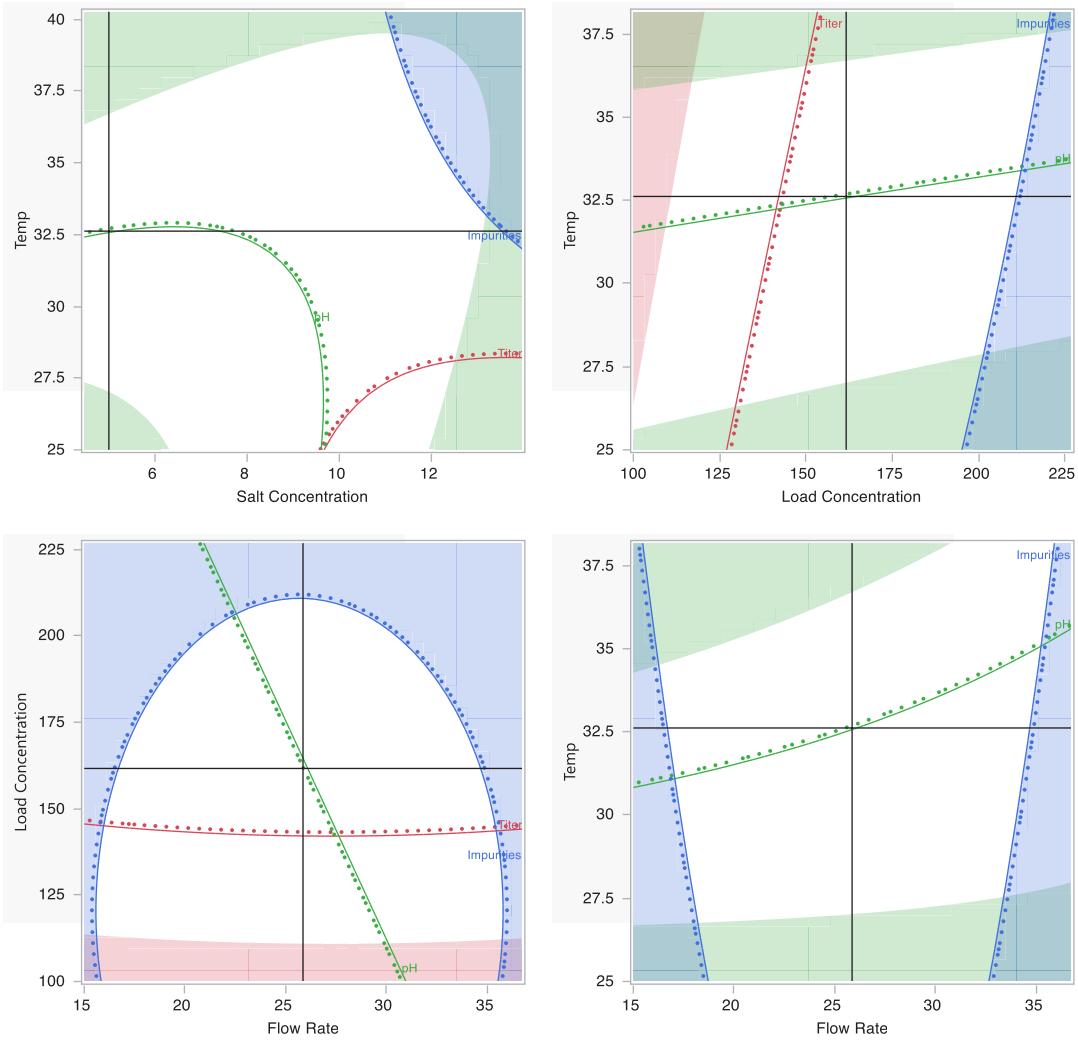
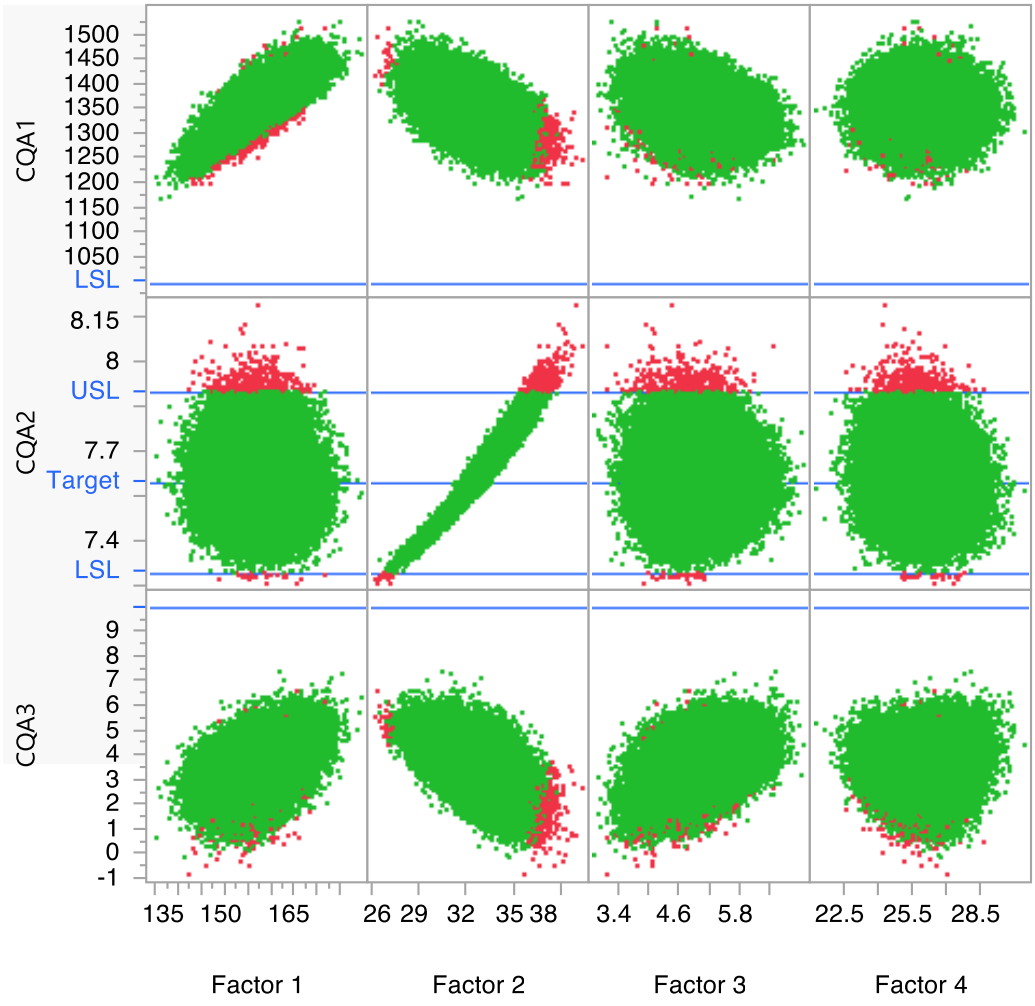


Figure 5-8. *Graphical Representation of Simulated Response Values for the Production Bioreactor Unit Operation*

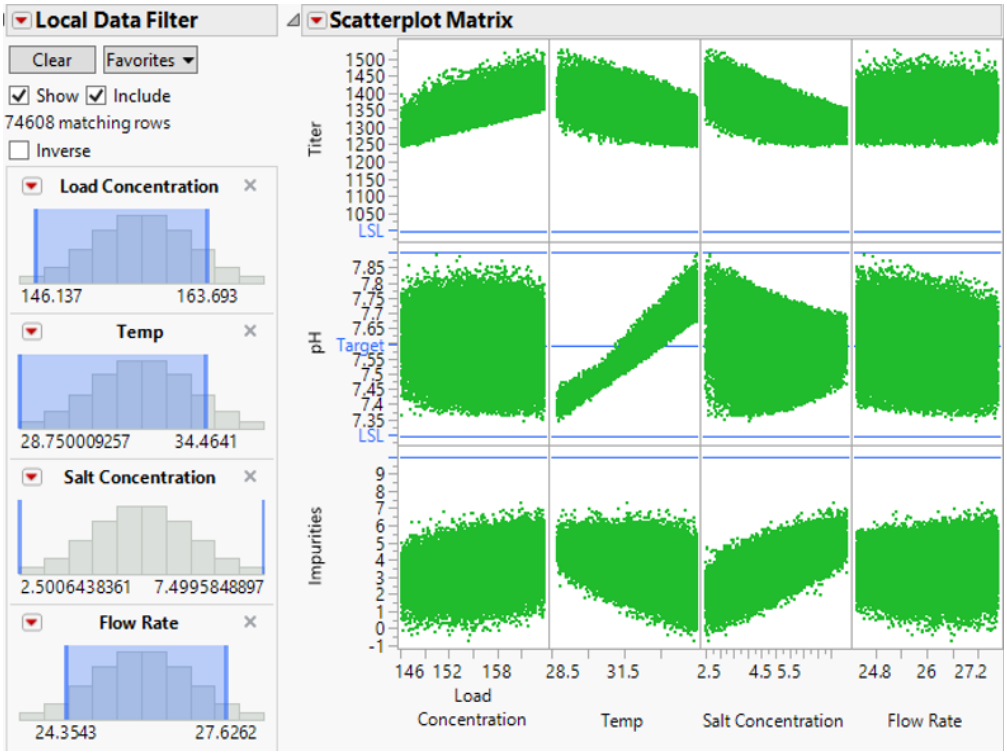


from the computer experiment as an interpolator to predict and visualize the results of the experiment. A data table is created with the design and the estimated overall failure rate is reported in PPM. Figure 8 is a graphical representation of the simulated response values for the production bioreactor unit operation. Green dots represent acceptable CQA values and red dots represent CQAs that are outside the acceptable range (Table 26). This approach satisfies the requirement to demonstrate assurance of quality per the ICH definition of design space.

The design space for the production bioreactor shown in Figure 9 is multidimensional. To define limits of the design space that can be translated to clear manufacturing instructions, predictive models for each response were used to set limits. This is achieved by examining the sensitivity to changes in CQAs by increasing the standard deviation by a factor of 1.5 to 2. While performing margin analysis by varying the factors, representative critical factors can be evaluated by increasing their standard deviation and examining impact on CQAs. Proven acceptable ranges (PAR) are identified by the range in standard deviation of critical factors that limits failure PPM limits to <100.

Risk Assessment 3 and Control Strategy: A final risk assessment was conducted after the completion of the process characterization studies to define the control strategy for the commercial manufacturing process. Commercial-scale batch performance was used to verify process performance and demonstrate the control strategy at scale. The risk assessment identified unit operations

Figure 5-9. *Design Space for Production Bioreactor*



that had an impact on product quality and process performance. Unit operations that were identified to impact product quality were then included in defining the design space for the upstream process.

Scaled estimates as noted in Table 27 are used to identify process parameters linked to product quality and process performance. Scaled estimates are scaled based on the ranges tested in the DOEs so that they measure change in the response value by half-range. The full effect of each factor or interaction is calculated by doubling its scaled estimate. Scaled estimates for quadratic terms measure change over the full range and do not need to be doubled to calculate their full effect. Classification of parameters was based on their potential impact to product quality, the likelihood of a parameter to exceed acceptable limits, and the ability to detect and/or correct a failure if it occurred. Tolerance (%) was calculated using the following equations. For CQAs, terms with tolerance values ≥20% were identified as CPPs, as shown in Table 27. For productivity measures, terms with tolerance values ≥20% were identified as PPs (Process Parameters, not shown here). PPs do not impact product quality but are important to ensure successful and reliable commercial manufacturing operations.

Equation 1. *Tolerance.*

$$Tolerance\ (\%) = \frac{|Full\ Effect|}{(Upper\ Spec\ Limit - Lower\ Spec\ Limit)}$$

The proposed control strategy for the production bioreactor unit operation ensures that the process delivers a product that meets its specifications, and a consistent and robust commercial manufacturing process. Product quality is ensured by operating the process within the limits of the design space (i.e., all quality-linked process parameters [CPPs] must operate within the defined PARs). Process consistency is ensured by controlling PPs within established limits and by monitoring relevant process attributes.

A summary of the control strategy for the production bioreactor is presented in Figure 10. Here, quality-linked process parameters must be controlled within the design space and in-process quality attributes must be within specified limits to ensure drug safety and efficacy. The control of PPs ensures that commercial success criteria and yield are met.

Table 5-27. *Scaled Estimates*

Term	Estimate	Prob>Itl	Multiplier	Full Effect	% Tolerance	CPP
Speed(100,200)	-0.017007585	<.0001 *	2	-0.034015169	56.7	CPP
Temp(250,300)	0.0654243417	<.0001 *	2	0 .1308486833	218.1	CPP
Time(5,10)	0.0176018576	<.0001 *	2	0.0352037153	58.7	CPP
Pressure(15,30)	.0143259292	<.0001 *	2	0.0286518585	47.8	CPP
Speed*Temp	-0.001554387	**0.0190 ***	2	-0.003108773	**5.2**	**non-critical**
Temp*Temp	0.0159445836	<.0001 *	1	0.0159445836	26.6	CPP
Temp*Time	-0.037915132	<.0001 *	2	-0.075830263	126.4	CPP
Time*Time	0.0138420999	<.0001 *	1	0.0138420999	23.1	CPP
Temp*Pressure	-0.025754387	<.0001 *	2	-0.051508773	85.8	CPP
Time*Pressure	0.0346654026	<.0001 *	2	0.0693308051	115.6	CPP

Applicability of Design Space to Multiple Operational Scales and Bioreactor Configurations: Engineering Design Space

The design space previously described is based on the quality-linked process parameters. These parameters are considered scale-independent variables and thus apply to all operational scales. However, other scale-dependent parameters must be considered for successful and consistent process performance when operating at various scales. The engineering design space includes bioreactor design characteristics and engineering parameters to ensure robust and consistent bioreactor performance to meet product quality targets.

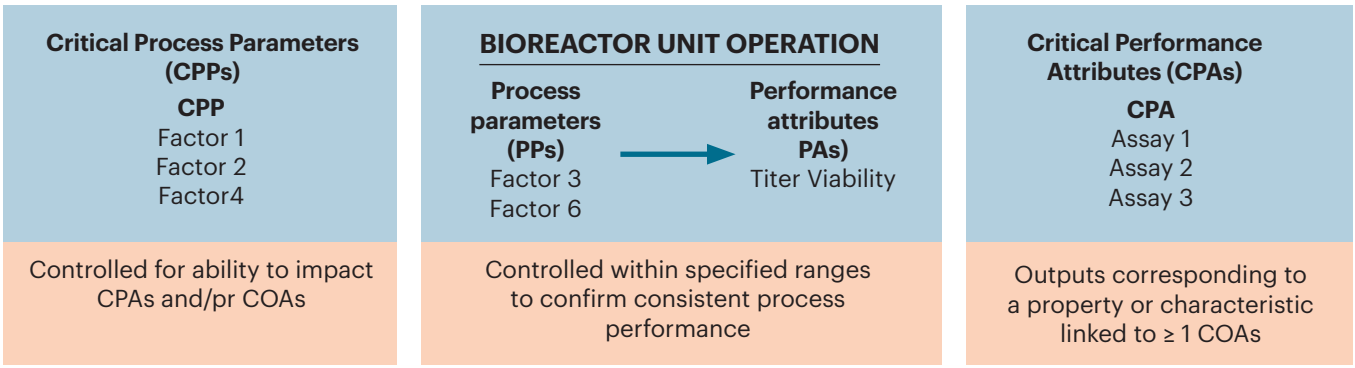
The engineering design space includes bioreactor design characteristics and engineering parameters for small scale bioreactors used for process development and large-scale bioreactors used for manufacturing. During early development, scale-up of the process from small-scale bioreactors used for development to larger-scale bioreactors used for manufacturing is of concern. However, as the scale for commercial manufacturing is established, the small-scale bioreactor model is also adjusted to ensure it is representative of the performance observed at large scale. Additionally, as a scale-down model is established it is qualified by demonstrating that process performance at the small scale is representative and predictive of large-scale manufacturing. This ensures that the process characterization executed using the scale-down model is applicable to large-scale manufacturing.

The following section describes methodology for the selection of parameters used to scale up and scale down the manufacturing process for A-Gene and qualification of the scale-down model.

DEVELOPMENT OF A SCALE-DOWN MODEL

The levels of productivity and product quality that are achievable depend primarily on bulk mixing, oxygen mass transfer, and hydrodynamic conditions, which in turn are affected by bioreactor design, impeller type, and process operation. To accomplish successful scale-up, reasonable similarity between these conditions must be maintained across scales. It follows that creating a successful scale-down model requires that any limitations observed at the large scale are also reflected at the small scale, even if these need to be deliberately imposed. For example, a limitation in the maximum permissible oxygen transfer at large scale may be deliberately imposed at the small scale to reflect equipment capability at the large scale. In some cases, the bioreactor scale or configuration utilized for manufacture of toxicology, clinical, and commercial material is not known early in development. In this case, the small-scale model for early development may use prior knowledge from other programs or vendor recommendations regarding operating parameters. As information regarding manufacturing

Figure 5-10. *Overview of Control Strategy for Production Bioreactor*



scale and bioreactor configuration becomes available, the scale-down model can be adapted to ensure it remains representative and predictive of large-scale operation.

Table 28 describes primary and secondary reactor design features that should be evaluated to assess the bioreactor capability to support a high-density mammalian cell culture for recombinant protein expression. This matrix was developed based on published literature, bioreactor engineering industry best practices, and extensive prior experience with cell culture operations at multiple scales. Secondary parameters have a lesser impact on bioreactor performance capability, whereas primary design parameters have a direct impact.

Because the A-Gene process does not involve high density cultivation, this affords us more flexibility in the selection of engineering parameters since the range of parameters can achieve favorable conditions for growth and productivity.

A thorough discussion of scale-up and scale-down criteria and related bioreactor design is available in the A-Mab case study.[8]

ESTABLISHING A SCALE-DOWN MODEL AND DESIGN SPACE APPLICABLE TO MULTIPLE OPERATIONAL SCALES

Since clinical and commercial demand for A-Gene was expected to be manageable with production at 200-L scale, disposable bioreactors were chosen as the hardware platform for commercial manufacturing. This was advantageous because most configuration details for preassembled disposable bioreactors that impact scale-up were readily available from vendors and could be used to establish a scale-down model early in development.

Establishing the scale-down model involves matching mass transfer and energy dissipation across scales, as well as the impact of engineering design parameters. The maximum mass transfer may be matched by matching the maximum achievable mass transfer coefficient (kLa) across scales. Since the kLa depends on mechanical energy input as well as sparging rates, matching the kLa essentially involves selecting the appropriate agitation rate, gas flow, and sparger type.

As mentioned earlier, initial small-scale bioreactors used for development may not be based on scale-down models of larger-scale bioreactors and use of vendor or literature references to select appropriate agitation and sparging parameters. However, as the specific knowledge regarding the large-scale bioreactor system becomes available, the small-scale model may be revised to impose limitations observed at large scale. For A-Gene, the small-scale bioreactor parameters were adjusted to lower the maximum achievable oxygen mass transfer. This was achieved by appropriately lowering the maximum gas flow rates. At the same time the agitation rate was increased to mimic the increased hydrodynamic shear (any increase in oxygen transfer was offset by lower gas flow rates). A template of bioreactor characteristics for various scales of operation for A-Gene is shown in Table 29. This represents the engineering design space for A-Gene.

QUALIFICATION OF SCALE-DOWN MODEL FOR PRODUCTION BIOREACTOR AND ENGINEERING DESIGN SPACE

To demonstrate the applicability of the scale-down model to predict large-scale production bioreactor performance, process and product quality attributes are assessed. The

Table 5-28. *Impact of Production Bioreactor Engineering and Process Parameters on the Manufacturing Process*

	BIOREACTOR PERFORMANCE INDICATORS														
DESIGN PARAMETERS	Hydrodynamic shear	Bubble shear	Power per volume	Mixing time	Culture heterogeneity	Gas transfer (kLa)	Superficial gas velocity	Gas holdup volume	CO_2 stripping time	Bioburden control	pH	DO	pCO_2	Temperature	Osmolarity
Bioreactor aspect ratio	P	P	S	P	P	P	S					P		P	
Baffles				P	P	S									
Impeller design/size	P	P	P	P	P	P	S								
Number of impellers	P	P	P	P	P	P	S								
Agitation rate	P	P	P	P	P	P	S	S	S		S	S	S	S	S
Gas composition, flow rates, control		P			P	P	P	P	P			P	P		
Sparger design and location	S	P	P		P	P	P	P	P		S	P	P		
Location of addition ports/tubes					P						S				
Feed addition rates					P						S			S	S
Vessel pressure										P	S	S	S		
Probe locations					S						P	P	S	P	S
DO control loop							S				S	P	S		S
pH control loop							S				P	S	S		S
Temperature control loop											S	S	S	P	
Foam control							P	P	S						

P=Primary design consideration expected to impact bioreactor capability. Impact assessment based on prior knowledge, engineering fundamentals, and/or modeling studies (e.g., Computational Fluid Dynamics).
S=Secondary design indirectly impacting bioreactor capability based on prior knowledge and engineering standard design.

initial scale-down model used for process development was qualified based on performance data collected from prior programs (X-Gene and Y-Gene). Both processes used the same cell host along with a similar cell culture and transfection process. For the qualification studies, scale-independent variables (pH, temperature, iVCC, DO, culture duration, etc) in the scale-down bioreactors were operated at the proposed target process values of commercial operations. For scale-dependent parameters (agitation, gas flow rates, pressure, volume, etc), operating conditions at small scale were established to match process performance at full-scale as described in this section.

Performance of the processes across scales was compared by examination of growth profiles (viable cell density, viability) as well as metabolites (lactate, ammonia). The primary options available to achieve this assessment included:

- Examination of process performance profiles one parameter at a time with simple statistical measures to assess comparability of the data. This may involve simple comparisons of datapoints across batches at the same normalized timepoint. Alternatively, the parameter profile may be fit to a model (curve) and the similarity of the curves for different batches may be compared using appropriate statistical methods. This mitigates the issue of not having data at corresponding

Table 5-29. *Template for Bioreactor Design and Engineering Characterization at Various Scales of Operation for A-Gene*

	2L Standard Development Model (Process 3)	2L Scale-down Model (Process Characterization)	200 L (Process 3)
Nominal volume (L)			
Working volume (L)			
Aspect ratio (H:D)			
Impeller design			
Number of impellers			
Baffles			
RPM			
VVMs			
Sparger design			
Average P/V (W/m^3)			
Max local P/V (W/m^3)			
Vs ($x10^{-3}$ m/s)			
KLa (hr^{-1})			
Mixing time (s)			
CO_2 stripping time			
Gas hold-up volume (L)			

culture times across scales. While not as sophisticated as multivariate methods, this approach may be sufficient depending on the number of parameters and quality attributes being compared.

- Multivariate analysis using principal component analysis (PCA) modeling. PCA transforms a large number of possibly correlated variables into a smaller number of uncorrelated variables called principal components that are formed with different loadings of the original variables. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. The strength of this approach can be viewed as revealing the internal structure of the data in a way that best explains the variance in the data. Thus, this multivariate approach represents a powerful means to assess if the correlation structure between key performance attributes and quality attributes in the scale-down model data is comparable to results from full-scale bioreactors. The resulting model is more sensitive than commonly used univariate comparisons (e.g., t tests) because it can detect observations that do not fit the predicted response patterns while resulting in fewer false-positive signals.

The A-Gene process has been successfully run in bioreactors from 2-L to 200-L working volumes and various design configurations. The primary design parameters described earlier (aspect ratio, impeller design and

Table 5-30. *Template of Process Performance and Product Quality for Various Scales of Operation for A-Gene*

	2L Development Model (Process 3)	22L scale-down model (process chacterization)	200L (Process 3)
Proportion of full capsids			
Capsid protein purity			
Vector genome sequence (inclusive of ITRs and all other components and the GOI itself)			
Vector genome species			
Potency			
Infectious titer			
Residual plasmid DNA			
Residual E1A oncogene DNA			
Capsid degradation and modification			
Capsid PTMs			

number, baffles, addition port location, and sparger design) were verified during A-Gene process scale-up from 2-L to 200-L bioreactors to ensure that the bioreactors could support the A-Gene process. Similarly, process engineering parameters (P/V, superficial gas velocity, kLa, mixing time, and gas holdup volume) were measured and confirmed to meet A-Gene process requirements. Information from 2-L scale-down characterization studies (using Process 3) provides additional support to the notion that wide ranges of engineering parameters can be used and have been demonstrated to be acceptable, both in terms of process performance and product quality.

Table 30 allows comparison of process performance across all scales and determination of whether product quality is within the predicted design space. These results should demonstrate whether the design space defined using scale-down data accurately predicts performance at various operational scales.

Downstream Process Description and Characterization

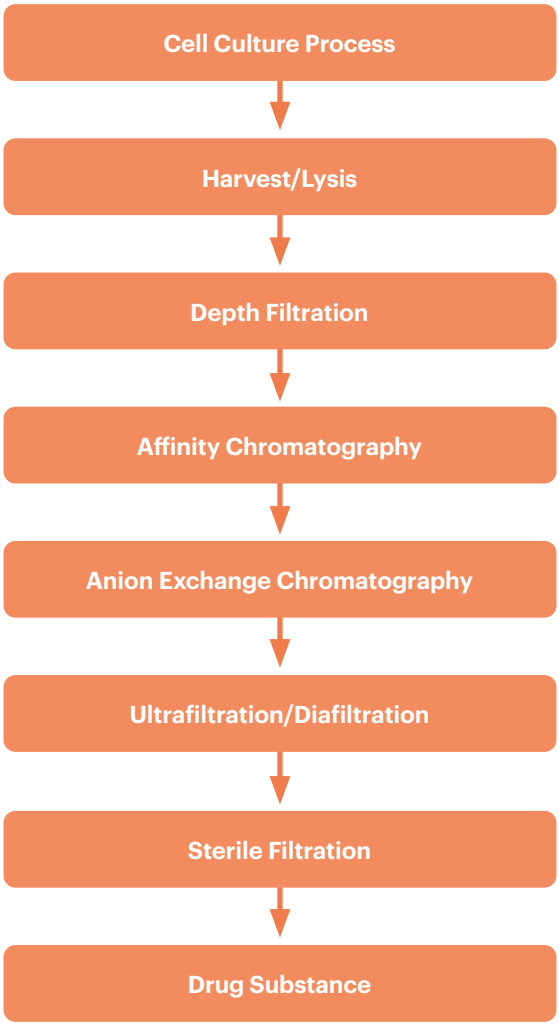
Summary

The commercial manufacturing process for downstream purification steps are designed to remove and/or control process-related impurities such as host cell proteins, residual unencapsulated DNA, process additives (endonucleases), and product-related impurities such as aggregated vectors and empty capsids that are generated during the upstream process. The scalable AAV purification process contains orthogonal steps designed to remove impurities and concentrate the viral vectors before final formulation. Formulation focuses on the identification of suitable buffers and excipients to allow for long-term storage of AAV product as well as suitable conditions for delivery to patients.

AAV process development activities often rely heavily on empirical determination of process raw materials and set points. Historically, process development often emphasized the definition of setpoints and conditions for the process through well controlled single variable experiments. However, these experiments provided limited or no information as to the robustness of the bioprocess to deliver the specified product. Additionally, the interactions of separate input variables are poorly understood and/or the impact of perturbations to the system are unknown.

QbD is a more systematic, goals-focused approach that leverages both historical knowledge and results through experimental design (DOE) and utilizes quality risk management during the development cycle. A QbD approach can increase process robustness through knowledge of both what quality attributes are critical and what process parameters are the most relevant to those attributes; facilitate process transfer across facilities and scale, and thus decrease regulatory burden; facilitate control strategies to enable more consistent products; streamline lifecycle management; and decrease the likelihood of failure across all stages of processes by reducing the likelihood that high-risk issues escape attention during downstream development.

Figure 5-11. *AAV Purification Process Flow Diagram*



For example, when mapping the parameters that impact the quantity of empty capsids in the final dose, the pH of the AEX elution step, column loading, and starting empty capsid ratio could all be considered. As such, an experimental design that explores these conditions can identify the impact that the parameter has on the CQA and aid in the further development of ranges in which the step could be performed and produce material that meets the predefined QTPP within normal variation from upstream processes. All steps are performed within a risk assessment and management system that uses previous process knowledge to define the stages. As

Table 5-31. *Overview of Downstream Process Steps*

Downstream Step	Purpose of Step
Lysis	Nucleic acid degradation
Depth Filtration	Remove cell debris, host cell proteins, large aggregates Prevent fouling of subsequent downstream processes
Affinity exchange chromatography	Remove impurities (e.g., host cell proteins [HCPs], unpackaged DNA, protein aggregates, viruses)
Anion exchange chromatography	Remove impurities (e.g. HCP and hcDNA) Remove empty capsids Enrich viral vector
Tangential flow filtration (ultrafiltration/ diafiltration)	Exchange in the final formulation buffer
Sterile filtration	Bioburden control

with any risk-based approach, CQAs or CPPs should be continuously updated or monitored to ensure all process knowledge is captured. For additional information on QbD principles, please refer to Chapter 4.

The downstream process entails the clarification and purification of A-Gene with a goal to generate the final clinical product with high potency, purity, and titer. For this case study, assumptions are a yield from the 200-L suspension cell culture of ~$1x10^{11}$ vg/mL, with a final concentration of ~$1x10^{13}$ vg/mL.

The downstream manufacturing process for A-Gene comprises several steps that are presented in the flow diagram Figure 11. The purpose of each step and the scope of information included in the case study are summarized in Table 31. Detailed step descriptions and process performance analyses are presented in the sections that describe each step. The purification process consists of:

- Harvest (in upstream processing)
- Nucleic acid degradation by enzymatic treatment
- Capture by affinity-based purification
- Polishing by anion exchange chromatography
- Concentration
- Formulation (for more information, please refer to Chapter 6)
- Fill-Finish (refer to Chapter 6)

Throughout these steps, output parameters include yield, empty/full capsid ratio, aggregates, and other impurities (e.g., HCPs, host cell DNA).

Process Understanding Based on Prior Knowledge

Utilizing extensive prior knowledge, an initial risk assessment for our hypothetical A-Gene product was conducted to identify which downstream process steps potentially impact product quality. For details of how to conduct a risk assessment, refer to Chapter 4.

Based on the results of the risk analysis, for the purposes of this case study in this chapter, only a subset of quality attributes are considered:

- Aggregate
- % Full particles
- Host cell DNA
- Residual HCP
- Residual unpackaged DNA (e.g. rep/cap, helper)
- Capsid protein purity

By contrast, extensive prior knowledge has demonstrated that the distribution of viral proteins (VP1, VP2, and VP3) is minimally impacted by downstream processing and is mainly influenced by the upstream process conditions. Based on this assessment, viral protein ratios were not included in the testing for characterization studies of the downstream process steps. Viral clearance and process residuals (e.g., affinity ligands, Benzonase) were also included in the downstream process discussion. In an actual study, the examples and approaches described here would include all relevant product quality and material attributes.

VIRAL CLEARANCE

For early phase clinical studies viral clearance is generally not required for HEK293-transient plasmid transfection process platforms. Viral clearance studies are required for AAV manufacturing platforms that do have a relevant process virus, such as baculovirus, HSV or adenovirus based systems, and should include at least viral clearance studies on process relevant virus for phase 1/2 and additional model viruses for a BLA. The focus of this chapter is on manufacturing requirements for earlier stage studies. Viral clearance studies may be required for marketing applications. In general three model viruses are sufficient. As noted in the introductory section of this document, the reccomendations made here should not be taken as regulatory guidance.

Viral Safety Risk Assessment

An outline of the risk assessment conducted to assure viral safety is summarized here:

- A-Gene is produced in HEK293 cells using animal component–free (ACF) growth medium, nutrient feeds, and supplements. In addition, HEK293 is a well characterized cell line used for the production of other clinical monoclonal antibody products.
- The A-Gene master cell bank (MCB) and working cell bank (WCB) were characterized and shown to be free from adventitious virus contaminants.

Measures are in place to ensure the safety of raw materials used in the manufacturing process. Any animal-derived components used in the medium preparation of the research cell bank, the MCB, and the media for cell cultivation are sourced from low BSE-risk countries that have bans in place against ruminant-to-ruminant feeding.

BATCH HISTORY

The downstream platform process did not require any significant changes to accommodate the increased productivity of the cell culture process or facility changes made through the development life cycle. The only changes made to the downstream process represent scale increases to match the upstream process scales. The A-Gene batch history is summarized in the upstream process section.

Terms in the Downstream section

Process inputs are measurements that can be directly manipulated or controlled and are classified based on their impact on process performance and product quality. Process inputs are classified as described below:

- Critical Process Parameters (CPPs) are inputs controlled for their ability to impact in-process CPAs and/or drug substance CQAs. CPPs must be controlled within specified acceptance criteria to ensure that drug substance CQAs are achieved. Confirmed excursions are investigated for product quality impact and could lead to lot rejection.
- Process Parameters (PPs) are inputs controlled for their ability to impact in-process PAs. PPs must be controlled within specified ranges to confirm consistent process performance. PPs have either designated action ranges or alert ranges. Excursions are investigated according to established procedures.

Monitored Parameters (MPs) are input parameters that are unrelated to product quality, do not have established ranges, and are used to monitor the manufacturing process. MP results are reported, trended, and monitored.
Process outputs are measurements that cannot be directly manipulated or controlled, such as in-process measurements, and are indicators of process performance and product quality. Process outputs are classified as described below:

- Critical Performance Attributes (CPAs) are outputs corresponding to a property or characteristic linked to one or more drug substance CQAs. CPAs are in-process results and have designated acceptance criteria. Confirmed excursions are investigated for product quality impact and could lead to lot rejection.
- Performance Attributes (PAs) are outputs monitored to confirm process performance and consistency. PAs are in-process results and have either designated action ranges or alert ranges. Excursions are investigated according to established procedures.

Monitored Attributes (MAs) are outputs unrelated to product quality that do not have established ranges and are used to monitor the manufacturing process. MA results are reported, trended, and monitored.

Downstream Process Characterization

The following sections describe the approaches used to identify parameters linked to product quality and process performance that serve as the basis for defining the design space for each process step. The classification of process parameters used in this section is based on the decision logic presented in the Control Strategy section.

LYSIS AND CLARIFICATION

The lysis and clarification unit operation, consisting of a non ionic surfactant treatment followed by filtration, bridges the production bioreactor and affinity capture (AC) chromatography steps. The production bioreactor step provides a consistent product pool containing $\leq 1x10^{13}$ viral genomes/mL. The lysis and clarification operation consistently provides a process stream at pH 5.0±0.2 to the affinity capture chromatography unit operation.

Table 5-34. *Lysis and Clarification Step Linkages*

Input: Eluate from Production Bioreactor	Output to Affinity Capture (AC) Chromatography
Viral genome concentration $\leq 1x10^{12}$ vg/mL	Viral genome concentration $\leq 1x10^{12}$ vg/mL
pH >4.0	pH=7.0±0.2
Aggregate <3.1%	Aggregate <3.1%
HCP typically > 10^6 ng/mg	10^5-10^6 ng/mg
% full AAV capsid=15%	% full AAV capsid=15%
Capsid protein purity >90%	Capsid protein purity >90%

Table 5-35. *A-Gene Lysis Parameters*

Parameter/Step	Description	Value	Range	Units	Comments
Surfactant addition	10% (w/v) surfactant	2.86	N/A	% (w/v)	Pre-addition volume spike basis (e.g., if 100 L of bulk harvest, add 2.86 L of Triton stock for final volume of 102.86 L)
Nuclease addition	Endonuclease, Grade I	10	N/A	U/mL	≥99% purity
pH adjustment	2 M tris base, 0.001% (w/v) poloxamer 188	Target 7.0	6.8-7.2	pH	-
Hold conditions	Temperature	Target 37	35-39	°C	Recommend lysis hold within the SUB for better control Hold time starts when the following conditions are met: (1) all lysis components are added; (2) pH adjustment target is reached; and (3) temperature reaches 37°C±2°C
	Time	Target 4	4-6	hr	
	Agitation rate	Target 6	5-7	P/V (W/m^3)	
Other comments	Above addition values should give a final lysis condition of 0.25% (w/v) non ionic surfactant, 10 U/mL Endonuclease, and pH 7.0±0.2				

Table 5-36. *A-Gene Depth Filtration Process Parameters*

Parameter/step	Description	Value	Range	Units	Comments
Filter	+ charge, cellulose fiber and diatomasceous earth media	N/A	N/A	N/A	Larger sizes (≥0.1 m²) require a POD holder
Pre-use flush	Water for injection (WFI)	Target 50	40-60	L/m²	If running the depth filter and sterile filters in-line, the media pre-use flush must be done prior to connecting the sterile filter
		Target 300	250-350	LMH	
Load	Challenge	≤500	N/A	L/m²	
	Flux	Target 150	100-200	LMH	
Recovery flush	50 mM sodium phosphate, 350 mM sodium chloride, 0.001% (w/v) poloxamer 188, pH 7.4 ± 0.3	Target 10	8-12	L/m²	Recovery flush to be collected with the filtered pool
		Target 150	100-200	LMH	
Pressure limits	Differential pressure	≤30	N/A	psid	
	Inlet pressure	≤50	N/A	psig	
Other comments	Measure both back pressure and differential pressure during operation. Ensure that neither limit for the filter unit is exceeded. It is recommended to run the depth and sterile filters in-line at the same time.				

Table 5-37. *A-Gene 0.2 μm Filtration Process Parameters*

Parameter/step	Description	Value	Range	Units	Comments
Filter	0.5/0.2 μm	N/A	N/A	N/A	
Challenge	Load challenge	≤250	N/A	L/m²	Operational flowrate is applicable if depth and sterile filtration operations are performed separately. If both operations are performed simultaneously, use depth filter operational flowrate.
		Target 250	200-400	LMH	
Recovery flush	50 mM sodium phosphate, 350 mM sodium chloride, 0.001% (w/v) poloxamer 188 pH 7.4±0.3	Target 10	8-12	L/m²	Parameters are applicable if depth and sterile filtration operations are performed separately. If both operations are performed simultaneously, use depth filter parameters.
		Target 300	200-400	LMH	
Pressure limits	Differential pressure	≤30	N/A	psid	
	Inlet pressure	≤50	N/A	psi	
Other comments	Measure both back pressure and differential pressure during operation. Ensure that neither limit for the filter unit is exceeded. It is recommended to run the depth and sterile filters in-line at the same time. No pre-use flush is required for the sterile filter.				

Table 5-38. *Comparison of Lysis Step Performance at Various Scales*

Process	Scale	Scale Factor	Viral Genome Titer (vg/mL)	Yield (%)	Aggregate (%)	Purity (%)
Scale-down model (n=6)	100 mL	1	$1.0\pm0.4\times10^{11}$	95±2	2.6±0.1	95.3±1.2
GLP Toxicology (n=3)[a]	50 L	500	$1.0\pm0.4\times10^{11}$	92±3	2.7±0.3	96.5±1.0
Clinical and commercial (n=3)[b]	200 L	2000	$1.0\pm0.4\times10^{11}$	94±3	2.5±0.2	97.1±1.5

[a]Agitation rate is based on bioreactor scale-down model mixing ranges. The USP process uses a mass transfer model and growth performance to scale the mixing setpoints in rpm for the bioreactors. The rpm ranges established for the USP process along with their corresponding power by volume (W/m^3) values is assessed across the lysis step.
[b]Same as the small-scale and toxicity process.

Step Description

The lysis step is a chemically induced, cell membrane disruption process enabling the release of AAV particles from the host cell into the media for further capture and purification. Lysis operation is initiated by the addition of a pre-determined amount of a stock solution of a surfactant and generally between 10-100 units/mL of an endonuclease to the production bioreactor, and a pH adjustment to pH 7.0 with 2M Tris, 0.001% (w/v) Poloxamer 188. Conductivity is an important parameter that should be controlled. The total bioreactor content of A-Gene product and lysis chemicals should be incubated at 37°C for ≥4 hours. Agitation of the bioreactor content must be maintained during the incubation period. The resulting material post-incubation is considered crude lysate. Table 35 lists all components and volumes required for execution of the lysis step.

Depth Filtration and Bioburden Filtration

Depth filtration step uses a size exclusion method for separating lysed cell debris from rAAV particles prior to rAAV chromatographic capture. The depth filtration step is followed by filtration across a dual layer (0.5/0.2 µm) filter for the removal of any fine particulates and bioburden/endotoxin reduction. Table 36 and Table 37 list the operational details required for the execution of the depth filtration and bioburden reduction steps, respectively.

Viral safety is required for gene therapy products. The risk of viral contamination can be mitigated through the screening of raw materials, testing process intermediates, and/or evaluating the effectiveness of viral removal/inactivation during manufacturing processes. The exposure of AAV process intermediates produced in bioreactor detergent treatment is traditionally used for viral clearance in gene therapy manufacturing.

Prior Knowledge

Lysis by surfactant treatment has been used extensively to manufacture gene therapies as well as many other therapeutic agents. Moreover, the process conditions have remained essentially unchanged for these products and throughout the A-Gene development process. Thus, experience gained from the characterization of past studies constitutes prior product knowledge and may be applied directly to the A-Gene process. Because this is not a purification step, worst-case conditions have been identified to assess the stability of the AAV vector during the lysis process. These worst-case operating conditions involve holding the AAV vector at a higher concentration, at the highest surfactant concentration, and at a longer time and higher temperature than routinely specified in manufacturing. Following the worst-case surfactant treatment, the product was tested for aggregation by SE-HPLC and viral genome titer by ddPCR.

Table 5-39. *Depth Filtration Scale Comparison*

Parameter	Scale-down model	200 L
Total filter area (m^2)	0.0023	0.77
Scale-down factor	335	1
Flow-rate (LMH)	≤150	≤150
Feed pressure (psig)	≤18	≤18
Load factor (L/m^2)	≤250	≤250

Table 5-40. *Lysis: Impact on Product Quality Study Design Rationale*

Process parameter	Normal manufacturing target or range	Worst case study conditions	Scientific rationale
Surfactant Concentration	2.86±0.1	2.76	Lowest concentration is expected to result in insufficient lysis, so lowest concentration was chosen to ensure conditions for sufficient lysis during the study.
A-Gene concentration	≤1×10^{11} vg/mL	1×10^{11} vg/mL	The maximum gene concentration in the study was set at 1×10^{11} vg/mL to assess potential aggregation
Time	60-240 minutes	240 minutes	Longer hold times are expected to result in greater aggregation.
Temperature	35-39°C	40C	Higher temperatures may result in capsid instability, greater aggregation, and changes to the purity. We studied a temperature above the normal operating condition to ensure that the AAV vector was stable under normal operating conditions.
Agitation Rate	5-7 P/V (W/m^3)	5 P/V (W/m^3)	Lower agitation rates may result in insufficient lysis

Scale-Down Model

Scale-down models have been used to characterize the process performance of the lysis and clarification step. Table 38 compares the scale factors, yield, aggregate, in lab-scale experiments and large-scale manufacturing. To qualify the model and ensure proper performance at full scale, mixing studies were executed across all scales to ensure efficient mixing within the established time limits. The lysis for this process is performed in the production bioreactor so that the bioreactor SDM qualification performed during upstream process development can be used to support the use of 2-L bioreactors as a SDM for the 200-L scale bioreactors for the lysis step. The data indicate that the process is consistent and comparable across all manufacturing scales and that the laboratory model is representative of full-scale manufacturing operations. Process flow, operating pressure, and load capacity are key scale-down parameters for this unit operation. Table 39 contains a comparison of the SDM filtration process with the 200 L scale process.

Characterization Studies to Assess Impact to Product Quality

Table 40 summarizes the process parameters that were used in the study and the rationale for the selection of these parameters.

The experiment was executed in triplicate in a 100-mL laboratory model (results not shown) and the results are summarized in Table 41. Over time, there was a steady decrease in monomer content while the percentage of aggregates increased, consistent with results obtained with other gene therapies under comparable process conditions. Based on product quality considerations, no critical process parameters were identified for this step.

Hold Time Study

Acceptable hold time stability for the detergent-treated solution after completion of the inactivation, adjustment to pH 5.0 and depth filtration was performed.

Table 5-41. *Product Quality Results Template for Worst-Case Scenario Studies*

Process Hold Time (minutes)	Titer (vg/ml)	Aggregate (%)	Purity by CE-SDS (%)
0	1×10^{11}	<3.1%	>99
30	1×10^{11}	<3.1%	>99
60	1×10^{11}	<3.1%	>99
90	1×10^{11}	<3.1%	>99
120	1×10^{11}	<3.1%	>99
150	1×10^{11}	<3.1%	>99
180	1×10^{11}	<3.1%	>99
240	1×10^{11}	<3.1%	>99

A solution containing the maximum A-Gene concentration of $1x10^{11}$ vg/mL was held at the maximum hold temperature of 25°C for 7 days (data not shown) and demonstrated that this process intermediate can be held at 25°C for 7 days without significant degradation or impact to product quality.

Summary of Process Parameter Classification and Ranges

Depth filtration range was established based on a worst case scenario to support lysis studies. The worst case scenario included high load challenge and flowrate developed from prior process history.

AFFINITY CAPTURE CHROMATOGRAPHY

The affinity step is linked to the performance of the AEX chromatography steps. The affinity capture (AC) chromatography step is linked to the harvest step as shown in Table 44.

Step Description

The purpose of AC chromatography is to remove impurities while capturing the product (maximize product yield). This step uses an immobilized resin that binds the viral vector from the harvested cell culture fluid (clarified harvest) to maximize yield and remove impurities (e.g., HCPs, unpackaged AAV DNA, aggregates, viral contaminants). AC chromatography is an inherently robust processing step with a rich platform performance history that supports the proposed design space. Process

Table 5-42. *In-Process Hold Study Results Template*

Time (days)	Aggregate (%)	Purity (%)
0	<3.1%	>98
1	<3.1%	>97
3	<3.1%	>98
5	<3.1%	>96
7	<3.1%	>96

impurities such as HCP, DNA, and small molecules are removed in the flow through or wash. A low pH buffer elutes the viral vector and sets up the subsequent anion exchange step. AAV capsids are then eluted into a single-use bag containing a predetermined amount of a neutralizing base such as 2M Tris.

Input parameters for affinity capture chromatography include the AAV vector load concentration and load challenge to the resin. The input load concentration would be roughly 1×10^{11} vg/mL and the loading is generally in the range of 1×10^{13} vg/mL resin to 1×10^{14} vg/mL resin, depending on the resin and its flow properties. Interactions that may impact quality attributes include flow rate, elution pH, elution buffer conductivity, and collection UV criteria. To analyze these variables, univariate and/or multivariate studies are needed.

It is important to consider that the separation of AAV particles from viral impurities must be tied to each specific AAV serotype. Some serotypes work well

Table 5-43. *Acceptable Ranges and Criticality Assessment for Lysis and Depth Filtration Step*

Operating Parameter	Acceptable Range	Classification	Rationale	Control strategy
Surfactant concentration	2.76-2.96	CPP	Triton X-100 concentrations <2.76% (w/v) may result in insufficient clearance	Batch record procedure
Time	60-180 min	PP	Longer times lead to aggregation, shorter times may result in incomplete inactivation	Batch record procedure
A-Gene concentration	≤1×10^{11} vg/mL	MP	No effect seen on stability or inactivation	Batch record procedure
Temperature	35°C to 40°C	PP	Higher temperatures may result in capsid instability, greater aggregation, and changes to the purity	Temperature control

Table 5-44. *AC Chromatography Step Linkages*

Input from Lysis and Clarification	Output to Affinity Capture
Vector concentration ≤1×10^{11} vg/mL	Vector concentration ~1×10^{12} vg/mL
pH 7.5±0.5	pH 7.0-8.5
Aggregate <3.1%	Aggregate <3.1%
Purity ~95%	Purity ~95%
HCP ~10^{5}-10^{6} ng/mL	~200 ng/mL but may range from 100 to 1200 ng/mL
Unpackaged DNA ~1,300,000-2,000,000 ng/mL	may range from 100 to 1,000 ng/mL

with commercially available resins, but this step must be optimized for each gene therapy product because the interaction of the resin with the serotype and the impurities are complicated. This will entail optimization of the appropriate wash and elution strategy to maximize removal of viral impurities.

For development and manufacturing purposes, the columns are packed to a bed height of 10 to 20 cm. The column is purged of storage buffer and equilibrated. The clarified harvest is then loaded. After loading to 1×10^{13} to 1×10^{14} vg/mL, the column is washed with equilibration buffer. The viral vector is eluted from the column with a low pH elution buffer. The start of collection is based on absorbance at 280 nm and 260 nm and is ended by the absorbance at 280 nm and 260 nm or based on specified column volumes. The elution occurs into a container or bag that has a predetermined amount of neutralizing buffer such as 2M Tris to ensure that the affinity elution pool is neutralized immediately. The column is then cleaned, regenerated, and re-equilibrated prior to starting the next load cycle. Upon completion of the processing of the entire harvest, the column is washed with and stored in storage buffer until the next use.

Outputs for affinity capture chromatography include process-related impurities (e.g., HCP, unpackaged DNA), 14product-related impurities (aggregates, full-empty ratio), and process attributes (yield). More in-depth characterization of post-translational modifications or additional degradation by low pH exposure may be expected at this stage, and it is important to assess additional quality attributes by more advanced techniques such as mass spectrometry to determine conformational changes in the AAV vector.

Table 5-45. *Parameters Included in the Multivariate Study for AC Chromatography*

Parameter	Potential Interactions	Range
AAV vector load	• Flow rate • Elution pH • End collection • Bed height (univariate)	10^{13}-10^{14} vg/mL resin
Flow rate	• Vector load • Temperature • Bed height (univariate)	100-500 cm/hr
Elution buffer pH/conductivity	• Vector load • End collection	pH 2-3
End collection	• Elution buffer • pH • Vector load	1-3 CVs

Table 5-46. *Linkages from Clarified Harvest to Affinity Chromatography*

Input From Clarified Harvest	Output to AC
Vector concentration	1×10^{13} to 1×10^{14} vg/mL
pH	7.0-8.5
Conductivity	17±0.5 mS/cm
Aggregate percentage	≤3%
HCP concentration	≤200 ng/mL
Unpackaged DNA concentration	≤1000 ng/mL

Table 5-47. *Design and Results*

Parameter	Testing Range	Potential Interactions	Scientific Rationale
Vector load concentration	1×10^{10} to 1×10^{11} vg/mL	None	• We expect no impact or interaction • It is possible that an extended load volume due to a decrease in titer could cause displacement of impurities during the load phase, which would result in lower pool impurities • We further evaluated the 2 feedstocks used in the multivariate study via spiking and dilution studies over a range of titers ($1x10^{10}$ vg/mL to $1x10^{12}$ vg/mL)

Scale-Down Model

Typical scale-down models are columns that have a bed height of 10 cm and a column diameter of 1 cm. Additional scale-down experiments with different column heights (10 cm, 20 cm and 30 cm) to ensure pressure flow properties scale appropriately and consistency of product quality attributes were performed. This allows flexibility to transfer the technology to different manufacturing locations and contract manufacturing organizations.

Risk Assessment to Plan Process Characterization Studies

A risk assessment tool that is used to identify parameters that must be included in the design of process characterization studies, which include DOEs and univariate approaches. Details of how to conduct a process risk assessment (PRA), are shown in chapter 4. In this chapter for the sake of brevity, only the results are shown.

Multivariate DOE Studies

Based on the results of a risk assessment (details not shown here) parameters included in the A-Gene multivariate study for affinity capture chromatography include the AAV vector load, bed height, flow rate, elution buffer pH, and end collection (Table 45). Linkages to the next step are shown in Table 46. For additional details on how to conduct a risk assessment, refer to chapter 4.

CHROMATOGRAPHY

Univariate Studies

Based on the risk assessment results and prior knowledge, load concentration was not expected to interact with any other process parameter of this step, thereby enabling it to be studied as a univariate process variable (Table 47). Results of the study showed that no impact was observed on step performance or product quality over the range tested (data not shown). Thus, this parameter was classified as a MP and included along with multivariate study results to fully describe the knowledge space for the affinity step.

Process Ranges Based on Platform Knowledge

Based on the risk assessment, the process parameters that were considered as not requiring further investigation are listed in Table 48. For these parameters, the extensive process knowledge and modular process performance claims justify the proposed acceptable ranges.

Summary of Process Parameter Classification and Ranges

Results of affinity step characterization studies demonstrated that this step does not impact the distribution of product variant CQAs (e.g., VP ratios). Moreover, this step was shown to have robust process performance even when challenged with a wide range of feed stream inputs (HCP, DNA, and titer).

The affinity operating conditions influence the HCP and unpackaged DNA levels in the resulting product pool. Because subsequent steps (AEX) can reduce HCP and DNA to safe and consistent levels, the acceptable output levels from the affinity step are linked to the operating conditions of these subsequent steps. A model defining this linkage is given in the Linkage of Unit Operations section.

Risk analysis, process characterization studies. and process performance history demonstrated that the

Table 5-48. *Process Parameter Ranges Supported by Prior Knowledge and Module Process Performance Claims*

Parameter	Prior knowledge	Acceptable range	Scientific Rationale
Bed height	X-Gene	10-20 cm	Platform knowledge shows no significant effect on product quality or process performance. There is potential at low bed height, high vector load, and high flow rate to decrease yield and increase product pool impurities. Acceptable range is 8-20 cm.
	Y-Gene	9-11 cm	
	Z-Gene	12-18 cm	
Eq/wash pH	X-Gene	pH 6.6-7.6	Platform knowledge shows no significant effect on product quality or process performance. Therefore, the proposed buffer pH range should not affect the performance of this affinity resin. Acceptable range is pH 6.6-7.6.
	Y-Gene		
	Z-Gene		
Eq/wash composition	X-Gene	(70-140%) Tris, NaCl	Platform knowledge shows no significant effect on product quality or process performance. Therefore, the proposed composition ranges should not affect the performance of this affinity resin. Acceptable range is 60-140% Tris, NaCl concentration.
	Y-Gene		
	Z-Gene		
Start collection parameter	X-Gene	Start 0.1-1.0 OD	Platform knowledge shows no significant effect on product quality or process performance. The elution phase elutes the product and does not separate the product from impurities. Therefore, the only potential impact to the process is decreased yield or collection of additional equilibration buffer in the product pool, but due to the steepness of the starting part of the elution peak neither of these outcomes will occur. Acceptable range is 0.05-1.0 OD.
	Y-Gene	Start 0.3-1.0 OD	
	Z-Gene	Start 0.05-0.5 OD	
Eq/wash volumes	X-Gene	(90%-110%)	Platform knowledge shows no significant effect on product quality or process performance. Acceptable range is 60-140%.
	Y-Gene	(90%-110%)	
	Z-Gene	(60%-140%)	

affinity step does not have any critical process parameters (CPPs). Only three parameters were linked to CQAs (vector load challenge (vg/mL resin), elution buffer pH, and elution buffer conductivity) and were classified as PPs based on control capabilities to operate within the proposed design space. The classification of process parameters is summarized in Table 49.

Reuse/Lifetime Resin Studies

Column lifetime studies using the scale-down model for A-Gene established that the useful lifetime of the affinity resin is expected to be at least 20 cycles. The data supporting this conclusion are shown in Table 50.

Anion Exchange Chromatography

Ion exchange chromatography is used to reduce the residual impurities and enrich viral vectors. IEX requires process development and optimization that depends on the initial feed stream, serotype, and the target needed for product quality. Targeted yield percent may vary depending on the capsid serotype and the indication, but higher yield percentages may be more difficult to achieve.

Table 5-49. *Variables, Ranges, Controls, and Parameter Classification*

Parameter	Range Studied	Justification	Control	Classification
Vector load	1×10^{13} to 1×10^{14} vg/mL resin	Multivariate	Batch procedures, skid control	PP
Elution buffer pH	2.5-3.5	Multivariate	Batch procedures	PP
Flow rate	100-300 cm/hr	Multivariate	Skid control	PP
End collection	2.0-3.2 CV	Multivariate	Skid control	MP
Temperature	15-30°C	Multivariate	Environmental control	MP
Resin lifetime (Resin A)	<30 cycles	Univariate	Column use log	MP
Load concentration	1×10^{10} - 1×10^{11} vg/mL	Univariate	Titer analysis	MP
Bed height	8-20	Modular	Column use log	MP
Eq/wash pH	6.6-7.6	Modular	Batch record procedure	MP
Eq/wash composition	60-140% of target	Modular	Batch record procedure	MP
Start collection parameter	0.05-1.0 OD	Modular	Skid control	MP
Eq/wash volumes	60-140% of target	Modular	Skid control	MP

Notably, there is a triangular association between cost, speed, and quality. While two of these may be attainable, the third aspect may require additional development.

Depending on the target, one may need to use ultracentrifugation rather than IEX for enriching empty capsids from full capsids in particular. For example, if there is a very low percentage of full capsids, IEX may not be sufficient for purification because the peak will be buried within a larger peak of empty capsids. In that case, ultracentrifugation may provide a better alternative to increase the percentage of full particles. However, careful evaluation of the IEX step can still lead to a successful empty/full separation step that provides high yield, acceptable enrichment, and manufacturability.

The design space for the AEX step is linked to the performance of the affinity capture chromatography step. This section describes the use of prior knowledge to design A-Gene process characterization studies and support a modular approach to impurities clearance.

Input parameters to be included in the multivariate

Table 5-50. *Hypothetical Affinity Resin Lifetime Study*

Reuse cycle number	Yield (%)	HCP (ng/mL)	DNA (ng/mL)	Aggregate (%)
2	75	220	1000	2.2
4	70	180	1000	2.4
6	75	300	1000	2.0
8	70	150	1,100	1.9
10	70	190	900	2.5
15	70	240	1,100	2.1
20	68	250	900	2.2

Table 5-51. *Anion Exchange Chromatography Step Linkages*

Input from affinity capture chromatography	Output to small virus retentive filtration
Vector concentration $5x10^{12}$ to $5x10^{13}$ vg/mL	Vector concentration $1x10^{12}$ to $1x10^{13}$ vg/mL
pH ~7.0-8.5	pH 7.0-8.5
Aggregate <3%	Aggregate <2%
HCP ~200 ng/mL	HCP < 10 ng/mL
Unpackaged DNA 100-1000 ng/mL	DNA <50 ng/mL
Full capsids ~20-30%	Full capsids ~40-50%

study for AEX include AAV vector load, % full capsids, bed height, flow rate, elution buffer pH, and end collection. Outputs for AEX include process-related impurities (e.g., HCP, DNA) and product-related impurities (e.g., aggregate, full-empty ratio), and process attributes (e.g., yield) (Table 51). Other outputs that may need to be considered depending on serotype include deamidation, oxidation, and glycosylation.

Step Description

The final purification step in the A-Gene process is AEX chromatography, which is operated in the flow-through mode to bind impurities such as HCP, DNA, empty capsids, and endotoxins to the resin while the AAV flows through the column. The AEX step can be operated in the bind-elute mode or the flow-through mode depending on the target for impurity clearance. Operating AEX in the flow-through mode may be useful to further enhance impurity clearance (HCP, DNA) with an affinity pool with 60% full AAV capsids. The bind-elute mode is preferable for affinity pools with <20% full AAV capsids. The full capsid will bind to the resin while the empty capsids, HCP, and DNA impurities will flow through. The full capsid can then be eluted using a salt step or linear gradient.

The column is packed with AEX resin to a height of approximately 10 cm. Prior to loading, the affinity capture chromatography product pool is adjusted to the appropriate pH and conductivity. Following equilibration and loading, the column is washed with equilibration buffer to collect the A-Gene product based on absorbance of 280 nm. The entire batch is typically processed in one cycle, but multiple cycles are acceptable where the AEX product pools are combined for subsequent processing. If multiple cycles are required, the column is regenerated and re-equilibrated prior to subsequent cycles. After the final cycle, the column is regenerated, cleaned, and stored.

Table 5-52. *Scale-up Parameters for AEX Chromatography Step*

Column parameters	Laboratory scale	Pilot scale (200 L)	Manufacturing scale (200 L)
Bed volume (mL)	9.5	3142	3142
Bed height (cm)	9-11	9-11	9-11
Diameter (cm)	1.1	20 cm	20
Linear flow rate (cm/hr)	150	150	300
Residence time (min)	4	4	4
Vector load concentration (vg/mL of resin)	$1×10^{13}$	$1×10^{13}$	$1×10^{13}$
Scale-up factor	1	83	83

Table 5-53. *Process Performance for the AEX Chromatography Step at Different Scales*

Product quality attributes	Laboratory scale		Commercial scale (200 L)	
	Minimum	Maximum	Minimum	Maximum
HCP (ng/ml)	10	25.6	8	15.1
DNA (ng/ml)	50	110	10	63.0
Yield (%)	85	95	95	100
Aggregate (%)	0.8	1.0	0.2	0.8
% Full Capsids	40%	50%	40%	50%

Table 5-54. *Parameters Included in the Multivariate Study for AEX*

Parameter	Potential interactions	Range
AAV vector load	• Flow rate • Load pH • Load conductivity • Bed height (univariate)	10^{12} to 10^{13} vg/mL resin
Flow rate	• Vector load • Temperature • Bed height (univariate)	75-225 cm/hr
Load pH/conductivity	• Load conductivity • Vector load	pH 7.6-8.6 Conductivity 17±3 ms/cm

Scale-Down Model

A scale-down model for the AEX step was established following standard scale-down/up considerations for chromatography: the column size was scaled based on column diameter, with constant bed height, linear velocity, protein load, and load volume/column volume ratio across the scales. This scale-up approach ensures that residence time and mass transport are constant across scales. Volumes of the equilibration, wash, and other buffers are based on column volume, thereby ensuring the same amounts are used proportionally at laboratory and production scales. A summary of the scale-up parameters is presented in Table 52.

As shown in Table 53, the laboratory-scale AEX chromatography step performance is comparable to the full-scale manufacturing (200 L) scale process, including the quality attributes of the AEX product. The residence time of the product on the columns and the elution profiles were comparable in both the laboratory- and full-scale production processes. Furthermore, by visual inspection the chromatograms were consistent and comparable for the individual small-scale purification runs.

Risk Assessment to Define Process Characterization Studies

A risk assessment approach was used to categorize all AEX chromatography step process parameters into three groups: parameters warranting multivariate evaluation, secondary parameters whose ranges could be supported by univariate studies, and parameters that did not require new studies, but instead would employ ranges based on knowledge space or modular claims established from prior knowledge. For details on how to conduct a risk analysis, refer to chapter 4.

Each process parameter was assessed based on the potential impact on quality attributes or process attributes and impact assessed. Platform process development and process characterization knowledge from other gene therapies, manufacturing history, and scientific knowledge were used to rank each process variable in the initial risk assessment and set the ranges for evaluation.

Multivariate DOE Studies

Based on this risk assessment, parameters included in the A-Gene multivariate study for AEX chromatography

include the AAV vector load, load flow rate, elution pH, and conductivity (Table 54). Linkages to the next step are shown in 55.

Univariate Studies

Based on the risk assessment results and prior knowledge, vector load concentration was not expected to interact with any other process parameter of this step, thereby enabling it to be studied as a univariate process variable (Table 56). Results of the study showed that no impact was observed on step performance or product quality over the range tested (data not shown). Thus, this parameter was classified as a MP and included along with multivariate study results to fully describe the knowledge space for the affinity step.

Process Ranges Based on Platform Knowledge

Based on the risk assessment, the process parameters that were considered as not requiring further investigation are listed in Table 57. For these parameters, the extensive process knowledge and modular process performance claims justify the proposed acceptable ranges.

Summary of Process Parameter Classification and Ranges

Results of AEX chromatography step characterization studies demonstrated that this step does not impact the distribution of product variant CQAs (e.g., VP ratios). Moreover, this step was shown to have robust process performance even when challenged with a wide range of feed stream inputs (HCP, DNA, titer, and full capsids). The AEX operating conditions influence the HCP and DNA levels in the resulting product pool. Since subsequent steps cannot reduce process-related impurities to safe and consistent levels, the acceptable output levels from the AEX steps are linked to the operating conditions of these subsequent steps.

Table 5-55. *Parameters Included in the Multivariate Study for AEX*

Input from affinity step	Output to ultrafiltration/ diafiltration
Vector concentration	1×10^{12} to 1×10^{13} vg/mL
Load pH	8.1±0.5
% Full Capsids	20-30%
Load conductivity	17±3 mS/cm
Aggregate percentage	≤2%
HCP concentration	≤10 ng/mL
DNA concentration	≤100 ng/mL

Risk analysis, process characterization studies, and process performance history demonstrate that the affinity step does not have any CPPs. Four parameters were linked to CQAs (vector load, flow rate, load pH, and conductivity) and were classified as PPs based on control capabilities to operate within the proposed design space. The classification of process parameters is summarized in Table 58.

Reuse/Lifetime Resin Studies

Column lifetime studies using the scale-down model for A-Gene established that the useful lifetime of the affinity resin is expected to be at least 10 cycles. The resin lifetime study (Table 59) showed no yield loss with extended use and is also consistent with change in process-related impurities such as HCP and DNA.

Table 5-56. *Design and Results*

Parameter	Testing range	Severity rating	Potential interactions	Scientific rationale
Vector load concentration	1×10^{12} to 1×10^{13} vg/mL		None	No impact or interaction is expected. An extended load volume due to a decrease in titer would only potentially cause displacement of impurities during the load phase (resulting in lower pool impurities). The two feedstocks that were used in the multivariate study were further evaluated by spiking and dilution studies to cover 1×10^{12} to 1×10^{13} vg/mL titers.

Table 5-57. *Process Parameter Ranges Supported by Prior Knowledge and Module Process Performance Claims*

Parameter	Prior knowledge	Acceptable range	Scientific rationale
Bed height	X-Gene	9-11 cm	Platform knowledge shows no significant effect on product quality or process performance. There is potential at low bed height, high vector load, and high flow rate to decrease yield and increase product pool impurities. Acceptable range is 8-18 cm with process control of vector load and flow rate within specified ranges.
	Y-Gene	9-11 cm	
	Z-Gene	10 -18 cm	
Load pH	X-Gene	pH 7.8-8.8	Platform knowledge shows significant effect on product quality or process performance. Therefore, the proposed buffer pH range should be carefully evaluated for anion-exchange resin between pH 7.8-8.8.
	Y-Gene		
	Z-Gene		
Load conductivity	X-Gene	20 ± 6 mS/cm	Platform knowledge shows significant effect on product quality or process performance. Therefore, the proposed composition ranges should be carefully evaluated for load conductivity.
	Y-Gene		
	Z-Gene		
Start collection parameter	X-Gene	Start 0.1-1.0 OD	Platform knowledge shows no significant effect on product quality or process performance. The elution phase elutes the product and does not separate the product from impurities. Therefore, the only potential impact to the process is decreased yield or collection of additional equilibration buffer in the product pool, but due to the steepness of the starting part of the elution peak neither of these outcomes will occur. Acceptable range is 0.05-1.0 OD.
	Y-Gene	Start 0.3-1.0 OD	
	Z-Gene	Start 0.05-0.5 OD	
Flush volumes	X-Gene	(80%-120%)	Platform knowledge shows no significant effect on product quality or process performance. Acceptable range is 60-140%.
	Y-Gene	(80%-120%)	
	Z-Gene	(60%-140%)	

ULTRAFILTRATION/DIAFILTRATION

Step Description

Ultrafiltration/diafiltration (UF/DF) is the dedicated step to concentrate the viral vector to the target concentration and place it in the final formulation buffer. The UF/DF step is accomplished using an ultrafiltration membrane that is retentive to the viral vector and permeable to buffer species. A poloxamer excipient is added to the diafiltration buffer. A parameter to consider within the risk analysis is the loading capacity, which would be measured in vg/m^2. The AEX pool is loaded onto a 30-kDa membrane with a screened channel and concentrated to a specified concentration of 5×10^{12} vg/mL. At this concentration, the product is diafiltered into formulation buffer. Upon the completion of diafiltration, the product retentate pool is recovered from the system and the system is flushed with an appropriate volume of formulation buffer. This flush is used to dilute the product retentate pool to the specified concentration. The TFF membrane is used for a single cycle and then discarded.

Prior Knowledge

From past process knowledge of UF/DF, it is known that AAV vectors are available at fairly low concentrations on a total protein basis. Therefore, the viscosity of the AAV solutions is similar to the buffers they are formulated in (1-1.5 cP) with little propensity of intermolecular interactions that affect the filtrate flux. It is important to identify optimal operating conditions that minimize

Table 5-58. *Variables, Ranges, Controls, and Parameter Classification*

Parameter	Range studied	Justification	Control	Classification
Vector load	1×10^{12} to 1×10^{13} vg/mL resin	Multivariate	Batch procedures, skid control	PP
Load pH	8.2-9.6	Multivariate	Batch procedures	CPP
Load conductivity	16-18	Multivariate	Batch procedures	CPP
Flow rate	75-225 cm/hr	Multivariate	Skid control	PP
Temperature	15-30°C	Multivariate	Environmental control	MP
Resin lifetime (AEX)	<10 cycles	Univariate	Column use log	MP
Load concentration	1×10^{12} to 1×10^{13} vg/mL	Univariate	Titer analysis	MP
Bed height	9-20	Modular	Column use log	MP
Eq/wash pH	6.6-7.6	Modular	Batch record procedure	MP
Eq/wash composition	60% to 140% of target	Modular	Batch record procedure	MP
Start collection parameter	0.05-1.0 OD	Modular	Skid control	MP
Eq/wash volumes	60% to 140% of target	Modular	Skid control	MP

Table 5-59. *AEX Resin Lifetime Study*

Reuse Cycle Number	Yield (%)	HCP (ng/mL)	DNA (ng/mL)	Aggregate (%)
2	85	20	120	1.2
4	82	10	100	1.4
6	86	50	80	1.9
8	85	13	110	1.9
10	88	19	100	1.5
15	80	41	110	2.0
20	82	26	120	1.2

the number of pump passes through the membrane. In general, a flux vs TMP excursion that provides the right balance of filtrate flux and TMP is chosen so that channel-induced shear effects are minimized.

Scale-Down Model

The TFF operation consists of an ultrafiltration step, which concentrates the product, followed by a diafiltration step, which is used to exchange the product into the appropriate formulation buffer. The key scale-down parameters for TFF are the membrane pore size (specified in terms of a molecular weight cut-off), cross flow rate, membrane load, bulk concentration, transmembrane pressure (TMP), and diafiltration volumes (DV). Table 61 contains a comparison of the scale-down model for the tangential flow filtration (TFF) process.

Risk Assessment to Define Process Characterization Studies

A risk assessment approach was used to categorize all Tangential Flow Filtration (TFF) step process parameters into three groups: 1) parameters warranting multivariate evaluation, 2) secondary parameters whose ranges could be supported by univariate studies, and 3) parameters

Table 5-60. *TFF Process Parameters*

Parameter	Value	Comments
UF/DF membrane molecular weight cut-off	30 kD	
Material of construction	Composite regenerated cellulose	
Temperature	15°C to 25°C	
Equilibration pH	8.5±0.2	
Equilibration conductivity	17±2 mS/cm	
Membrane Load	≤1×10^{17} vg/m^2	
Transmembrane pressure (TMP)	20±5 psig	
Cross flow rate	4±2 LMM	
Cbulk	(5±1)×10^{12} vg/mL	Concentrate and diafilter at this concentration
UF/DF pool concentration	(3.5 ± 0.5)× 10^{12} vg/mL	
Diafiltration volumes (DV)	≥6 DV	Manufacturing: 6±1 DV
Retentate pool pH	7.3±0.2	Buffer exchanged product

which did not require new studies, but instead would employ ranges based on knowledge space or modular claims established from prior knowledge.

Each process parameter was assessed based on the potential impact on quality attributes or process attributes and impact assessed. Platform process development and process characterization knowledge from other gene therapies, manufacturing history, and scientific knowledge were used to rank each process variable in the initial risk assessment and set the ranges for evaluation. The TFF ranking results are summarized in Table 62.

Process Characterization Studies

Input parameters for TFF include loading (vg/m^2), transmembrane pressure (psig), and virus concentration (1x10^{12} to 1x10^{13} vg/mL). The tangential flow filtration step parameters requiring characterization based on the risk assessment are summarized in Table 62. The DF buffer conductivity will be evaluated using a univariate study, while membrane load, DF buffer pH, flow rate, TMP, UF concentration, and DV will be evaluated using DOE study designs. The study outputs used to measure the performance of these studies are listed in Table 63.

Table 5-61. *Tangential Flow Filtration Scale Comparison*

Parameter	Scale-down model	200 L
Total membrane area (m^2)	0.0088	0.44
Scale-down factor	50	1
Membrane pore size (kDa)	30	30
Membrane load (vg/m^2)	≤1×10^{17}	≤1×10^{17}
C_{bulk} (vg/mL)	5×10^{12}	5×10^{12}
TMP (psig)	20±5	20±5
Cross flow rate (LMM)	4±2	4±2
Diafiltration volume	6 DV	6 DV

Tangential Flow Filtration Univariate Studies

Three parameters were evaluated individually in one factor at a time (OFAT) studies. These include DF buffer conductivity, flow rate, and TMP. The flow rate and TMP were evaluated in the multivariate studies as well, but the goal was to optimize the ranges for these two parameters

Table 5-62. *Summary of Tangential Flow Filtration Risk Assessment Results*

Parameter	Study Plans	Set-Point	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Membrane load (vg/m2)	DOE	≤1.2×10^{15}	2.0×10^{15}	1.0×10^{16}	2.6×10^{16}	4.22×10^{16}	1.0×10^{17}	-	-	-
TFF DF buffer pH	DOE	7.3	6.9	7.1	7.3	7.5	7.7	-	-	-
TFF DF buffer conductivity (mS/cm)	DOE	19.7	15	18	21	-	-	-	-	-
Flow rate (LMM)	DOE	5	2	3	4	5	6	-	-	-
TMP (psig)	DOE	25	15	20	25	30	35	-	-	-
End of UF product concentration (vg/mL)	DOE	5e+12	4e+12	4e+12	5e+12	6e+12	7e+12	-	-	-
DV	DOE	8	5	6	8	10	11	-	-	-
Hold time TFF pool (15-25°C)	Hold study	≤12 hrs	0	6	9	12	24	36	48	-
Temperature during hold time TFF pool (15-25°C)	Hold study	15-25°C	25	-	-	-	-	-	-	-
Hold time TFF pool (2-8°C)	Hold study	≤30 days	0	1	4	7	14	21	30	40

prior to executing the DOE studies. In addition, studies were performed to evaluate poloxamer clearance across the TFF membrane.

TFF Diafiltration Buffer Conductivity

The impact of DF buffer conductivity was assessed by targeting selected NaCl concentrations and comparing the performance of the DF step using DF buffer with concentrations of 150 mM, 180 mM, and 210 mM NaCl. This study is important to establish bounds on the diafiltration buffer conductivity without reducing the resolution of the characterization study.

Flow Rate and Transmembrane Pressure Optimization

Flow rate and TMP will be evaluated through an optimization study examining 5 levels of each parameter and comparing resulting flux curves to identify the TMP-independent operation ranges. These results will help to define the ranges to be evaluated in the DOE studies. The outline of this study is provided in Table 64.

Ideally, this study is performed during process development. Initial process development to identify flux vs TMP must be the first study performed. However, this study must be repeated during process characterization using different membrane lots at different permeabilities to ensure that the range chosen during development stands valid as a function of membrane lot variation.

Poloxamer Sieving Rates

The sieving rates of poloxamer across the TFF process will be evaluated in order to model the co-concentration of poloxamer throughout this unit operation. For this purpose, the sieving coefficients of poloxamer at different feed flow rates is measured using formulation buffer with different concentrations of poloxamer. Another measurement is made using the AEX pool (load material to UF/DF step) to ensure that measurements with the buffer are comparable to the measurement in the presence of the

Table 5-63. *Tangential Flow Filtration Study Responses*

Process Performance Responses	Analytical Responses
Product pool turbidity (NTU) Product pool pH Product pool conductivity Osmolality Product pool volume Process time Appearance	Residual affinity ligand Residual antifoam Residual Surfactant Aggregates; submicron Aggregates; subvisible Vector genome titer Poloxamer-188 concentration Sodium phosphate concentration (pH and conductivity) Sodium chloride concentration (pH and conductivity) Vector genome recovery

AAV. The poloxamer sieving rate study design is shown in Table 65. Experiments were performed under conditions of total recycle to measure sieving coefficients of poloxamer. All experiments were conducted at a TMP of 20 psig.

Tangential Flow Filtration Multivariate Studies

Due to the number of factors to be studied, the TFF step was evaluated in two stages. First, a randomized fractional factorial design (FFD) screening study examining all six parameters, followed by a randomized 1.5 axial central composite design (CCD) focused on the four factors demonstrating the most significant estimates of effect. These parameters include membrane load, DF buffer pH, flow rate, TMP, UF concentration, and DV. Table 66 summarizes the design for the FFD screening study. The full study design is not shown here.

This study was designed allowing estimation of all 2-factor interactions and includes eight center-point runs, resulting in a total of 40 runs.

Summary of Parameter Classifications and Ranges

Results of TFF step characterization studies demonstrated that this step does not impact the distribution of product variant CQAs (e.g., VP ratios). Moreover, this step was shown to have robust process performance even when challenged with a wide range of feed stream inputs (listed in table 64) or a range of diafiltration buffers between 150 and 210 mM, indicating that this step is unlikely to go out of control within normal manufacturing ranges of pH and conductivity. The TFF conditions influence the aggregate levels in the resulting product pool.

Risk analysis, process characterization studies and process performance history demonstrate that the critical process parameter was directly related to membrane load challenge. Given the fairly high fluxes of the membrane at different feed flow rates and TMP, parameters were linked to CQAs (vector load, flow rate, load challenge, TMP, and conductivity) and were classified as PP based control capabilities to operate within the proposed design space. The classification of process parameters is summarized in Table 67.

Table 5-64. *Flow Rate and TMP Optimization Study Design*

	Level 1	Level 2	Level 3	Level 4	Level 5
Flow Rate (LMM)	2	3	4	5	6
Transmembrane Pressure (psig)	15	20	25	30	35

Table 5-65. *Poloxamer Sieving Rate Study Design*

Load material	Flow rate (LMM)	Flow rate (LMM)	Flow rate (LMM)
Formulation buffer with 0.001% poloxamer	2	4	6
Formulation buffer with 0.01% poloxamer	2	4	6
AEX pool with 0.001% poloxamer	2	4	6

Table 5-66. *TTF DOE Screening Study Design: FFD*

Level	Membrane load (vg/m²)	Diafiltration buffer pH	Flow rate (LMM)	Transmembrane Pressure (psig)	UF Concentration (vg/mL)	DV
-	5E+15	7	2	10	3E+12	4
0	5E+16	7.3	4	15	6E+12	8
+	9.5E+16	7.6	6	20	9E+12	12

Table 5-67. *Variables, Ranges, Controls, and Parameter Classification for TFF step*

Parameter	Range studied	Justification	Control	Classification
Membrane Loading	1×10^{15} to 1×10^{17} vg/m²	Multivariate DOE	Batch procedures, skid control	CPP
Final Concentration (vg/mL)	3×10^{12} to 7×10^{12}	Multivariate DOE	Batch procedures, skid control	CPP
Feed Flow Rate (LMM)	2 – 6	Multivariate DOE	Batch procedures	PP
Transmembrane Pressure (psig)	10-20	Multivariate DOE	Batch procedures	PP
Temperature	15-30°C	Multivariate DOE	Environmental control	MP
Load concentration	1×10^{12} to 2×10^{13} vg/mL	Univariate	Titer analysis	MP
Diafiltration buffer pH	7.3 – 7.6	Multivariate DOE	Batch procedures	MP

SUMMARY OF DOWNSTREAM PROCESS DESIGN SPACE

Worst-case linkage studies are part of the BLA-enabling studies with the intent to run each unit operation within the scope of the DOE at the worst-case scenario (including hold time) concurrently to determine whether the quality attributes are impacted. Hold times, not only at each step but also cumulatively, are important to consider in the worst-case linkage studies. Importantly, it is possible to perform worst-case linkage studies on a smaller (i.e., not commercial) scale for cost considerations and to reduce product usage. A detailed description of process characterization studies may be reviewed in A-Mab.[10]

Control Strategy for Downstream Process:

For details on the control strategy, refer to chapter 7.

Endnotes

1. *Robert MA, Chahal PS, Audy A, Kamen A, Gilbert R, Gaillet B. Manufacturing of recombinant adeno-associated viruses using mammalian expression platforms. Biotechnol J. 2017;12(3).*
2. *Merten OW, Hebben M, Bovolenta C. Production of lentiviral vectors. Mol Ther Methods Clin Dev. 2016;3:16017.*
3. *Nouri F, Sadeghpour H, Heidari R, Dehshahri A. Preparation, characterization, and transfection efficiency of low molecular weight polyethylenimine-based nanoparticles for delivery of the plasmid encoding CD200 gene. Int J Nanomedicine. 2017;12:5557-5569.*
4. *van der Loo JC, Wright JF. Progress and challenges in viral vector manufacturing. Hum Mol Genet. 2016;25(R1):R42-52.*
5. *Gray SJ, Choi VW, Asokan A, Haberman RA, McCown TJ, Samulski RJ. Production of recombinant adeno-associated viral vectors and use in in vitro and in vivo administration. Curr Protoc Neurosci. 2011;Chapter 4:Unit 4 17.*
6. *Bi X, Liu LF. A replicational model for DNA recombination between direct repeats. J Mol Biol. 1996;256(5):849-858.*
7. *Boissy R, Astell CR. An Escherichia coli recBCsbcBrecF host permits the deletion-resistant propagation of plasmid clones containing the 5'-terminal palindrome of minute virus of mice. Gene. 1985;35(1-2):179-185.*
8. *CMC Biotech Working Group. A-Mab: a case study in bioprocess development (chapter 3). CASSS website. https://cdn.ymaws.com/www.casss.org/resource/resmgr/imported/a-mab_case_study_version_2-1.pdf. Updated October 30, 2009. Accessed February 16, 2021.*
9. *ICH. ICH Q5A (R1) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. ICH website. https://www.ema.europa.eu/en/ich-q5a-r1-quality-biotechnological-products-viral-safety-evaluation-biotechnology-products-derived. Updated January 1997. Accessed February 9, 2021.*
10. *CMC Biotech Working Group. A-Mab: a case study in bioprocess development (chapter 4). CASSS website. https://cdn.ymaws.com/www.casss.org/resource/resmgr/imported/a-mab_case_study_version_2-1.pdf. Updated October 30, 2009. Accessed February 16, 2021.*

Chapter 6

Drug Product

Alliance *for* Regenerative Medicine

NIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 6 | Contents

Chapter Summary

The vector manufacturing process culminates with the formulation and vialing of the purified vector product. While this stage may come temporally at the end of the process, it is important to consider the desired drug product characteristics, including fill-finish, formulation, characterization, container closure integrity testing (CCIT), and long-term storage and stability, at the commencement of the gene therapy development process. It is important to note that the final product has the greatest value, and thus mistakes at this stage may significantly impact the gene therapy's success regarding product misformulation, contamination, or improper packaging.

It is of the utmost importance to begin the gene therapy development process while considering the "end goal" (as outlined in the TPP and QTPP). In this chapter, we have outlined the best practices for fill-finish, formulation, characterization, CCIT, and long-term storage and stability that can be used to develop a robust final gene therapy product; however, the information here is in no way exhaustive. Each process will differ based on the specific product, its characteristics, administration route, titer selection, final formulation buffer, and container choice, among others.

Key Points

The final stage of the vector manufacturing process is the formulation and vialing of the purified vector product. It is critical that formulation and vialing be considered at the beginning of the gene therapy development process in order to develop a robust final product.

- After all the effort that goes into GMP bulk gene therapy production, the final product has the greatest value. Mistakes at this point are the costliest and can lead to product aggregation, contamination, or instability, among other adverse consequences.
- Fill-finish refers to the immediate outcome from the upstream and downstream processes. Because the product container is going to be used for the long-term storage of the product, it must be robust and be able to maintain product integrity at the defined storage conditions. The most important critical quality attributes (CQAs) during this stage include aggregation, potency, identity, sterility, and CCIT.
- In the preclinical stage, potency is generally the most important CQA during the formulation stage. At a clinical stage of development, CQAs should cover the four Food and Drug Administration defined categories of tests: safety, purity, identity, and potency. Within these categories, specific CQAs include but are not limited to physical titer, genetic identity, aggregation state, infectivity, full to empty particles, biological activity or potency, and/or immunological activity.
- CQAs should be monitored in a longitudinal manner to ensure stability of CQAs over time during long-term stability studies.
- Several factors may impact stability during long-term storage, including temperature fluctuations, diluents, container constituents, and other environmental considerations.
- When choosing an appropriate container and container closure, it is imperative to consider the container's compatibility with the product, the intended route of administration, and long-term storage conditions (e.g., ability to withstand cryopreservation).

Introduction

The final stage of the vector manufacturing process is the formulation and vialing of the purified vector product. It is important to ensure the use of excipients that allow vector stability under the anticipated storage conditions as well as excipients and resultant formulations that are compatible with the expected route of administration.[1] After the considerable amount of effort that goes into GMP bulk gene therapy production, the final product has the greatest value (and thus has the most to lose as a product failure). Mistakes at this point can lead to product aggregation, contamination, or instability, among other adverse consequences.

There is a holding step between the bulk drug substance (DS) and drug product (DP) stages. During this time, there will be GMP measurement to ensure that the DP can meet the requirements as specified by regulatory agencies. Analytics related to the safety profile, potency, and titer are performed prior to formulation and fill-finish (i.e., product vialing).

Fill-Finish

Fill-finish refers to the immediate outcome from the upstream and downstream processes. Because the product container is going to be used for the long-term storage of the product, it must be robust and be able to maintain product integrity at the defined storage conditions. During the fill-finish stage, CQAs that are typically at the forefront include aggregation, potency, identity, sterility, and CCIT. This section will discuss these CQAs in more detail.

The main differences between fill and finish for gene therapy products compared to more traditional biologics include the batch size, which is relatively small for gene therapy, and the time to process the batch. Typically, gene therapy products should be processed quickly, between 4 and 5 hours, due to potential instability at room temperature.

CONCENTRATION AND VECTOR AGGREGATION

Vector aggregation that may occur during vector concentration (depending on AAV serotype) can contribute to unexpected loss during handling, altered biodistribution, and increased immunogenicity after clinical administration. The typical concentrations of purified AAV vectors (10^{11} to 10^{13} vg/mL) correspond to dilute solutions of drug substance (~1-100 µg/mL), and nonspecific adsorption of vectors to plastics, glass, metal, and other surfaces during storage/handling of the vector may occur. However, inclusion of a surfactant may help to prevent vector losses.[1]

Certain clinical programs may require high doses to be administered in a relatively small volume.[2] It has been reported that recombinant AAV2 undergoes aggregation in a concentration-dependent manner for titers >$1x10^{13}$ vg/mL when formulated in physiologic ionic strength buffers. Elevated ionic strength can prevent aggregation to titers up to $5x10^{13}$ vg/mL.[2] However, slightly elevated ionic strength formulations compatible with direct parenteral injection can limit the AAV2 vector titers to approximately $2x10^{13}$ vg/mL.

Serotype appears to affect vector aggregation. AAV2 vectors are prone to aggregation at concentrations >$1x10^{13}$ to $2x10^{13}$ vg/mL in parenteral-compatible buffers, but recombinant AAV8- and AAV9-based vectors can be prepared in neutral physiologic buffers at much higher concentrations. Thus, use of AAV8- and AAV9-based vectors can facilitate gene therapy applications that require a limited volume but a high dosage.[3]

GENE THERAPY DELIVERY

Specific gene therapies may be delivered through different routes of clinical administration, such as intravenous, intraocular, subretinal, intramuscular, or cell therapy (e.g., T-cells). Different formulations are developed to suit the clinical administration and preserve the potency and stability of the product.

For example, for gene therapies targeting the central nervous system (CNS), various routes of administration are possible (e.g., intraparenchymal, intracerebroventricular, cisternal intrathecal, or lumbar intrathecal). Each of these routes has various advantages and disadvantages. Intraparenchymal delivery requires a relatively low dose compared with systemic or cerebrospinal fluid (CSF) administration. In addition, delivery into the immune-privileged site of the brain reduces the impact of potential preexisting immunity to AAV serotypes. Mouse

models have shown that intraparenchymal delivery into various areas of the brain lead to widespread enzyme distribution and biochemical and histological correction in large areas of the brain. Delivery into the CSF (intralumbar or cisternal) requires a somewhat larger dose and may result in effects to non-CNS areas. Intravenous delivery of CNS-targeting gene therapies appears to be possible, although the efficiency appears to be limited in older mice. Further, accumulation of sialic acid within the CNS, which is an inhibitor of AAV9 transduction, may limit efficacy of this approach.[4]

ASEPTIC PROCESSING AND STERILE FILL-FINISH

Facilities, equipment, procedures, and personnel must be appropriate to ensure aseptic processing and sterile fill-finish. In addition, it is important that filling processes be qualified prior to the actual product fill.[1]

Sterilization of each component of a drug product, regardless of form, must be carried out prior to aseptic processing/fill-finish. Various methods may be used, such as heat sterilization in an autoclave, radiation sterilization (especially useful if the component is heat-sensitive), filter sterilization, and ethylene oxide gas sterilization for heat- and moisture-sensitive components.

CHOICE OF CONTAINER

Ideally, the container should be selected during phase 1 or 2 of clinical development while considering commercialization factors, such as scalability. Failure to select a container without considering commercialization may lead to problems and delays in later stages of development.

Several options exist for gene therapy containers, and pros and cons of various vials and bags must be considered while keeping in mind the particular characteristics, including mode of administration/delivery, for the DP. It is also important to consider and mitigate shear effects when dispensing. For example, open filling from a needle is a break in the closed system handling of a BSL-2 virus. Thus, this would create a greater demand for facility containment capabilities than what would be needed when dispensing a protein product.

Titer of a gene therapy product must be measured so that the fill-finish can be done in a titer-appropriate manner with consideration of both material and size. Because AAV is charged and will adhere to certain substances, thereby causing drug product loss, appropriate vial material is crucial. Vial types may include glass, cryo-vial plastic, and various polymers (e.g., Crystal Zenith). For some vectors, moving away from glass vials to polymeric vials can decrease potential safety issues by ensuring safe containment of the vector.[5] Bags are an important consideration for AAV and are typically used in the context of cell therapy to ensure aseptic connection and workflow integration.

Formulation

In the preclinical stage of formulation, potency is generally the most important CQA. At a clinical stage of development, CQAs may include physical titer, genetic identity, aggregation state, infectivity, full to empty particles, biological activity or potency, and/or immunological activity.[5] Agencies generally look at a combination of route of administration and CQAs in a longitudinal manner to ensure stability of CQAs over time. Therefore, it is very important that formulation is considered early during the development process and often so that drug stability is not sacrificed.

Formulation is a critical component of the process because it includes stability, potency, purity, and safety. However, it is often overlooked. Formulation should be considered early during the process, as early as the process development stage. It is largely determined by two factors: the clinical administration route and the process development stage stability analysis, including for AAV-specific gene therapy aggregation, stability, and potency.

Aggregation can be measured by dynamic light scattering (DLS). Light scatters from the moving macromolecules. This motion imparts a randomness to the phase of the scattered light, such that when the scattered light from two or more particles is added together, there will be a changing destructive or constructive interference. This will cause time-dependent fluctuations in the intensity of scattered light, which are measured by a fast photon counter and are directly related to the rate of diffusion of the molecule that is correlated with particles' hydrodynamic area (AAV viral particle or aggregate size).

Expansion of the CQA list beyond infectivity is important to determine the route of viral vector

degradation in different buffers and under different extrinsic conditions, and eventually, to enact improvements in formulation. Some viruses have a temperature threshold, above which the virion structure is abruptly affected. Temperatures for the storage and shipment of viral vectors could be -40°C, -70°C, or below, which can complicate the cold supply chain. Bulk material or drug product may require shipment on dry ice, and there are additional factors that must be considered, such as the need for containers with low permeability to carbon dioxide vapor due to the typical instability of viruses at low pH. Therefore, temperature studies are required in order to determine the threshold effect as well as the impact of cumulative excursions near a threshold.[5]

Formulation buffers are also important considerations. Luxturna, a currently available AAV vector-based gene therapy for the treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy, is administered via subretinal injection. A single-dose, 5-mL vial of Luxturna contains 5 x 10^{12} vector genomes (vg) per mL, 180 mM sodium chloride, 10 mM sodium phosphate, and 0.001% Polaxmer 188 (pH 7.3). Following dilution, each 0.3-mL Luxturna dose contains 1.5 x 10^{11} vg. The diluent, which is supplied in a 1.7-mL extractable volume per vial in 2-mL vials, contains sterile water containing 180 mM sodium chloride, 10 mM sodium phosphate, and 0.001% Poloxamer 188 (pH 7.3).[6]

The Luxturna active substance (bulk drug product) is formulated and shipped frozen on dry ice (to maintain a temperature of ≤-65°C) to a filling site, where it is processed into the final product by filtration and filling into Crystal Zenith vials. The finished product is then shipped at ≤-65°C to the secondary packaging and labelling site in insulated shipping containers in semi-finished vials with primary labels applied.

Zolgensma, an AAV vector-based gene therapy for pediatric patients <2 years of age with spinal muscular atrophy with biallelic mutations in the SMN1 gene, is available in 5.5- and 8.3-mL vials with a concentration of 2.0 x 10^{13} vg/mL. Zolgensma also contains 20 mM tris (pH 8.0), 1 mM magnesium chloride, 200 mM sodium chloride, and 0.005% Poloxamer 188. The IV dosage is determined by body weight, with a recommended dose of 1.1 x 10^{14} vg/kg. The Zolgensma product is shipped and delivered frozen (≤-60°C) in clear vials and is stored refrigerated. Zolgensma is stable for 14 days after receipt when stored between 2°C and 8°C.[7,8]

Characterization

During preclinical stages of development, characterization focuses mainly on potency. During clinical development, several CQAs must be considered. It is important that gene therapy design is "smart" (e.g., considering how large the payload is), and various elements of design will impact the final drug quality. Specific CQAs during characterization include safety and potency considerations. Endotoxin testing is recommended on the final container product to ensure an appropriate level of endotoxin, which is defined as five endotoxin units (EU)/kg/bodyweight/hr) according to FDA guidance. The ratio of empty-full capsids should also be considered at this stage, and although the FDA does not provide specific guidance because it is related to the biology of the product, it is important for this CQA to maintain consistency in a longitudinal manner. Titer measurement is also important to consider due to lethality from liver toxicity related to high doses.

Drug product release is the ultimate goal for gene therapy manufacturing and gene therapy development. In order to do this, AAV vectors must be thoroughly characterized to ensure that they meet the predetermined specifications for vector identity, safety, purity, potency, and stability for every lot. The Biologics License Application (BLA) requires that all quality control assays be finalized and validated.[1] Biological products are complex and are often heterogeneous with complex mechanisms of action. Product characterization allows the manufacturer to determine the relationship between product quality attributes and safety and efficacy.

One challenge involved in the characterization phase is the cost of analytics.[5] The product yield from current manufacturing and purification processes is low, so the amount of product needed for complete in-process testing, product characterization, lot-release, and stability testing can consume a significant portion of clinical lots. In addition, current practices for qualifying reference standards rely on labor-intensive and variable analytics, and additionally are not uniform across the industry. It is important to note that reference standards for a given gene therapy are generated from designated clinical lots.

Table 6-1. *Example of Release Testing for a Clinical AAV Vector Product*

Crude Cell Harvest	Bulk Drug Substance	Drug Product
GENERAL		
	• Appearance	• Appearance
	• pH	• pH
	• Osmolality	• Osmolality
		• vg identity
IDENTITY		
	• Capsid	• Capsid
	• Payload sequencing	• Payload sequencing
POTENCY		
	• vg titer	• vg titer
	• Infectivity	• Infectivity
	• *In vitro* expression	• *In vitro* expression
PURITY		
	• SDS-PAGE silver stain	• SDS-PAGE silver stain
	• OD260/OD280	• Aggregates
	• Residual host-cell DNA	
	• Residual plasmid DNA	
	• Residual BSA	
	• Residual HEK293	
	• Residual benzonase	
	• Residual cesium	
	• Aggregates	
SAFETY		
• Adventitious viral testing	• Endotoxin	• Sterility
• Mycoplasmas	• Sterility	• Endotoxin
• Bioburden/sterility	• rcAAV (replication- competent AAV)	

The poor yield, along with the high variability inherent in the manufacturing of gene therapies and the required amount of analytical testing, can require the frequent generation of new clinical lots and reference standards. Thus, a significant portion of the product yield is easily consumed through required and necessary analytical testing. The use of QbD and DOE approaches can help to ensure that the analytics employed in this capacity are robust and meet the acceptable levels in order to confidently determine product characterization and quality assessment data.

Examples of tests that may be used for release testing of a clinical AAV vector product are shown in Table 1.[1]

For some gene therapy products, the empty-full capsid ratio must be considered prior to the product being placed into its vial/container. Some methods that may be used to analyze empty-full capsid ratio include spectrometry, ELISA, qPCR, analytical ultracentrifugation, ion exchange chromatography, and transmission electron microscopy.

The U.S. Food and Drug Administration defines characterization in four categories of tests, as shown in Table 1: safety, purity, identity, and potency.

The following sections take a closer look at tests contained within these categories.

Table 6-2. *Overview of Safety Testing.*[1]

Assay	Purpose of Assay	Time of Assay
Adventitious viral agents (AVA)	Demonstrate absence of AVA	Crude cell harvest
Mycoplasma	Demonstrate absence of mycoplasma	Crude cell harvest
Bioburden	• Ensure aseptic conditions throughout the manufacturing process • Ensure product conforms to recommendations of USP <71> and 21 CFR 610.12	Following each purification step to ensure
Endotoxin	Demonstrate absence of endotoxin in a manner appropriate for the intended route of administration	Final product
Sterility	Ensures product safety and aseptic, sterile product without detectable microbial contamination	Final product
rcAAV	Demonstrate absence of potential pathogenic derivatives of recombinant AAV	Bulk drug substance

SAFETY

The product must be tested for the presence of potentially unsafe impurities, which is done by assessing the quality of raw materials as well as process contaminants (e.g., column media, antibiotics, or other agents used). Safety testing should encompass assays to assess sterility, mycoplasma, adventitious viral agents, bioburden, replication-competent AAV (rcAAV), and endotoxin. For licensure, general safety should also be assessed. Table 2 shows an overview of safety testing. Sterility testing must be performed on the final product according to 21CFR 610.12.[1]

Bioburden assays should be performed at product stages, for example following each purification step, in order to ensure aseptic conditions throughout the entire manufacturing process. These assays must be performed in order to ensure the product conforms to the recommendations of the USP <71> and 21 CFR 610.12. These assays may be performed through the direct inoculation of the bulk or final test sample into two different types of media. The samples are incubated for 14 days at two different temperatures. In addition, results should be confirmed by a bacteriostasis/fungistasis test to demonstrate that the samples do not interfere with the growth of six organisms of varying classes of mold and bacteria.

Bacterial endotoxin can be assayed via a LAL/chromogenic method. In this test, precise amounts of FDA-licensed LAL reagents, chromogenic substrates, and controls are required. A variety of commercial products are available to measure endotoxins over a range of 1.0 to 0.1 EU/mL.

Recombinant AAV replication requires the presence of a helper adenovirus, in addition to wild-type AAV genes that are involved in virion construction and packaging. Therefore, rcAAV requires only the presence of a helper adenovirus for AAV replication within a permissive cell line. Replication-competent AAV should be assessed using an infectious center assay. This may be challenging for some AAV serotypes due to the low infectivity of specific serotypes in cultured cells. When developing new serotypes for clinical studies, assay development efforts should focus on addressing this challenge. Replication-competent AAV can be assessed through cell culture and qPCR.

In addition, commercially relevant production methods may use Herpes simplex virus (HSV) or baculovirus to manufacture AAV vectors. It is important to ensure the removal of these viruses because residual viral particles could elicit toxic or immune reactions. Methods to purify AAV from HSV-containing matrices generally use detergents such as Triton X-100 at concentrations as high as 1% w/v during harvest or low pH-induced flocculation of cellular and HSV proteins from denatured viral capsids. Although it appears that residual HSV proteins are detectable in final rAAV stocks, preclinical toxicology studies have shown that these levels were tolerated in animals, and no reactions have been reported in humans. Like HSV inactivation,

Table 6-3. *Overview of Purity Testing During GMP*

Assay	Purpose of Assay	Time of Assay
Residual host-cell protein	Ensure host cell protein is at an acceptable level	Purified bulk (DS)
Residual host-cell DNA	Ensure host cell protein is at an acceptable level	Purified bulk (DS)
OD260/OD280	Determine protein concentration	Purified bulk (DS)
Residual plasmid DNA	Demonstrate absence of residual plasmid DNA	Purified bulk (DS)
Residual BSA	Demonstrate absence of residual BSA in product	Purified bulk (DS)
Residual benzonase	Demonstrate absence of residual benzonase in product	Purified bulk (DS)
Residual substances from purification (e.g., cesium)	Ensure removal of purification substances	Purified bulk (DS)
Aggregates	Ensure aggregates are at an acceptable level so as to not affect dose or concentration	Formulated, vialed (DP)
SDS-PAGE silver stain	Visualize the VP1, VP2, and VP3 bands and ensure that there are no other proteins	Formulated, vialed (DP)

chemical lysis by Triton X-100 (0.5% w/v) at harvest results in disruption of the cell membrane and baculovirus envelope. Detergent inactivation results in inactivation of most baculovirus particles but does not disrupt the integrity of the nucleocapsids. Baculoviruses are known to transduce mammalian cells but are not able to replicate, and there is no evidence that baculoviruses harm humans unless injected intravenously at very high doses.[20]

PURITY

Purity is related to levels of cell-culture process impurities, such as residual host cell proteins and DNA, helper virus protein and nucleic acids, and helper plasmid DNA. During the early development process, tests for contaminants in the final product should be tested for. As development progresses, the manufacturing process should be validated to remove and to not introduce process-related contaminants. Identified contaminants should be removed, or appropriate limits should be set based on data from lots that were shown to be safe in preclinical and/or clinical studies. Initial specifications, including acceptance limits, may need to be refined based on manufacturing process experience gained throughout development.

Purity testing aims to characterize the capacity of the purification process to remove manufacturing components (such as cell culture media, helper virus, antibodies) as well as product isoforms (such as empty capsids). Table 3 describes assays used for purity testing during GMP runs. However, note that other factors may need to be considered during engineering runs.

A visual test should be conducted via S0026 based on USP <790> and <1790> to ensure that a product's visual liquid appearance is clear and the liquid is free from visible particulates using both white and blue backgrounds and appropriate liquid. Vials should be assessed for imperfections, such as cracks and loose caps.

Residual host cell protein (HCPs) can be evaluated via polyacrylamide gel electrophoresis and protein staining, reverse-phase high-pressure liquid chromatography, host-cell protein ELISA, and spectrophotometry. Residual host cell DNA may be assessed using qPCR with appropriately designed primers and probes, and helper plasmid DNA may be assessed through qPCR designed to analyze residual plasmid-derived DNA fragments.[1]

Residual benzonase should be analyzed through specific and sensitive methods as part of clinical testing. The data must demonstrate assay sensitivity, linearity, accuracy, precision, and specificity. Residual polyethyleneimine (PEI) may be assayed via UHPLC-CAD assays that use both linear and branched forms of PEI over a range of molecular weights. Residual iodixanol should be measured in AAV viral vector products purified by iodixanol gradient ultracentrifugation. Iodixanol

Table 6-4. *Overview of Identity Testing*

Assay	Purpose of Assay	Time of Assay
Capsid identity	Assess heterogeneity and confirm capsid identity (e.g., ELISA, western blot, empty-full capsid analysis, silver staining)	• Unpurified bulk • Purified bulk (DS) • Formulated, vialed (DP)
Payload identity	Ensure that the identity of the product completely matches that of the designed product	• Unpurified bulk • Purified bulk (DS) • Formulated, vialed (DP)

concentration can be assessed through semi-quantitative HPLC size-exclusion chromatography analysis.

Nuclease-resistant, AAV-encapsidated DNA impurities can be assessed by qPCR using primers and probes designed for relevant sequences in helper plasmids or high copy number genomic sequences.[1] The sensitivity to nuclease treatment performed prior to qPCR allows one to make a distinction between nuclease-sensitive residual DNA impurities and nuclease-insensitive–encapsidated residual DNA impurities. The total AAV capsids can be measured using capsid-specific ELISA assays, with the amount of empty capsid determined by comparing to capsid particle titer and vg titer. Monoclonal antibodies specific for a conformational epitope on an assembled AAV capsid that is coated onto strips of a microtiter plate can be used to capture AAV particles from the sample.

IDENTITY

Identity refers to characterization of molecular integrity of the gene therapy per design. It should include quantitative testing by phenotypic and/or biochemical assays to confirm cell identity and assess heterogeneity (Table 4). Methods to characterize identity may include capsid confirmation with ELISA or western blot, and/or empty-full capsid analysis. To confirm payload identity, sequencing, either next-generation sequencing or Sanger sequencing, of AAV may be carried out to ensure that it completely matches with the designed product. Appropriate references should be included in quality control assays to ensure that the results obtained are valid and reproducible.[9]

POTENCY

Potency is a biology- and product-specific characteristic. Potency tests primarily focus on determining vg concentration as well as functional activity. This step tends to be time-consuming due to the product-specific considerations that must be taken into account during assay development. Potency assays should be in place during early product development to demonstrate product activity, quality, and consistency throughout product development, generate data to support specifications for lot release, provide a basis to assess manufacturing changes, assess product stability, recognize technical problems, and collect sufficient data to support correlation studies (to link potency to functional activity).[10]

Formally, potency is defined as "the specific ability or capacity of the product, as indicated by the 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" (21 CFR 600.3(s)).

Potency tests are performed to measure product attributes that are associated with product quality and manufacturing controls throughout all phases of clinical study (Table 5). Measurements of potency are used to show that product lots meet defined specifications or acceptance criteria during all phases of clinical development as well as following market approval. Although no single test can adequately measure product attributes that predict clinical efficacy, data from well-controlled clinical investigations can provide evidence to show that a product has biological activity and is potent.[10]

The exact *in vitro* potency assay that is used depends on the gene therapy product and is highly centric around the biological process that the therapy is supposed to interefere with. Assays may range from TCID50, flow cytometry, protein expression, gene expression, enzymatic assays, etc. Particularly in earlier stages, nuclease-resistant genome concentration may be assessed by dot blot hybridization, ddPCR, or qPCR. It is important to note

Table 6-5. *Overview of Potency Testing*

Assay	Purpose of Assay	Assay Performed on
TCID50/Infectivity	Determine concentration at which 50% of cells are infected	• Unpurified bulk • Purified bulk (DS) • Formulated, vialed (DP)
Protein expression	Ensure protein levels	• Purified bulk (DS) • Formulated, vialed (DP)
In vivo animal testing (immunostaining)	Ensure appropriate dosing and potency	• Formulated, vialed (DP) for reference only

that when using PCR, a linearized DNA template is necessary in order to generate a standard curve because the use of supercoiled DNA standards will result in a significant overestimate of the vg concentration. Determination of AAV titer is an important consideration that has proven to be somewhat difficult in practice. Further, sample preparation is a key consideration in qPCR. It may be prudent to use viral material as the control, which can serve both as a standard curve as well as a control for sample preparation.

In addition to genome concentration, assays must be done to assess the functional activity of the vectors. In earlier stages of development, ELISA may be used to demonstrate transgene protein expression in a dose-dependent manner, whereas later stages of development (e.g., phase 3 clinical trial) require the establishment of a bioassay that quantifies the functional activity of the transgene product. Additionally, quantification of infectivity may be used as a supplemental approach to assess the functional activity of AAV vectors.

Potency assays should take into account the product's MOAs. However, this tends to be a large hurdle for gene therapies due to the multifaceted nature of MOAs.[5] Many gene therapies have complex MOAs that rely on multiple biological activities (transfection/infection, gene transcription, translation, action of translated protein), and the MOAs of many are not fully characterized. Thus, several stages of MOA must be captured within a single potency assay. We currently lack an *in vitro* to *in vivo* translation for biological assays to determine whether *in vitro* measurements (e.g., of potency, efficacy, cytotoxicity, and immunogenicity) are meaningful and predictive of physiological action.

Container Closure Integrity Testing (CCIT)

Although many containers are available, it is important to consider the container's compatibility with the intended route of administration as well as cryopreservation.

- Compatibility with route of administration:
  Depending on the route of administration, it may be important to be able to plug into a system aseptically. Drug products and containers must be specifically designed to allow such aseptic removal of drug. In addition, extractability studies may be required to ensure that the dose can be extracted from the container, both related to physical volume and maintenance of potency.
- Container closure cryopreservation compatibility:
  (-80°C is typical storage temp, container and closure may contract at a different ratio, which may compromise volume, aseptic conditions)

CCIT evaluates the adequacy of the container closure systems and the ability of the container closure to maintain a sterile barrier against potential contaminants (e.g., microorganisms, reactive gases, or other substances). While CCIT may not be performed during the early-stage development of biologics or vaccines, it is important to consider early during development for a gene therapy due to the accelerated timelines involved in bringing a gene therapy for a rare disease to market.

It is important that container closure systems maintain the sterility and product quality throughout the shelf life (until the expiration date) of the product. Regulatory

requirements mandate that the design of the closure system be qualified, which can be done in multiple ways. The selection of the appropriate method is based on the contents of the container as well as the container closure system itself.[11] Establishing a proper container closure system is vital for product and consumer safety. It is ideal to test it on the final product, but because of the requirement of a large number of vials for testing, it is challenging in gene therapy when every vial counts. In some cases, it may be possible to do placebo-based CCIT in order to preserve the gene therapy product. However, the ultimate proof of suitability of a container closure system and packaging is a full shelf life stability study.[12]

Container closure systems consist of both primary packaging components (those that come into direct contact with the product such as a vial or syringe) and secondary packaging components (those that are vital to ensure correct package assembly, such as aluminum caps over stoppers). Packaging materials must not interact physically or chemically with the product to avoid effects on safety, identity, strength, quality, and purity.[13,14]

CCIT testing will vary based on the specific containers and materials used, for example vials vs bags. Methods that may be used to assess container closure can include:

- Electrical conductivity and capacitance test
- Laser-based gas headspace analysis
- Mass extraction
- Pressure decay
- Tracer gas (vacuum mode)
- Vacuum decay

Storage and Stability

Several factors may impact long-term storage and stability. During stability testing, temperature fluctuation (stable temperature storage and freeze-thaw cycles), diluents (including serum and solutes with various pH), container constituents (glass, plastics, and steel), and other environmental considerations should be taken into account. AAV is relatively stable at -80°C, but this can translate into problems related to clinical administration. Maintaining the cold chain at -80°C with minimal excursions is difficult, particularly when "public-friendly" -80°C is not available. It is important to note that stability may differ between AAV serotypes.

Storage and stability must be evaluated in conjunction with the container and closure systems; storage and stability should be evaluated early in the process, and the stability of the container system should be known in advance (Table 6). Assays are required to verify stability and ensure that purified clinical vectors maintain their purity, potency, and safety profiles during storage and over the course of their potential use.[1] During early-phase clinical studies, stability studies can be performed concurrently with clinical use. Accelerated tests performed at 25°C and 37°C may be helpful to accelerate the stability studies and derive long-term stability data to inform shelf life and storage conditions.[15]

Many biologics are sensitive to pH, and pH may affect the structure of the AAV capsid. pH must be studied early during the pre-formulation/early formulation phase in order to determine the optimal pH range for product stability. pH must be analyzed for compatibility with both the container material, diluent, and vector. Note that pH may change during freezing due to crystallization of the buffer components, temperature dependence of pH, and change in the apparent acid dissociation constant as a result of the decrease in the polarity of the liquid phase due to freeze-concentration.[15] pH can affect stability based on serotype; whereas higher pH may result in increased stability in some serotypes, it may result in lower stability in others.

AAV DP are generally stored as a frozen solution; however, AAV formulations (and other aqueous biopharmaceuticals) are not completely frozen at -15°C to -25°C and consist of ice and a freeze-concentrated solution. The freeze-concentrated solution (which is liquid) contains all of the active ingredient and unfrozen water between ice crystals. Storage at -20°C may lead to destabilization via various routes, such as aggregation, pH changes, increased oxygen concentration, extensive ice/solution interface, and crystallization of cryoprotectors. When possible, storage at lower temperatures (below -65°C) should be used.[15]

Although storage at low temperatures may be practical, shipping and site storage of frozen biologics becomes more difficult. Therefore, it is desirable to have AAV formulations that are stable above 0°C. However, results of AAV stability at higher temperatures have been conflicting. Whereas some report stability of AAV vector in neutral phosphate-buffered saline with 5% sorbitol and 0.1% polysorbate 80 without losing transduction activity

Table 6-6. *Recommended Stability Parameters*

Parameter	Assay Recommendation	Time of Assay
Aggregation	• DLS	0, 3, 6, 12, 18, 24 and months
Potency	• Protein expression • TCID50	0, 3, 6, 12, 18, 24 and months
Identity	• Gene-specific physical titer (qPCR or ddPCR)	0, 3, 6, 12, 18, 24 and months
Sterility	• Refer to <USP71>	0, 3, 6, 12, 18, 24 and months
CCIT	• Appropriate test for product/materials (refer to CCIT section)	0, 3, 6, 12, 18, 24 and months

after 1 year at 2°C to 8°C, others have reported up to 40% loss in transgene expression after 7 weeks at 4°C for AAV1 in PBS with 0.5 mM $MgCl_2$.[15-17] A study of conditions commonly encountered in human gene therapy trials found that over a range of temperatures, pH, and environmental conditions, rAAV was found to be remarkably stable (4°C to 55°C), pH (5.5 to 8.5) in various container materials for up to >1 year. The exceptions included heating to 72°C and exposure to UV for 10 minutes.[18]

The Reference Standard Materials working group generated standards for AAV serotype 8. Three independent laboratories provided stability data on the AAV8 serotype (AAV8RSM) deposited to the American Type Culture Collection (ATCC), which required relabeling and container cap tightening 2 years after the original deposition due to labels detaching too easily and container caps that were not tightly closed. Following these corrective actions in 2016, AAV8RSM demonstrated consistent titers using qPCR, TCID50, and ELISA analyses compared to titers at deposition into ATCC in 2014, and capsid protein integrity (SDS-PAGE) was equivalent at 2 years after deposition at appropriate storage conditions (≤70°C).[19]

Conclusion

Starting from the beginning of gene therapy product development, it is critical to consider the "end goal" with regards to desired drug product characteristics and how to achieve that using standardized, informed design processes. In this chapter, we have outlined the best practices for fill-finish, formulation, characterization, and CCIT; however, it is in no way exhaustive. Each process will differ based on the specific product, its characteristics, administration route, titer selection, final formulation buffer, and container choice, among others. This chapter is a framework that can be used in the development of a robust final product.

CQAs typically considered during the fill-finish stage include aggregation, potency, identity, sterility, and CCIT. During formulation, major focus may shift to potency as the most important aspect, while still considering stability, purity, and safety. The characterization stage is broad, and the major focus continues to be on potency, although safety, identity, and purity are still considered. CCIT must ensure compatibility of the DP with route of administration and container closure cryopreservation compatibility. Finally, several variables must be evaluated in order to minimize factors that may impact long-term storage and stability, including temperature fluctuations, freeze-thaw cycles, diluents, container constituents, and other environmental considerations.

Currently, gene therapy is limited in supply due to a manufacturing bottleneck. However, progress in the gene therapy field is rapid, and once the manufacturing bottleneck is overcome, it will be prudent to focus on solving possible rate-limiting steps during the fill-finish stage. For example, automated filling technology utilizing engineered tubing to eliminate product loss, research on polymers used in vials, lyophilization of product, and increased temperature resilience of the DS are improvements that we look forward to in the next decade of AAV-based gene therapies. Such industrial improvements will ensure that the fill-finish process does not become a rate-limiting step in getting vital gene therapies to the patients who need them.

Endnotes

1. *Wright JF. Manufacturing and characterizing AAV-based vectors for use in clinical studies. Gene Ther. 2008;15(11):840-848.*
2. *Wright JF, Le T, Prado J, et al. Identification of factors that contribute to recombinant AAV2 particle aggregation and methods to prevent its occurrence during vector purification and formulation. Mol Ther. 2005;12(1):171-178.*
3. *Wright JF, Hauck B, Zhou S, Zelenaia O, Sumaroka M, High KA. Concentration of AAV8 and 9 vector to titers ranging from 1-6 x 10^{14} vg/mL: feasibility assessment for volume-limited dosing (abstract 547). Mol Ther. 2013;21(Suppl 1):S210-211.*
4. *Hocquemiller M, Giersch L, Audrain M, Parker S, Cartier N. Adeno-associated virus-based gene therapy for CNS diseases. Hum Gene Ther. 2016;27(7):478-496.*
5. *The National Institute for Innovation in Manufacturing Biopharmaceuticals. Gene therapy roadmap. National Institute for Innovation in Manufacturing Biopharmaceuticals website. https://niimbl.force.com/resource/1541788844000/NIIMBLGeneTherapyRoadmap. Accessed May 19, 2020.*
6. *Luxturna [prescribing information]. Philadelphia, PA: Spark Therapeutics, Inc.; 2017.*
7. *Zolgensma [prescribing information]. Bannockburn, IL: AveXis, Inc.; 2017.*
8. *US Food and Drug Administration. Summary basis for regulatory action. US Food and Drug Administration website. https://www.fda.gov/media/127961/download. Updated May 24, 2019. Accessed June 30, 2020.*
9. *US Food and Drug Administration. Guidance for industry: FDA guidance for human somatic therapy and gene therapy. US Food and Drug Administration website. https://www.fda.gov/media/72402/download. Updated 1998. Accessed June 14, 2020.*
10. *US Food and Drug Administration. Guidance for industry: potency tests for cellular and gene therapy products. . US Food and Drug Administration website. https://www.fda.gov/media/79856/download. Updated January 2011. Accessed June 14, 2020.*
11. *Ewan S, Jiang M, Stevenson C, et al. White paper: Container closure integrity control versus integrity testing during routine manufacturing. PDA J Pharm Sci Technol. 2015;69(3):461-465.*
12. *US Food and Drug Administration. Guidance for industry: container closure systems for packaging human drugs and biologics. US Food and Drug Administration website. https://www.fda.gov/media/70788/download. Updated 1998. Accessed June 18, 2020.*
13. *USP. Packaging and storage requirements. USP website. https://www.uspnf.com/sites/default/files/usp_pdf/EN/notices/2018/c659-proposed.pdf. . Accessed June 18, 2020.*
14. *ICH. Pharmaceutical development Q8(R2). ICH website. https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf. Updated August 2009. Accessed June 18, 2020.*
15. *Rodrigues GA, Shalaev E, Karami TK, Cunningham J, Slater NKH, Rivers HM. Pharmaceutical development of AAV-based gene therapy products for the eye. Pharm Res. 2018;36(2):29.*
16. *Wright JF, Qu G, Tang C, Sommer JM. Recombinant adeno-associated virus: formulation challenges and strategies for a gene therapy vector. Curr Opin Drug Discov Devel. 2003;6(2):174-178.*
17. *Howard DB, Harvey BK. Assaying the stability and inactivation of AAV serotype 1 vectors. Hum Gene Ther Methods. 2017;28(1):39-48.*
18. *Gruntman AM, Su L, Su Q, Gao G, Mueller C, Flotte TR. Stability and compatibility of recombinant adeno-associated virus under conditions commonly encountered in human gene therapy trials. Hum Gene Ther Methods. 2015;26(2):71-76.*
19. *Penaud-Budloo M, Broucque F, Harrouet K, et al. Stability of the adeno-associated virus 8 reference standard material. Gene Ther. 2019;26(5):211-215.*
20. *Penaud-Budloo M, François A, Clément N, Ayosu E. Pharmacology of recombinant adeno-associated virus production. Mol Ther Methods Clin Dev. 2018;8:166-180.*

Chapter 7

Process Control Strategy

Alliance *for* Regenerative Medicine

NIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 7 | Contents

Chapter Summary

A comprehensive control strategy for a pharmaceutical product (including gene therapy products) is the key to achieving process consistency, product quality, safety, and efficacy. The International Council for Harmonization (ICH) Q10 defines control strategy as "[a] planned set of controls, derived from current product and process understanding, which assures process performance and product quality. The controls can include parameters and attributes related to drug substance (DS) and drug product (DP) materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control."

The elements that contribute to control strategy are outlined in ICH guidelines ICH Q8 (R2), Q9, Q10, and Q11. These guidelines describe a quality paradigm in which a scientific and risk-based approach, using principles of pharmaceutical development, quality risk management, and quality systems, is used for quality product development, robust dossier submission and review, inspection, and post-approval changes to ensure both product quality and process consistency. A comprehensive control strategy enables the design of the appropriate process validation and continued process verification (CPV) program for implementation during the product life cycle. CPV monitoring and trending may identify areas of improvement in the process, leading to the evolution of the control strategy. The GMP control strategy spans from cell bank manufacturing to final dosage form delivered to a clinical site.

This section describes the approaches to developing a comprehensive control strategy and a detailed overview of the key elements of a control strategy for a gene therapy product.

Key Points

- The control strategy determines where in the process to place appropriate controls to consistently ensure product quality, safety, and efficacy.
- This is accomplished through an iterative risk assessment process that considers evolving product and process knowledge that lead to the identification of the critical quality attributes (CQAs) of the product and critical process parameters (CPPs) of the process.
- Adjustments to the control strategy (e.g., providing additional controls to further reduce risk, moving control points to optimum location within the process, or removing controls determined to be redundant or ineffective) can be made during this iterative process, as needed.

Elements of Control Strategy

The overall control of the process and product should be examined holistically and systematically. There are eight elements of control that the product team should consider for each product or process attribute to ensure a robust control strategy. These elements are depicted in Figure 1.

All product and process inputs and outputs are risk assessed, and the critical process controls that are linked to control of CQAs are categorized as established conditions.

A more detailed description of types of control that could be considered for each control element is provided in Table 1.

In the regulatory dossier (e.g., BLA), key control strategy elements focused on the control of CQAs, are filed in the respective DS and DP leaflets.

Figure 7-1. Elements of Control for Product Quality and Process Performance

Assessment of Product Quality Attributes

Iterative assessments of QA criticality are conducted during product development to identify CQAs. This process includes a review of prior knowledge and periodic reviews of available information in the public domain, including literature and publications.

Scoring of QAs for A-Gene DS and DP is performed taking into consideration the QA's relation to the QTPP and the potential impact of the DS attributes on safety and efficacy of the drug product. Based on prior knowledge and

Table 7-1. Elements of Control for Product Quality and Process Performance

Control Element	Definition	Type of Control
		PROCESS CONTROL
Element 1	In-process control of product quality attributes	• Direct in-process tests, or surrogates, of product quality attributes and their control limits • Product quality attribute in-process control demonstrated through process validation
Element 2	Control of process parameters	• Control implicit in the design of manufacturing process or unit operations • Manufacturing process controls • Process parameters that impact product quality or process performance attributes and control limits/acceptable ranges • Manufacturing hold time control limits • Manufacturing process development and history for understanding and application of acceptable ranges
Element 3	Control of process performance	• In-process tests of process performance and their control limits
		PRODUCT CONTROL
Element 4	Non-routine product characterization test-ing	• Elucidation of structure and other characteristics • Non-routine tests for characterization and demonstration of product comparability • Characterization included in reference standard or reference material qualification
Element 5	Product control through quality attribute testing	• Routine release test and acceptance criteria in product specification • Justification of specification • Analytical procedure and its validation • Product quality attribute control demonstrated through process validation
Element 6	Product control through stability testing	• Routine stability test and acceptance criteria in stability protocols • Stability data and conclusions
		FACILITY AND MATERIAL CONTROL
Element 7	Control of materials	• Specifications for raw materials • Manufacture and testing of cell banks; cell bank controls • Characteristics of incoming materials (such as raw materials, starting material, intermediates, primary packaging materials) that impact product quality attributes and their acceptable ranges • Compatibility with container closure system; container closure controls
Element 8	Facility and equipment controls	• cGMP and pharmaceutical quality systems for the manufacturing facilities • Environmental and equipment controls directly impacting product quality attributes

Table 7-2. Description of CQA Severity Scores

Score	Severity Score Definitions	
10	Major	Potentially serious impact to patient, may be life threatening or irreversible
7	Moderate	Moderate impact on patient – treatable AE[a], no permanent harm
5	Minor	Low impact on patient – temporary inconvenience/impairment
1	Negligible	No patient harm

[a]Note: impact to patient includes both safety and efficacy; lack of efficacy is considered an adverse event (AE).

compendial or regulatory expectations, certain attributes are not formally scored but are identified as obligatory CQAs. Control of these CQAs through routine testing with predefined acceptance criteria is considered mandatory.

Other QA categories (e.g., purity) are individually evaluated. When applicable, the data from relevant literature, prior knowledge, structure-function studies, stress studies, stability studies, and clinical data are used in the determination of QA criticality. Each category is scored separately based on the potential impact (severity) of the attribute and with respect to the uncertainty associated with the impact. In general, less experience or knowledge with a particular QA leads to a higher uncertainty score. A quality attribute is determined to be a CQA if, in the evaluation of the two categories, any of the scores exceeds the predefined criticality threshold that accounts for the combination of severity and uncertainty scores. The severity threshold matrix is provided in Table 4 below, where a red cell indicates a potential CQA.

Severity scores may be defined as provided in Table 2. The definitions are based on the potential impact of an attribute being outside of its acceptable range. Uncertainty scores may be defined as provided in Table 3. The criticality assignment matrix and criticality determination are shown in Table 4 and Table 5, respectively.

ELEMENT 1: IN-PROCESS CONTROL OF PRODUCT QUALITY ATTRIBUTES

For certain quality attributes, in-process testing can be selected as a means of control. The use of in-process testing provides opportunities for real-time monitoring of quality attributes. Table 6 and Table 7 show the in-process tests for A-Gene DS and DP, respectively.

Table 7-3. Description of CQA Uncertainty Scores

Score	Uncertainty[a]
10	Low confidence or no information
6	Medium confidence
4	High confidence
2	Prior knowledge (well established understanding)

[a]Could be based on relevant literature, prior knowledge, *in vitro* or *in vitro* S/F study, clinical data, etc.

Table 7-4. Criticality Assignment Matrix (Potential CQAs are in red)

		Severity			
		10	7	5	1
Uncertainty	10				
	6				
	4				
	2				

Table 7-5. Criticality Determination

Designation	Severity Score	Uncertainty Score
Potential CQA	≥7	Any value
Potential CQA	5	≥6
Non-CQA	5	<6
Potential CQA	1	10
Non CQA	1	<10

ELEMENT 2: CONTROL OF PROCESS PARAMETERS

On a high level, the A-Gene manufacturing unit operations are similar to the biologics manufacturing processes and include:

- Upstream unit operations focused on production of recombinant viral particles
- Downstream unit operations consisting of purification and polishing steps
- Formulation and fill-finish steps

Although in many cases, the relationships between process parameters and CQAs for gene therapy processes are complex and not fully understood, the control of process parameters itself can be directly leveraged from well-known and established process control strategies.

The most unique aspect of gene therapy manufacturing is a transient transfection step. The traditional transfection methods for rAAV rely on the transient transfection of HEK-293 cells facilitated by a transfection

Table 7-6. In-process Monitoring and Controls for A-Gene Drug Substance

Process Step	In-process Test	Acceptance Criteria or Action Limit	Rationale for Designation
AAV vector production (unprocessed bulk material)	Bioburden (in compliance with USP <61>, Ph. Eur. 2.6.12, JP 4.05)	< 1 cfu/mL	In-process control with acceptance criteria to demonstrate that the cell culture process is free of contaminants, including mycoplasma, bacteria, and adventitious viruses
	Mycoplasma (by direct inoculation agar and broth assays, use of indicator cells and fluorochrome stain in compliance with USP <63> and Ph. Eur. 2.6.7)	Not detected	
	Adventitious virus (by *in vitro* virus assay using indicator cell lines (MRC-5, Vero, HeLa); 28-day duration)	Not detected	
Harvest	ddPCR or qPCR		In-process titer monitoring for forward processing to affinity step
	Bioburden	Assess if exceeded: 10 cfu/mL	Standard assays to help ensure product safety and control during manufacture of DS
	Endotoxin	Assess if exceeded: 5 EU/mL	
Affinity Purification	qPCR		In-process titer monitoring for forward processing to AEX step
	Bioburden	Assess if exceeded: 10 cfu/mL	Standard assays to help ensure product safety and control during manufacture of DS
	Endotoxin	Assess if exceeded: 5 EU/mL	
Ion exchange Chromatography	Bioburden	Assess if exceeded: 10 cfu/mL	Standard assays to help ensure product safety and control during manufacture of DS
	Endotoxin	Assess if exceeded: 5 EU/mL	
Nanofiltration pool	qPCR		In-process titer monitoring for forward processing to UF/DF step
	Bioburden	Assess if exceeded: 10 cfu/mL	Standard assays to help ensure product safety and control during manufacture of DS
	Endotoxin	Assess if exceeded: 5 EU/mL	
UF/DF	Bioburden	Assess if exceeded: 10 cfu/mL	Standard assays to help ensure product safety and control during manufacture of DS
		Assess if exceeded: 5 EU/mL	

Table 7-7. In-Process Tests for A-Gene Drug Product

Process Step	In-process Controls	Acceptance Criteria
Pre-sterile filtration	Bioburden (in compliance with USP <61>, Ph. Eur. 2.6.12, JP 4.05)	<1 cfu/mL
Sterile filtration	Pre- and post-use filter integrity	Pass
Sterile filling	Fill weight check	1% to 2% of the target (filler capability)

agent. This method presents the biggest challenge for process control, as the transfection efficiency is highly dependent on multiple factors/process parameters, including pH, concentration of solution components, and kinetics of the transfection reaction.

In instances where clinical doses are high and the production process yields are not high enough, supplies going to the clinic need to be maximized, which may require pooling of multiple DS sub-lots into one DS lot prior to converting into drug product. This allows for a significant reduction in material that is used for release testing, stability, and retained samples, a critical consideration in gene therapy given the value of the DP.

ELEMENT 3: CONTROL OF PROCESS PERFORMANCE

Control of process performance (robustness and consistency) is established through defined batch instructions, in-process monitoring, and in-process testing to ensure that the selected unit operations are performing adequately to achieve the intended product quality. For example, in-process monitoring and batch instructions during cell expansion can include the following:

- Incubator CO2 concentration (%)
- Incubator temperature (°C)
- Incubator shaker speed (RPM) 1" throw
- Media equilibration temperature (°C)
- Media equilibration duration (hours)
- Media volume (mL)
- Inoculum volume (mL)
- Initial cell density (x106 viable cells/mL)
- Post-inoculation working volume (mL)
- Batch duration (hours)
- Final cell density ($x10^6$ viable cells/mL)
- Final cell viability (%)

Similarly, the drug product sterile filtration and filling process performance are established by in-process monitoring and through batch instructions. Some of these may be CPPs depending on impact to product quality, including:

- Bulk DS thaw temperature (controlled vs uncontrolled)
- Filtration pressure/flow rate
- Mixing speed
- Mixing time
- Hold time(s)
- Filling speed
- Capping pressure

ELEMENT 4: NON-ROUTINE PRODUCT CHARACTERIZATION TESTING

Some quality attributes are best evaluated via heightened characterization testing rather than by cGMP release testing. These tests are not necessary for routine testing to ensure product control and some are not GMP-compatible, but they do provide supportive information during product development and can contribute to the overall control strategy. For this relatively new modality, product characterization testing will be utilized to further define the additional elements of control strategy (e.g., release testing and stability). Table 8 highlights several analytical tools used for in-depth characterization of A-Gene.

ELEMENTS 5 AND 6: PRODUCT CONTROL THROUGH RELEASE AND STABILITY TESTING

In addition to process controls (elements 1 and 2), CQAs may be controlled with cGMP release and stability testing. Table 9 includes a panel of release and stability tests that may be appropriate for an AAV gene therapy.

Table 7-8. Heightened Characterization Testing Panel

Quality Attribute	Analytical Test(s)	Rationale
Capsid size	SEC-MALS DLS	Properly formed capsids are expected to have a consistent size
Capsid proteins	rCGE RP-HPLC SDS-PAGE	Properly formed viral particles are expected to have a consistent ratio of VP1, VP2, and VP3 proteins
Molecular mass	ESI-MS	Properly formed viral particles are expected to have a consistent mass
Primary capsid sequence	Peptide mapping by LC/MS	Evaluating the primary capsid structure ensures capsid identity and enables characterization of posttranslational modifications
Particle content	AUC	The ratio of empty, intermediate, and full viral particles can be assessed as part of manufacturing consistency. Full particles are the API while empty particles are process-related impurities
Vector genome sequence	NGS	Confirmation of the ITR and transgene sequence of the viral vector ensures identity; as bioinformatics tools advance, NGS may also be useful for sequence variant analysis and characterization of impurities
Residual impurities (e.g., PEI, anti-foam)	Varied (e.g., RP-HPLC)	Confirmation of the removal of residual process-related impurities from the product may be used to support process development and process validation

ELEMENT 7: CONTROL OF MATERIALS

Raw materials for gene therapy are classified as any component or reagent intended for use in the production of ATMPs, including those that may or may not appear in the finished product. A formal risk assessment is required for all raw materials used in the manufacturing process that establishes the relationship between raw material attributes, process performance, and product quality attributes. All raw materials can be divided into the following categories:

- **Starting materials** are materials that are the starting point at which cGMP process manufacturing principles are applied.
- **Ancillary materials** are components, reagents, or materials used during the manufacture of a gene therapy product that are not intended to be part of the final product. These are materials used as processing and purification aids or agents that exert their effect on the therapeutic substance.
- **Excipients** are components or reagents used in the formulation of the final gene therapy product.

Starting materials, ancillary materials, and excipients can be available as commercial off-the-shelf (COS) or as custom materials.

- **Custom materials** are materials that are part of the final product that are genetically modified and custom manufactured per contracted specifications (e.g., plasmids that are not off-the-shelf or commercially available).
- **Commercial off-the-shelf** refers to materials that are commercially available.

Material Risk Assessment

STARTING MATERIALS

cGMP starting material should be used wherever possible, but the use of materials that are classified as High Quality is acceptable, taking into account the clinical trial phase of the final product, manufacturing process controls, and QC testing. The rationale for their use and qualification strategy must be documented in the

Table 7-9. Drug Substance and Drug Product Release Testing Panel

Quality Attribute	Analytical Test(s)	Rationale	Used for DS, DP and/or S
CHARACTERISTIC			
Clarity	Appearance	Compendial	DS, DP, S for both
Coloration	Appearance	Compendial	DS, DP, S for both
Visible particles	Appearance	Compendial	DP, S
Sub-visible particles	Sub-visible particles	Compendial	DP, S
pH	pH	Compendial	DS, DP, S for both
Osmolality	Osmolality	Compendial	DS, DP
Extractable volume	Extractable volume	Compendial	DP
Viral particle titer	SEC-HPLC; ELISA	Measures total viral particles	DS, DP, S for both
IDENTITY			
Capsid identity	Peptide map by RP-HPLC; ELISA	Ensures intended capsid is present	DS, DP
Vector genome identity	qPCR, restriction map, sequencing	Ensures intended vector genome is present	DS, DP
POTENCY			
Vector genome titer	qPCR, ddPCR	Vector genome concentration used for dose determination	DS, DP, S for both
Potency - infectivity	Infectious virus titer (TCID50)	Infectious virus titer for lot-to-lot comparison May not be needed if quantitative expression or functional assay is in place	DS, DP, S for both
	Relative infectivity	Alternative to TCID50 Measure delivered DNA by ddPCR, relative to a reference standard	DS, DP, S for both
Potency - expression	Cell-based assay with mRNA or immunoassay readout (RT-qPCR, ELISA, Western blot, etc.)	Demonstrates that product can infect cells and express protein of interest May not be needed if a quantitative functional assay is in place	DS, DP, S for both
Potency - activity	Cell-based assay with readout relevant to the therapeutic MOA (e.g., enzymatic activity assay)	Best expressed as relative potency when compared to an assay standard May be suitable to replace individual infectivity and expression assays as it encompasses both and adds a functional readout	DS, DP, S for both
PURITY			
Capsid protein purity	rCGE; RP-HPLC; SDS-PAGE	Provides a measurement of capsid protein purity	DS, DP, S for both
Particle content	UV260/UV280 ratio	Provides an indirect measurement of the percentage of full viral particles	DS, DP
Particle aggregation	SEC-HPLC	Provides a measurement of capsid aggregation	DS, DP, S for both

continued on next page

Table 7-9. Drug Substance and Drug Product Release Testing Panel

continued from previous page

SAFETY			
Endotoxin	Endotoxin	Compendial	DS, DP, S for both
rcAAV	rcAAV cell-based assay	Ensures the product does not contain rcAAV	DS
Bioburden	Bioburden	Compendial	DS
Sterility	Sterility (CCITa can be used as a surrogate on stability)	Compendial	DP, S
PROCESS-RELATED IMPURITIES			
Residual HCP	ELISA	Ensure control of impurities	DS
Residual affinity ligand	ELISA	Ensure control of impurities	DS
Residual benzonase	ELISA	Ensure control of impurities	DS
Residual BSA	ELISA	Ensure control of impurities	DS
Residual host cell DNA	qPCR	Ensure control of impurities	DS
Residual plasmid DNA	qPCR	Ensure control of impurities	DS

risk assessment along with the controls and mitigations required that ensure the quality, safety, efficacy, and traceability of the material.

EXCIPIENTS

In general, excipients should comply with the guidance in USP-NF General Chapter <1078> Good Manufacturing Practices for Bulk Pharmaceutical Excipients. In March 2015, the Official Journal of the European Union adopted "Guidelines on the formalized risk assessment for ascertaining the appropriate good manufacturing practice for excipients of medicinal products for human use."

ANCILLARY MATERIALS

The following risk classifications were based on the ancillary material qualification risk classifications per USP 1043, "Ancillary Materials for Cell, Gene, and Tissue-engineered Products." Both the USP and the EMA "Guideline on Good Manufacturing Practice Specific to Advanced Therapy Medicinal Products" provide a general overview of the materials used in the manufacture of ATMP and include the guidance on establishing a risk-based approach for materials used in ATMPs throughout the product lifecycle.

Tier 1

- Tier 1 materials are low-risk, highly qualified ancillary materials suitable for use in manufacturing that are either a licensed biologic, an approved drug, or an approved or cleared medical device.
- Examples include injectable monoclonal antibodies, cytokines, vitamins.

Tier 2

- Tier 2 materials are low-risk, well-characterized ancillary materials suitable for use in manufacturing that are produced under relevant cGMPs.
- Examples include recombinant growth factors, cytokines, sterile process buffers, USP-grade chemicals.

Tier 3

- Tier 3 materials are moderate-risk ancillary materials that require a higher level of qualification.
- These may include custom materials that are defined as high quality (or similar terminology) and have been manufactured under a quality system following the principles of GMP with regards to manufacturing, QA oversight, and QC testing.
- The rationale for their use and qualification strategy

Table 7-10. Examples of Materials

Starting Materials	Ancillary Materials	Excipients
• Bacterial, insect, or mammalian cell line carrying the gene to produce the therapeutic material • Host or packaging cell lines of bacterial, insect, or mammalian origin • Virus seed stocks • Virus master banks • Transgene plasmids • Plasmids • Cell lines	• Sterile process buffers • Media components (growth factors, cytokines, vitamins) • Animal-derived (including human) extracts • FBS	• Nonionic surfactant (Pluronic F-68, Polysorbate 80, Polysorbate 20) • Polyols (glycerol, sorbitol, mannitol, polyethyleneglycol) • Amino acids • Mono- and divalent salts

must be documented in the risk assessment along with the controls and mitigations required that ensure the quality, safety, efficacy, and traceability of the material.
- This tier classification may also be materials produced for *in vitro* diagnostic use or reagent grade that were not intended for use in the production of gene therapy products.
- Upgrade of the manufacturing processes may be necessary in order to use the material in manufacturing gene therapy products.
- Examples include diagnostic-grade chemicals, novel polymers, and process buffers.

Tier 4

- Tier 4 materials are highest-risk ancillary materials that require extensive qualification prior to use in manufacturing that are not produced in compliance with cGMPs.
- Developers in the early stages of development should evaluate the necessity of these materials and explore alternative substances or sources.
- Examples include FBS, animal-derived (including human) extracts, animal-derived polymers, scaffolds, and hydrogels.

SPECIFICATION DEVELOPMENT

Specification development refers to testing for starting materials, ancillary materials, and excipients, and may include testing for the following quality attributes:

- Identity (e.g., visual inspection of material labels, analytical methods [sequencing, mass spectrometry, ELISA, chromatography, restriction mapping, gel electrophoresis, etc., that identify material active ingredients)
- Purity (e.g., HPLC technologies, gel electrophoresis, UV260/280, bioanalyzer)
- Safety (e.g., sterility or qualified RMM, mycoplasma or qualified RMM, bioburden, endotoxin, adventitious agent testing including specific viral testing or qualified RMM)
- Functionality (where appropriate)

RETAIN STRATEGY AND STABILITY

The retain strategy for *in vivo* gene therapy product materials is performed using a risk-based approach that factors in both business and quality risk assessments. Materials used in the manufacture of a GTMP product may be placed in a stability program depending on the stage of development.

USE PERIODS

If a supplier has provided a use period or expiry date, the supplier's date may be used if determined to be suitable. Suppliers may have different review dates for the same material based on their manufacturing process and stability profile. If a supplier review date is not provided for a material, a documented risk assessment should be used to establish an appropriate review or expiry date.

LIFECYCLE APPROACH: ANCILLARY MATERIALS CONTROL STRATEGY

In cases when ancillary materials are chosen for their ability to provide a particular biological function in producing the therapeutic product, performance testing becomes an essential component of their overall qualification. This is especially true when the ancillary material plays a critical role in modulating a complex biochemical effect and has an impact on product manufacturing yield, purity, or final product potency. These ancillary materials tend to be complex substances or mixtures, are frequently biologically sourced, and can exhibit significant lot-to-lot variability. As a result, these ancillary materials usually have no simple identity test, nor can they be easily characterized by physical or chemical tests. The development of well-defined performance assays for complex ancillary materials will not only ensure process reproducibility and final product quality, but in many cases will satisfy the identity testing criteria in accordance with 21 CFR 211.84(d)(11).

In some cases, the initial qualification of an ancillary material for use in manufacturing should be the investigation of the effect of the amount of the ancillary material on the desired response (increased yield, purity, or potency of the therapeutic product). The amount of the ancillary material used in manufacturing should be chosen to consistently yield the desired effect while minimizing issues by removing the ancillary material in subsequent processing steps. Such testing frequently assesses the important functional attribute expected of the ancillary material in a scaled-down or simulated manufacturing process. Examples include:

- If an ancillary material is added to the culture media because it promotes cellular proliferation or the secretion of a critical therapeutic agent, the assay could demonstrate that each lot of ancillary material produces the expected rate and amount of cellular proliferation or the expected level of secreted therapeutic agent.

Figure 7-2. Risk Assessment Milestones

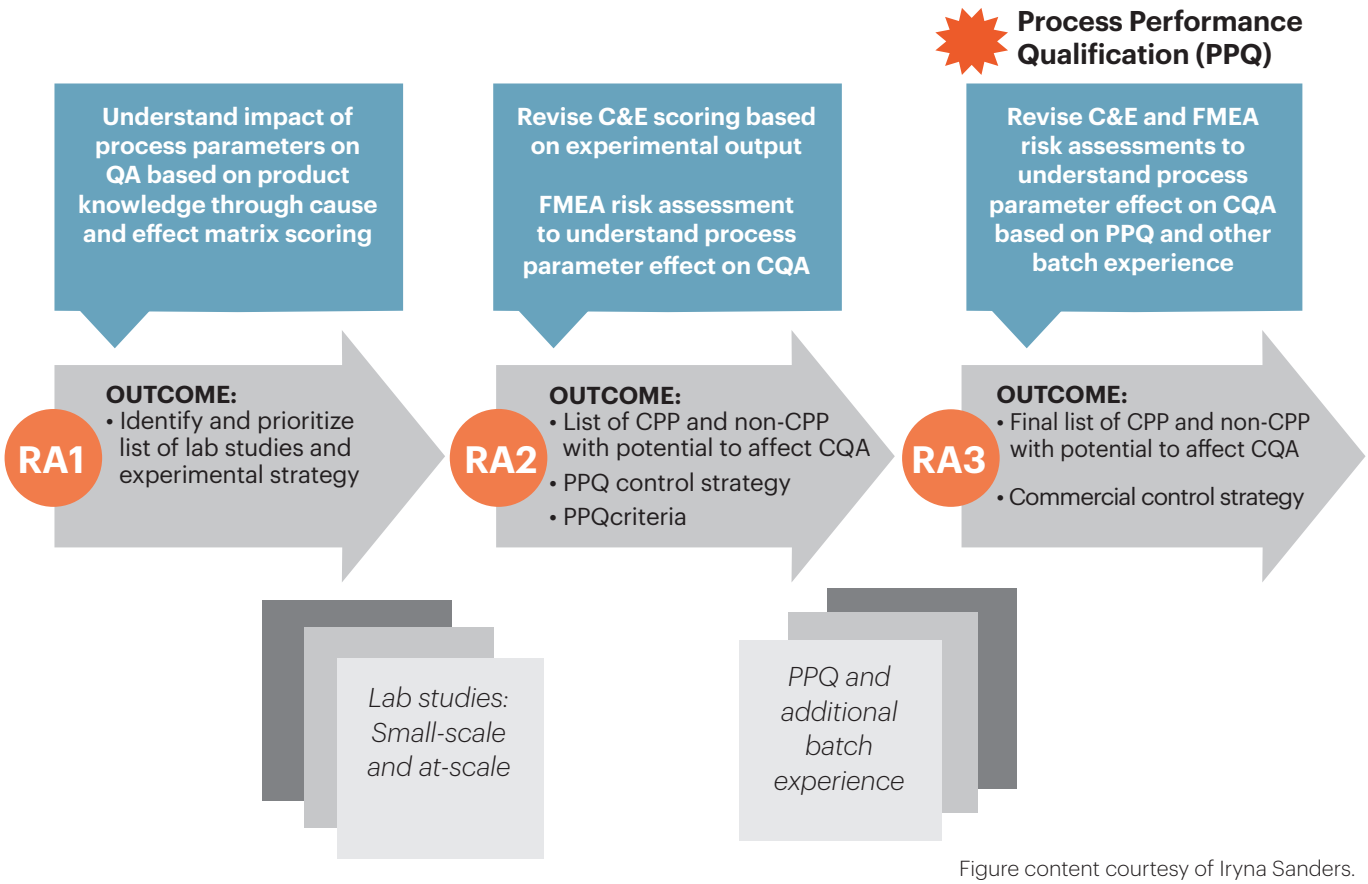


Figure content courtesy of Iryna Sanders.

- If a deoxyribonuclease is used to degrade cellular DNA, new lots could be tested for the ability of the deoxyribonuclease to degrade DNA.
- If a particular type of density gradient material is used to purify a vector, new lots of the material used to make the gradient could be shown to purify the vector to an acceptable level.
- If a plasmid or viral vector is used in the production of a gene therapy vector (e.g., helper function), new lots of the helper vector could be shown to produce the expected amounts of the gene therapy vector.
- The requirement to assess lot-to-lot effect on process performance for ancillary materials may require mitigation for Tier 3 and Tier 4 materials.

Process Risk Assessment

To develop a manufacturing process that consistently and reproducibly operates and delivers product that meets the desired quality, the approach used is based on understanding the relationships between process inputs and output attributes in each unit's operation over the entire manufacturing process. For this, multiple systematic risk assessments are conducted throughout the development lifecycle to identify process steps, material attributes, equipment design, and operation parameters that would be most likely to impact DS and/or DP QAs. The three major risk assessment milestones are depicted in Figure 2, but additional risk assessments can be completed based on program needs. Tools such as C&E tables and FMEA tables can help facilitate risk assessment discussions.

Risk Assessment 1 is recommended to occur in parallel with the decision to commence late-stage development and the initiation of moving the project to the commercial manufacturing scale or site. Many risk assessment tools can be used for teams to evaluate the current process and product understanding to prioritize process characterization activities. The tool shown here, as an example, is the use of C&E tables. These tables have all process inputs listed as rows and all process outputs (including product quality attributes) listed as columns. One relationship score is given for each input and each output. The scores can be ranked with numerical values representing high, medium and low relationships. A high score is neither good nor bad; it only defines the strength of the relationship.

Table 7-11. Example of Typical Scoring Criteria for Process Parameters

10	Strong relationship known based on the data in hand or experience
9	Do not know but expect there is a strong relationship
6	Known medium to low relationship
4	Do not know but expect there is a medium to low relationship
1	Know there is not a relationship

Table 7-12. Example of Typical Scoring Criteria for Attributes

10	Direct impact on product safety and/or efficacy is expected or established
7	Moderate or indirect impact on safety and/or efficacy; direct impact on process efficiency
5	Low or unlikely impact on product safety and/or efficacy; moderate or indirect impact on process efficiency
1	No impact on product safety and/or efficacy; low or unlikely impact on process efficiency

Process Parameter (PP) = Process Input, "x"

Process parameters refer to machines, materials, measurements, processes, people, and environments. This broad definition is appropriate for first-round risk assessments in which the team may choose to look at a variety of inputs, some of which may be non-numerical (e.g., the same raw materials from two vendors). Table 11 shows an example of typical scoring criteria for process parameters.

Attribute = Process Output, "y"

Attributes refer to physical, chemical, or microbiological properties or characteristics of a material (or process). Attributes include both product quality attributes (QA, CQA) and process performance attributes (PPA).

Table 12 shows an example of typical scoring criteria for attributes.

Table 7-13. Extract of C&E Risk Assessment for the Gene Therapy AAV Affinity Chromatography Process

	Unit of Measure	Effluent pH	Process Time	Resin Capacity	Resin Lifetime	Poros AAVX Step Recovery	Elution Pool Volume	Total
Attribute Score		5	7	7	7	7	7	
Bed Height	cm	1	10	10	1	4	10	250
Column Reuse #	Cycle #	1	1	9	10	10	9	278
Integrity - Asymmetry	Not Specified	1	1	1	1	1	4	61
Integrity - HETP	plates/m	1	1	1	1	1	4	61
Mass Loading on Resin	VG/mL	1	10	1	9	10	9	278
Number of Cycles Per Batch	Not specified	1	10	1	4	1	1	124
Process Temperature	°C	1	1	1	1	1	1	40
Differential Pressure	psi	1	1	1	1	1	1	40
Volumetric Challenge	L/m^2	1	1	1	1	1	1	40
Equilibrium Buffer pH	pH	10	1	1	1	1	1	85
Equilibrium Conductivity	mS/cm	4	1	1	1	1	1	55

The C&E matrix involves a team-based assessment of the relationship between process parameters and attributes. The process attributes are scored first and then the relationship between each given process parameter is scored, enabling a total relationship score for each process parameter. Team members will typically discuss the nuances of score, but this process enables a prioritization of parameters to be included in further process characterization studies.

The output of Risk Assessment 1 is used to guide process development activities and includes prioritization of process steps and parameters for optimization and characterization. Experimentation is carried out with univariate and multivariate studies (e.g., DOE) as appropriate using scale-down models to establish parameter-attribute relationships and identify robust operating conditions and acceptable process ranges. Results from the DOE studies provide an understanding of the multidimensional relationships between input process parameters and output quality attributes. Additionally, clinical manufacturing experience provides understanding of process performance and process control at various operational scales.

The information gathered following Risk Assessment 1 serves as the basis for Risk Assessment 2, which occurs prior to PPQ. During this risk assessment, the C&E tables are revised to capture increased product and process understanding. FMEA tables are also used during this risk assessment to understand control of process parameters as it relates to product quality and to develop a strategy for PPQ (e.g., defining parameters that must be demonstrated to be in control during production). These assessments contribute to a robust product and process understanding that ensures an appropriate control strategy and is validated during PPQ. The following risk categories are defined in the FMEA tables, and scores relative to the risk are shown in Table 14:

- **Severity (S):** scored based on the potential impact of the step on the CQA in context of the overall process.
- **Occurrence (O):** scored based on the probability of the critical process parameters exceeding the acceptable range.
- **Detection (D):** how well can the failure of the critical process parameters be detected prior to completion of the step.

Table 7-14. Example Scoring Rubric for FMEA

Score	Severity	Occurrence	Detection
9	Failure to meet DS or DP specifications or quality target leading to lot rejection. Complete failure of process step to meet intended purpose	>20% Very frequent	No way to detect excursion; not tracked and not alarmed.
7	Potential for variation outside specified ranges or limits for product quality and/or consistency, or variation outside historical ranges where specifications or quality targets are not defined. Investigation needed prior to product release.	~5% to 20% Frequent	Difficult to detect excursion, and not until after it has impacted the process.
5	Potential variation within specified ranges or limits for product quality and/or process performance attributes, or variation within historical ranges where specifications or quality targets are not defined.	~1% to 5% Occasional	Excursion can be detected, but not until after it has impacted the process.
3	No impact on product quality. Potential for minor variation in process performance attributes (e.g., yield).	<1% Rare	Excursion is usually detected and corrected prior to impacting the process.
1	No impact to process performance attributes or product quality.	0% Never observed	Excursion is obvious and always detected prior to impacting the process.

The acceptable output range for a QA at each step corresponds to the range that has been proven to work based on DOE studies and prior knowledge. Where linkages between steps exist, the acceptable output of a step is based on what the downstream process steps can handle.

During Risk Assessment 2, the relationships between process parameters and CQAs are better defined, which enables the categorization of process parameters. This categorization is based on the potential impact on CQAs and is reevaluated throughout the development lifecycle via subsequent risk assessments that build on cumulative process and product understanding. The final determination of criticality for process parameters is based on the ICH Q8 definition as a process parameter whose variability has an impact on a CQA and, therefore, should be monitored or controlled to ensure that the process produces the desired quality. Some process parameters that do not significantly impact product quality but are important to ensure consistent process performance can be identified at this step and considered for further monitoring or control during manufacture.

Following PPQ and prior to regulatory submission, Risk Assessment 3 is performed, in which both the C&E and FMEA tables are updated with the current process and product understanding. During this risk assessment, the final commercial control strategy is holistically pieced together using the outputs of the iterative risk assessments and the 8 Element Wheel shown in Figure 1.

Clinical Supply Chain Strategies

Control strategies for the entire clinical supply chain (all manufacturing, packaging, labeling, and distribution steps) should be the sponsor's responsibility up to and including the delivery of the investigational product to the clinical site where patient dosing will occur. At the point where it is delivered to the site, accountability should shift to the responsibility of the investigator and follow current GCPs.

SHIPPING AND DISTRIBUTION

In addition to DP stability and storage under cGMP, DP shipping and handling studies are typically performed using appropriate temperature ranges to ensure product quality is maintained. Shipping studies may involve shipping representative cryopreserved buffers

or product in shipping container(s) and packaging. For example, a shipping study may involve shipment of the representative product to and from a potential clinical site for confirmation of shipment temperatures and also for testing and analysis, if necessary. A shipping study may also be conducted to ensure distribution plans for movement of the bulk DP from the site of manufacture to the distribution vendor are acceptable.

Shipping studies should ensure that all processes have been tested in advance of an actual vendor transfer and clinical shipment to ensure the temperature can be properly maintained and monitored allowing for documentation of shipment data.

PACKAGING AND LABELING

The term labeling designates all labels and other written, printed, or graphic matter on an article's immediate container, or on or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term label designates that part of the labeling on the immediate container. In this section, the primary label refers to a physical label that is affixed to the primary DP container. Secondary label (also referred to broadly as labeling) refers to the label affixed to the secondary container in which the primary labeled container is placed.

Packaging and labeling of gene therapy will require a different approach from typical product labeling as the product must be placed in required cryogenic storage soon after manufacture and cannot be thawed to be labeled at a later date due to limitations on freeze-thaw cycling of these products. For this reason, in most cases, a minimal text primary label will be affixed to the product at the time of DP manufacture, after visual inspection but prior to placement into frozen storage. The primary label text strategy will need to define label content based on regulatory requirements and also based on the strategy to label the primary container at the time of DP manufacture.

Due to limited supply for gene therapy, an on-demand clinical supply model strategy should be considered. This model should include plans for labeling of the secondary container. A trigger for shipment of supply should be determined to ensure distribution controls for supply management.

This overall label strategy may require regulatory approval as part of the regulatory submission, especially due to limitations that may exist based on labeling of the primary container at the time of DP manufacture.

DOSE PREPARATION AND ADMINISTRATION

Dose administration of a gene therapy product involves loading the prepared dose into a delivery system such as a syringe or bag/infusion set and injecting or infusing the prepared dose into a subject. Specific development studies should be done on representative DP material (example: low-strength samples in the exact clinical formulation) to support the various steps in the dose preparation and administration process and to demonstrate that these steps are sufficiently robust and consistent so that the product can be administered without negative impact on the quality of the product. These studies might include activities such as:

- DP storage and handling conditions
- DP vial thaw, in-use shelf life, and storage conditions
- Dose preparation procedure, component compatibility, and in-use stability of dosing solutions

Recommended Readings

US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. Draft Guidance — established conditions: reportable CMC changes for approved drug and biologic products. Food and Drug Administration website. https://www.fda.gov/media/92242/download. Published May 2015. Accessed January 26, 2021.

ICH. Pharmaceutical development Q8(R2). ICH website. https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf. Published August 2009. Accessed January 26, 2021.

ICH. Pharmaceutical quality system Q10. ICH website. https://database.ich.org/sites/default/files/Q10%20Guideline.pdf. Published June 4, 2008. Accessed January 26, 2021.

ICH. Development and manufacture of drug substances (chemical entities and biotechnological/biologic entities) Q11. ICH website. https://database.ich.org/sites/default/files/Q11%20Guideline.pdf. Published May 1, 2012. Accessed January 26, 2021.

Chapter 8

Comparability

Alliance for Regenerative Medicine

NIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Chapter 8 | Contents

continued on next page

Contents, continued

Introduction

This chapter provides guidance to sponsors on the best practices and recommendations in managing manufacturing changes during early development by establishing basic concepts in comparability appropriate for gene therapy products, with a focus on adeno-associated virus (AAV)–based products. Making changes to the manufacturing process/product is an inevitable part of process development with the end goal of improving the product, and therapeutic developers should be prepared to institute robust comparability plans to minimize delays in commercialization.

Sponsors and developers are encouraged to begin the process of constructing a comparability plan as early as possible in product development. Taking into consideration both existing guidance documents on well-characterized biologics and other regulatory and guidance documents that are informative for implementing post-approval changes, sponsors should put an early and specific focus on gathering development and clinical data to support the rationale and support comparability testing associated with any manufacturing change. This chapter explores comparability as an end-to-end concept, provides best practice recommendations on managing minor and major manufacturing changes, and delineates circumstances that may require a formal comparability study.

Gene therapy products, including AAV products manufactured using manufacturing platforms, have seen a tremendous growth in recent years. However, the most promising products are often generated in small scale or a scale not suitable for commercial manufacturing. As a result, some manufacturers may be challenged to identify suitable commercial-scale manufacturing locations and processes prior to initiating their pivotal study. This sometimes involves technology transfer from an academic environment to contract manufacturing organizations (CMOs) and/or the introduction of multiple process changes that require comparability studies. For example, a major manufacturing change might include introduction of a new manufacturing platform using suspension cells rather than adherent cells, the use of new cell lines, or a manufacturing site transfer.

A key element of establishing comparability is to understand the most relevant critical quality attributes (CQA) for AAV products, which may include empty-full ratios, capsid post-translational modifications, multiplicity of infection (MOI), infectivity, residual impurities such as protein, and potency assays. The most challenging aspect of establishing comparability for AAV products is the lack of suitable potency assays, which are useful in measuring the biological activity of the product, and measurement tools that are used to quantify the virus dose and strength with sensitivity and accuracy.

At first glance, a typical approach for establishing comparability of AAV products includes measurement of identity, purity based on residual DNA and protein, and potency. Although a straightforward exercise on the surface, it is extremely challenging to demonstrate analytical comparability based on existing quality attributes that have questionable correlation with *in vivo* safety and efficacy. In addition, it may be challenging to establish comparability based on analytical methods that exhibit a high degree of variability and are neither qualified nor validated. For example, when measuring total residual DNA, PCR may provide different results depending on which target sequence is selected or the type of assay used (e.g., PCR vs ddPCR). Therefore, it is important that analytical comparability studies are conducted side-by-side with a set of the following: 1) well-defined quality attributes that are correlative to quality and efficacy, and 2) measurement tools that are quantitative and suitably sensitive. Additionally, robust method-bridging studies for significant method changes and enhancements are useful to support development, as well as future comparability exercises.

Many gene therapy products are discovered and developed in academic laboratories or small biotechnology companies with expertise in science and innovation but limited experience in bringing these products to market. Indeed, scale-up to commercial size and quality control is a laborious and complex process. As a result, the process may include avoidable risks that lead to unnecessary costs and delays later in development. Like most biotherapeutics, gene therapies need to be produced in a living system. The parallels with recombinant antibody production during the 1990s and 2000s, with regard to the upstream challenges of robust production levels, are important to understand where the industry currently is, and where it needs to strive to be.

Scaling up introduces challenges into the gene therapy development process. For example, while early on the highest titers were achieved with adherent cells in either roller bottles or cell stacks, similar results are now achievable in suspension adapted HEK293 cells. While this was sufficient to support early clinical trials and could supply market production for small patient population indications, the deficiencies in scalability with this platform are a significant limitation. The delivery of three plasmids to one cell is a relatively inefficient process. For larger-scale manufacturing efforts, transient delivery of plasmid requires excess quantities of DNA, adding to the overall cost of production and purification. Moreover, transient delivery of rep/cap genes in the presence of helper genes can also contribute to product heterogeneity, including vector capsids lacking a transgene. These empty capsids represent a significant proportion of virus produced in transient transfection assays or other manufacturing platforms used for AAV production.

Availability of current Good Manufacturing Practice (cGMP)-compliant manufacturing facilities, which are characterized as multi product facilities, also influence the process of scale-up manufacturing and tech transfer from academia to CMO. Currently, CMOs have limited capacity for manufacturing and typically serve several competing clients simultaneously, which creates logistical complexity in conducting comparability studies.

This chapter provides researchers and early developers information on potential risks and insights into how to minimize these risks, and outlines a pathway for easier translation of research into later-stage product development and commercialization. Common but avoidable problems related to manufacturing control and comparability of pre-post changes in products will be addressed. To this end, the workshop "Comparability in Cell & Gene Therapies," organized by ARM and USP in 2019, gathered more than 120 experts actively engaging in debates relating to different aspects of comparability for cell and gene therapeutic products to highlight significant challenges, identify different CQAs, and discuss processes to evaluate CQAs.

Regulation

Developers and sponsors are encouraged to consult existing regulations and guidance documents and engage regulatory authorities early and often as they lay out long-term plans for regulatory filing and clinical evaluation. In the United States, the applicable regulations are more explicit and defined for post-approval changes. These applicable regulations and guidance documents are not necessarily applicable to how manufacturers are expected to manage manufacturing changes during IND phases, but are considered to be good practice.

REPORTING REQUIREMENTS FOR POST-APPROVAL CHANGES

Regulatory requirements for managing manufacturing changes are described explicitly in 21 CFR 601.12, including the implementation of minor, moderate, and major changes and reporting requirements for these changes to licensed products.

All post-approval process changes should be monitored and tracked by the manufacturer through a quality management system. Gaps in reporting may occur when the sponsor's quality system does not trigger a regulatory filing or identify the change; in some cases, the manufacturer may not be aware that the change occurred. Definitions of major, moderate, and minor changes according to the ANDA Submissions – Prior Approval Supplements Under GDUFA Guidance for Industry are shown here.[1]

> **Major change:** a change that has a substantial potential to have a major effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or efficacy of the drug product. A major change requires the submission of a Prior Approval Supplement (PAS) and approval by the FDA before distribution of the drug product made.
>
> **Moderate change:** a change that has a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product. Depending on the nature of the change, either a Changes Being

Table 8-1. *Examples of Changes and Associated Risk Categories*

Example	Risk Category
Changes to tubing, bags, or plastic culture dishes	Low
Changes in critical raw materials, reagents, and ancillary materials	Moderate to high
Changes to production cell substrate (*in vivo* gene therapy)	Moderate to high
Changes to cell differentiation, selection,	
transfection/transduction steps, or allogeneic bank qualification	Moderate to high
Overall manufacturing change (e.g., vector sequence change including in the gene of interest or regulatory sequences)	Moderate to high

Effected in 30 Days (CBE-30) or Changes Being Effected (CBE-0) supplement must be submitted to the FDA for a moderate change.

Minor change: a change that has minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product. The applicant must describe minor changes in its next annual report.

While minor changes can be reported in annual reports, the manufacturers cannot implement major changes requiring PAS until the change is reviewed and approved by the agency. The current timeline for review of PAS involving manufacturing changes is 4 months. Moderate changes may be implemented under a CBE-30 supplement or a CBE-0 supplement. The timeline for CBE-30 and CBE-0 review is currently 6 months.

The categorization of major, moderate, and minor changes and requirements for comparability assessment for gene therapy products is the major topic of discussion in several guidance documents, summarized below.

Recently published guidance entitled "Chemistry, Manufacturing, and Controls Changes to an Approved Application: Certain Biological Products," which is currently in draft form, is arguably the most informative resource about what constitutes major, moderate, and minor changes of biological products, including cell and gene therapy products.[2] This guidance is intended to assist applicants and manufacturers of certain licensed biological products in determining which reporting category is appropriate for a change in chemistry, manufacturing, and controls (CMC) information to an approved biologics license application (BLA) as specified in 21 CFR 601.12 (i.e., post-approval changes).

Examples of post-approval manufacturing changes and recommended reporting categories are described in the Appendix of this guidance document, including a table of frequent manufacturing changes and recommended reporting categories. It is meant to serve as a guide to assist applicants and the FDA to identify reportable post-approval changes and determine appropriate reporting categories.[3]

Categorization of minor, moderate, and major changes depends on many factors, but must be determined based on the available product knowledge and potential risk to product quality. Changes that have very low risk of impacting product quality are considered minor, while changes that could potentially impact product quality are categorized as moderate or high risk. In practice, it is extremely challenging to define appropriate reporting categories for major and moderate changes in cell and gene therapy product manufacturing due to the difficulty in assessing the potential impact of these changes on product quality. For this reason, manufacturers are encouraged to consult appropriate offices before implementing moderate or major manufacturing changes. Table 1 provides examples of changes and potential risk categories.

REPORTING REQUIREMENTS OF MANUFACTURING CHANGES DURING THE IND PHASE

Although the reporting requirement after post-approval changes is well defined by regulatory agencies, the regulations for reporting changes during the IND phase are less well defined. Generally, manufacturers

should report major changes in amendments and minor changes in annual reports based on current FDA policies. This recommendation is summarized in a recent draft guidance covering the gene therapy products. "The CMC information submitted in an IND is a commitment to perform manufacturing and testing of the investigational product, as stated. We acknowledge that manufacturing changes may be necessary as product development proceeds, and you should submit information amendments to supplement the initial information submitted for the CMC processes (21 CFR 312.23(a)(7)(iii)). The CMC information submitted in the original IND for a phase 1 study may be limited, and therefore, the effect of manufacturing changes, even minor changes, on product safety and quality may not be known. Thus, if a manufacturing change could affect product safety, identity, quality, purity, potency, or stability, you should submit the manufacturing change prior to implementation (21 CFR 312.23(a)(7)(iii))."[4]

In some cases, FDA reviewers may require additional information in support of minor changes reported in the annual report if it is deemed to be major and could potentially impact the product quality. For complex changes, IND holders are encouraged to have early consultation with the agency to determine the category of change. If it is categorized as major based on manufacturer assessment and consultation with the FDA, then a comparability study may be required. It is advisable to develop a comparability study design with the agency prior to the implementation of a major manufacturing change.

UNDERSTANDING CRITICAL QUALITY ATTRIBUTES OF GENE THERAPY PRODUCTS DURING IND PHASE

Central to establishing product comparability is sponsor knowledge of product-specific CQAs that are relevant to the safety and biological activity of the product, as they are understood at the time of submission. CQAs form the backbone and primary reference point for a comparability plan, and the associated metrics will evolve over the course of development. For example, tolerance limits of a product CQA may be broad during early development when manufacturers are still gaining information about their product, and will narrow as information increases and reproducibility improves. In addition, the list of CQAs may be revised as product knowledge increases. Defining product characteristics that are relevant to the clinical performance of the gene therapy may be challenging during early stages of product development when product safety and quality are not sufficiently understood. Therefore, manufacturers should evaluate many product characteristics during early clinical development to aid in the identification and understanding of CQAs and ensure the ability to assess manufacturing process controls, consistency, and stability as development advances. This is especially important for sponsors of gene therapy products who are pursuing expedited development programs. CQAs may be used to specify key characteristics of the drug substance (DS) and drug product (DP) including, but not limited to, specifications for a later-phase clinical study or BLA, and are required to demonstrate product comparability by analytical methods.

IMPORTANCE OF ESTABLISHING COMPARABILITY

In all life science industries, the initial product envisioned by the inventor undergoes substantial revision and evolution as it translates from the scientific bench to the patient. Most manufacturers of cell and gene therapy products make changes at some point during development through the post-approval phase. Changes made to the manufacturing process may potentially impact the product's critical characteristics and therefore its clinical outcomes. In addition, minor changes in growth conditions of the common producer cell lines for gene therapy products (e.g., environmental cues such as extracellular matrix components and spatial organization of signaling molecules) may have profound impact on the cellular machinery that facilitates viral production. Thus, failure to detect the potential impact of these changes during late-phase clinical trials or post-approval could potentially affect product quality, effectiveness, and ultimately commercial success of the product.

To address these potential pitfalls, manufacturers are strongly encouraged by the FDA and other health authorities to define and implement a plan of action to understand the CQAs that could potentially affect product quality and the clinical outcomes early during the product development cycle. However, for a variety of business and logistical reasons, product developers often introduce major manufacturing changes late in the

product development life cycle. One example is when the productivity of the initial manufacturing process (function of titer and downstream yield) is sufficient for early phase trials, but not for later phase trials and/or the commercial phase (cost of goods). As such, manufacturers are encouraged to introduce major manufacturing changes early during the product development life cycle when possible, and to demonstrate that the product is comparable before and after the implementation of changes. The fundamental reason for this expectation of product comparability comes from the sponsor's reliance on clinical data generated with the product manufactured prior to the proposed change to demonstrate product safety and effectiveness. Thus, the clinical data may originate from a product manufactured using a different platform, at another manufacturing site, or even in another country.

Although regulatory authorities have established somewhat-defined expectations on how to demonstrate comparability, the risk is carried by manufacturers, who are responsible for changes made to product manufacturing processes that adversely impact the clinical effectiveness of the product.

RISK FACTORS THAT AFFECT PRODUCT COMPARABILITY

Risk factors that affect product comparability are dependent on the manufacturing change(s), its impact on product quality attributes, and the timing during the product development life cycle. The complexity of changes introduced during manufacturing also poses a risk. For example, if a developer or manufacturer introduces multiple changes simultaneously, there is an increased risk of impact to product quality. It is well understood that the relative risk associated with process and product changes is substantially increased during later phases of clinical trials, as well as when product knowledge is not comprehensive, particularly in the case of lack of understanding of how a given CQA relates to product safety and efficacy.

Challenges Associated with Comparability Studies in AAV Product Manufacturing[5]

PRODUCT COMPLEXITY

Gene therapy products represent a novel and complex class of biological products and are often heterogeneous mixtures. Furthermore, these therapies encompass a wide spectrum of products, each with unique mechanisms of action, material qualifications, challenges in establishing specifications, manufacturing facilities, product shipping/handling procedures, and storage conditions. Quality analytics methods and product understanding enable manufacturing changes with minimal impact on product quality, and it is important to note that the minimum level of testing for phase 1 INDs is not sufficient to understand complex biologic products.

INCOMPLETE PRODUCT KNOWLEDGE

Performing comparability in early phases is difficult because establishing comparability requires product knowledge and understanding of CQAs and critical process parameters (CPPs). In most cases in early development, developers have not established their CPPs because their process has not been characterized yet, and process characterization is not undertaken until process lock is ready. If CQAs, CPPs, and key process parameters (KPPs) are well known and correlations between CQAs and product quality, safety, and efficacy can be demonstrated, then testing of the product CQAs pre- and post-change and comparing the results using an acceptable statistical method may be sufficient. The ability to produce a consistent product is dependent on CPP control and CQA monitoring, along with other factors that define the overall quality of the product.

In the context of identifiable CQAs, CPPs and KPPs are limited for gene therapy products. In some cases, the manufacturing process is optimized based on a limited number of variables using manufacturing scales that are not representative of commercial product manufacturing. Because the establishment of comparability studies relies heavily on the analytical similarity of critical attributes that are informative in assessing product quality, safety, and efficacy, the limited knowledge of CQAs, CPPs, and

KPP for gene therapy product manufacturing further complicates the exercise of establishing comparability.

INADEQUATE CQA ASSAYS

In some cases, the analytical methods used to measure critical attributes that reflect product safety and efficacy evolve during the course of the development process and the development of product understanding. Establishing analytical comparability relies heavily on the availability of suitable methods that are qualified and/or validated. Additional requirements for assay qualification and validation are discussed later in the chapter in the Detailed Analytical Procedure section. Further, assays used for measurement of product quality changes may change during the product development cycle. These changes introduce additional challenges in taking advantage of historical data that are often collected using different assays, and methods should aim to analyze samples side-by-side.

SMALL BATCH SIZE

In contrast to biotechnology products for which it is practical to manufacture a reasonable number of batches, at-scale production of AAV products is challenging due to limited resources and manufacturing capacity at CMOs. The lack of sufficient manufacturing experience and representative clinical and or commercial products, and the small batch size of gene therapy products complicates the establishment of analytical similarities based on available statistical tools. Further, there tends to be a major imbalance between the number of batches manufactured before and after process changes. In some cases, more data may be available prior to the change, while for others, more data may be available post-change. It is important to note that analytical results may not be available for materials for which the initial emphasis was on gaining clinical experience and establishing clinical efficacy, rather than on planning for future analytic comparability.

SIDE-BY-SIDE COMPARISONS OF PRODUCT QUALITY

A well-defined comparability study should rely upon comparisons of key quality attributes of the product before and after major changes have been introduced. Heightened characterization methods may utilize side-by-side comparison, whereas other times release data may be used without side-by-side comparisons for other studies. In general, a side-by-side comparison is valuable to remove inter assay variability and to better home in on the true differences of the product. Methods that involve separations, CGE, HPLC, and other purity-based methods are best suited for this analysis as well as potency type methods. However, a side-by-side comparison for gene therapy products may be impractical. As a result, manufacturers may rely upon historical data to establish product comparability. Unfortunately, historical data are often not collected by measuring applicable attributes, or using qualified or validated methods. The lack of information for historical lots thus necessitates a side-by-side comparison of attributes for the product manufactured before and after changes using materials manufactured using the old process via a common analytical method.

ESTABLISHING STATISTICAL SIGNIFICANCE

Establishing comparability using a small number of batches via an appropriate statistical tool is challenging. Due to a number of factors, such as the timeline for implementation of the proposed changes, cost of manufacturing, and limited capacity at CMOs, it is a common practice to establish comparability based on two to three (or fewer) lots or batches. The limited number of samples poses a significant challenge to establishing comparability based on well-defined statistical methods and predefined comparability criteria. As a result, the application of conventional methodology used to establish comparability or even similarity for products is not necessarily applicable to gene therapy products with the current state of technology. For additional information about the appropriate use of statistics to establish comparability for gene therapy products, please refer to the Statistical Strategy for Comparability Assessment section of this chapter.

COMPRESSED TIMELINE

In gene therapy development, it is common for many products to receive expedited program designation (e.g., regenerative medicine advanced therapy or breakthrough therapy designation). Therefore, manufacturers often have aggressive timelines to implement major manufacturing changes as part of establishing readiness to initiate pivotal or licensing trials. Though aggressive timelines make thoughtful product development changes and

establishing subsequent comparability challenging, these aggressive timelines do not relax the requirements for comparability for manufacturing changes.

LACK OF REFERENCE MATERIAL AND STANDARDS

The lack of commercially available reference material remains a challenge in establishing manufacturing comparability. Although reference material is traditionally used for assay validation, internal reference materials for gene therapy products could also be used to establish comparability for changes introduced to the analytical methods before and after any proposed changes. Manufacturers are encouraged to develop internal standards that can serve as benchmarks for establishing manufacturing control. Standards should be stored under the proper conditions to ensure their stability over time.

The challenges associated with comparability studies in AAV product manufacturing include (but are not limited to) product complexity, incomplete product knowledge, inadequate CQA assays, small batch size, establishing statistical significance, compressed timeline, and lack of reference material and standards. Considering that manufacturers risk adversely impacting the clinical effectiveness, safety, or quality of the product whenever changes are introduced to the manufacturing process, they require tools and guidelines for facing challenges related to establishing product comparability.

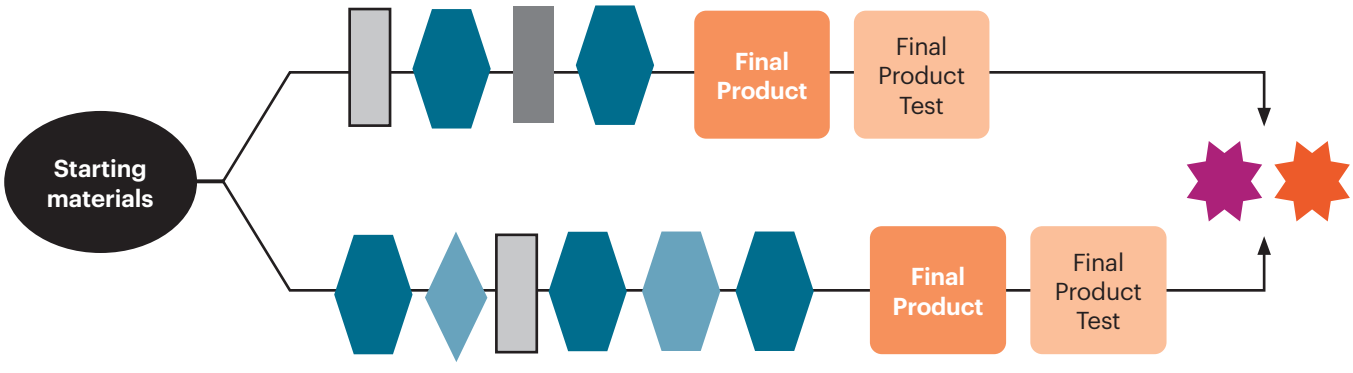
Tools to Establish Product Comparability

COMPARABLE PRODUCTS

Comparability is an essential part of the evolving process to ensure that data gathered is valid through development for marketing authorization and beyond. Comparability has become a routine exercise throughout the life cycle of biotechnological products. Currently, ICH Q5E is the most comprehensive guidance/guideline document that is available for establishing product comparability between gene therapy products.[6] In ICH Q5E, product comparability is defined as a conclusion that products are highly similar before and after manufacturing process changes with no predicted adverse impact on the quality, safety, or efficacy of the drug product. This conclusion is most often based on an analysis of product quality attributes. In some cases, in which subtle analytical changes are seen, nonclinical or even clinical/immunogenicity data may be indicated. The demonstration of comparability does not necessarily stipulate that the quality attributes of the pre-change and post-change product are identical, but that they are highly similar, and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product. In some cases, the regulatory agency may ask the IND sponsor or applicant to submit the comparability study for assessment and review prior to the data collection

Figure 1-1. *Schematic of Product Comparison*

Two processes that differ in the number and type of unit operations can be demonstrated to be comparable



and analysis. In most cases, product comparability cannot be established by demonstrating that a product manufactured by a new process meets a predetermined release specification. Fortunately, the minimal elements of a good comparability study for this emerging product class have been defined in several public presentations by FDA's CBER.[7] Importantly, meeting predetermined release specification is not sufficient to establish product comparability, and determinations of product comparability can be based solely on quality considerations if the manufacturer can provide assurance of comparability through robust analytical studies.

To perform comparability studies, statistical analysis and the generation of sufficient and robust data during both preclinical development and clinical trials play critical roles to demonstrate equivalence or superiority in a post-change product. However, where the relationship between specific quality attributes and safety and efficacy has not been established, and differences between quality attributes of the pre- and post-change product are observed, it might be appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise. The comparability study depends on the extent of the change and the stage in the product's development when the change takes place (for example pre- vs post-pivotal clinical trials). It is important to develop a comparability plan as early as possible in product development, preferably before a phase 1 trial.

If the knowledge of CQAs, CPPs, and KPPs is complete, the exercise of establishing comparability is straightforward, provided that there is substantial evidence that certain CQAs are linked to product efficacy and clinical outcome. In this case, it may be possible to establish product comparability based on a limited set of highly relevant attributes by comparing pre- and post-attributes using common analytical techniques with predefined comparability criteria for product comparability and a well-defined statistical method. In particular, predefined comparability criteria may be used for late-stage/high-risk programs.

If knowledge of CQAs, CPPs, and KPPs is incomplete, then a matrix-based approach is recommended. For products at the in-process and final release stages, all relevant attributes before and after the change (full/extended characterization) should be compared. Comparison of release specifications for the product before and after a change may not be sufficient due to lack of CQA/CPP knowledge. Matrix-based examples include:

- Analytical testing of product attributes, including release tests for the impacted product or product intermediate
- Additional in-process testing for the impacted product or product intermediates
- Side-by-side heightened characterization of the DS/DP/drug substance intermediate (DSI)
- Modulation of KPPs to ensure product manufacturing control
- Application of QbD if possible
- Product yield measurement at different stages of manufacturing

REQUIREMENTS FOR NONCLINICAL AND/OR CLINICAL STUDIES

Comparability studies based solely on *in vitro* studies is possible based on the strength of the data, extensive product knowledge, and measurement of attributes that are informative to assess both product quality and efficacy, and clinical outcome. This scenario is mostly applicable to changes introduced early during clinical studies, which permits manufacturers to collect additional clinical data using the new process.

In some scenarios, establishing analytical comparability may not be possible based on physiochemical and biological assays alone. In those cases, additional preclinical and/or clinical studies may be needed prior to the licensure of the product in order to be approved at-scale and using a process different from what was previously used to generate clinical results. Accordingly, IND holders may be required to conduct additional preclinical animal studies and/or bridging clinical studies prior to or after licensure. Reliance on *in vitro* analytical studies may be possible for AAV products, particularly if significant clinical data will be generated using the approved new process. However, when major changes occur very late in the product development cycle, establishment of product comparability based on preclinical animal models or a bridging clinical study may be unavoidable.

Table 8-2. *Examples of Manufacturing Changes*

Manufacturing Step	Process A	Process B	Rationale
Upstream	Adherent cell line	Suspension	Improvement in yield
Downstream Purification	Chromatography Method 1	Chromatography Method 2	Improvement in purity and yield

Essential Elements of a Comparability Study

Based on available information, certain components are considered by the FDA to be essential elements of a good, prospective comparability study for gene therapy products. In the United States, the comparability protocol prospectively describes the planned change to the manufacturing process in the form of a PAS, which when reviewed by the FDA will determine whether the planned change can be reported in a category that does not require a full comparability protocol. Typically, comparability protocols are submitted to the agency as PAS in support of lowering the reporting category from PAS to CBE-30 or CBE-0. The comparability study used to support manufacturing changes during the IND phase is not necessarily identical to comparability protocol that can be used to lower the required reporting category, but in principle it contains the essential elements of a good comparability study protocol.

A comparability study is defined as a prospective document that is submitted to the agency in support of the proposed manufacturing changes. The comparability study should include discrete essential elements as identified in detail below.

INTRODUCTION AND BACKGROUND

The introductory section provides an overview of the product, current regulatory status, and manufacturing steps. The background section provides information on why a comparability study is being submitted and details previous regulatory submissions related to the manufacturing changes. This section should contain a summary of the overall approach used to establish product comparability and what is reported in the comparability study.

DESCRIPTION OF CHANGE AND RATIONALE FOR INTRODUCING CHANGE

This section of the report should include a detailed description of changes reported in both text and tabular format. The changes should be reported in the context of major manufacturing steps (e.g., upstream or downstream purification steps). Examples of potential changes, along with the rationale for a change, are described in Table 2. It is critical to include all changes in the process and provide sufficient rationale for the introduced changes.

In some cases, manufacturers also report changes to analytical procedures or changes in the manufacturing facility. Each change should be described in detail under a separate heading.

CATEGORIZATION OF CHANGES

Manufacturing changes for gene therapy products can be categorized as minor, moderate, or major changes. Minor changes are defined as changes that do not have potential impact on product quality, while moderate and major changes could potentially have adverse impact on product quality and may require submission of a new IND or IND amendment (Table 3). The determination of minor vs major requires not only product knowledge but also an understanding of the relationship between CQAs/CPPs with product safety and efficacy. In the situation when the relationship between CQAs/CPPs and product quality is not understood fully, the FDA encourages the application of risk assessment principles. It is very important that the categorization of changes is conducted in consultation with regulatory authorities. Manufacturers may use risk assessment approaches, such as those described in Chapter 4, to prioritize CQAs for comparability studies.

Table 8-3. *Examples of Common Changes and Associated Risk Categories*

Changes	Description	Category	Notes
Buffer (like for like)	Supplier change	Minor	
Manufacturing platform	Completely different platform (e.g., change in cell line)	Major	Potentially requires a new IND; consult agency
Formulation	Final titer, buffers, excipients	Major	Affects dose
Manufacturing scale	Same process, scaling up or out	Moderate	
Manufacturing site	Site change for drug substance (same process)	Moderate	
Manufacturing site	Site change for drug product (no changes to DS or process)	Minor	
Change of purification process	Change from centrifugation to tangential flow filtration	Moderate	
Change of vector backbone	Completely different vectors and promoters	Major	Potentially requires a new IND; consult agency
Change of transgene	For example, different portion of same gene is used as transgene	Major	New product requires new IND; consult agency

COMPARISON OF CQAS

Potency

Potency, defined in 21 CFR 600.3(s), is interpreted to mean 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. Early development of multiple assays of potentially relevant product activities will not only facilitate development of a potency assay, but will also help ensure that the product is consistent and that early results are relevant for designing later studies and for licensure. Further, a suitable potency assay provides valuable information concerning overall product stability and is useful for establishing comparability of post-manufacturing process changes. Countries and/or regions may have different requirements to establish the potency testing strategy.

Purity

Purity, as defined in 21 CFR 600.3 and 21 CFR 610.13, refers to relative freedom from extraneous matter in the product, which in AAV products may include residual host cell DNA and proteins, empty AAV capsids, or AAV capsids containing helper virus DNA. Additionally, there could be present AAV particles containing the targeted genetic material that has undergone post-translational modifications such as (but not limited to) deamidation, phosphorylation, oxidation, all of which could have an effect on the transduction pathway. A commercial-scale product with an impurity profile that differs from those of previous noncommercial pilot batches could result in significant challenges in the establishment of product comparability and may require further process improvement to reach the predefined standards.

Strength

Strength generally refers to the number of AAV particles administered to the patient and the potency of the vector product. For products to be considered comparable, the overall strength of the product must be measured. Assays used to establish comparability in strength may measure, for example, vector infectivity, *in vitro* RNA and protein expression, and *in vivo* bioactivity.

Identity

For gene therapy, identity is defined in 21 CFR 610.14 as a test that distinguishes one product from other products manufactured in the same facility, relies heavily on the sequence information derived from the transgene and its fidelity using different sequencing platforms. Identity is not generally considered to be critical aspect of establishing product comparability but is important to verify product quality prior to release/distribution.

Safety

Safety is defined in 21 CFR 600.3 as the relative freedom from harmful effect to persons affected directly or indirectly by a product. For gene therapy products, safety is determined by testing the product for sterility, endotoxin, mycoplasma, and the presence of any adventitious agents that could be derived from the biological material/cell lines used. In addition, the absence of replication-competent virus must be established. Although some of these parameters may be included in comparability studies, measurements of safety may not be critical to establish product comparability but rather are used in establishing manufacturing control.

PREDEFINED APPROACH TO ESTABLISHING PRODUCT COMPARABILITY

The approach used to establish product comparability for genetically modified cells may involve side-by-side comparison of analytical data obtained from different manufacturing processes using the same starting materials. However, side-by-side comparisons may not be feasible in AAV-based gene therapy manufacturing. The common approach to establishing comparability after a major process change relies on comparison of critical attributes, with the highest relevance to product bioactivity, with a previously optimized process. This approach requires and mandates the use of analytical methods that are deemed comparable and appropriately qualified. Importantly, samples collected from reference lots (pilot or non-GMP lots) should be tested using the same assay under identical or similar conditions.

WELL-DEFINED ACCEPTANCE CRITERIA TO ESTABLISH ANALYTICAL COMPARABILITY

A risk-based approach can be used to determine comparability criteria for analytical comparability during process changes. However, selection of analytical methods and acceptance criteria may be the most challenging step in a comparability study. Predetermined acceptance criteria to establish comparability should rely heavily on historical product knowledge, manufacturing capacity, and robustness of the selected analytical methods. For example, analytical methods with a high degree of variability when measuring identical samples at different times, by different operators, or with a different facility or equipment, should be avoided.

In the comparability study submitted to regulatory authorities, the manufacturers propose predetermined acceptance criteria that is justified based on historical data, manufacturing capability, and assay variability. Historical data may be limited at early stages of development, for example, and deriving predetermined acceptance criteria from a limited number of lots may be acceptable. However, acceptance criteria during phase 3 studies may require additional justification based on a larger body of information collected during the product development life cycle.

DETAILED ANALYTICAL PROCEDURE

Analytical comparability for gene therapy products is solely dependent on the robustness of the analytical methods. Depending on the stage at which analytical comparability is performed, the analytical method must be in a state of control commensurate to the phase of the study. Assay qualification may be sufficient for early-phase analytics, but assay validation is recommended for changes in later phases. However, as a scientific matter and as good laboratory and manufacturing practice, it is highly recommended that manufacturers use validated assays whenever possible to measure CQAs, especially if they are used to collect information during clinical studies that determine product efficacy (e.g., a pivotal or licensing trial). The reason for this is simple: validation involves providing assurance that a given process or test can be performed reproducibly and accurately with a high degree of sensitivity, precision, and linearity, even in a worst-case scenario. Thus, the assay yields equivalent

results using the same sample when it is used by different operators in different lab environments, or using different instruments, so long as the assay parameters are controlled as specified in a protocol. Qualification sets a lower bar and requires that the assay can be performed with some reasonable degree of reproducibility by the manufacturers, under very controlled conditions, such as it being performed by a designated operator and using a specific instrument or a reagent lot.

As a result, manufacturers who choose to use a qualified (not validated) assay to collect critical information during phase 3 or pivotal studies could potentially collect data sets that are not fully representative of their product quality. This could potentially impact the usefulness of assay results that are relied upon to define meaningful specification/acceptance criteria to assess and verify product quality.

A typical approach to qualify and validate critical assays is as follows. For qualification, regulatory authorities generally expect demonstration of a reasonable degree of assay sensitivity, linearity, precision, and accuracy. For validation, these parameters must be complemented by assay ruggedness and robustness. For the purpose of this discussion, assay ruggedness is the reproducibility of the assay under a variety of variable test conditions that include different instruments, operators, and reagent lots. Robustness provides an indication of the assay's ability to perform under normal usage and without being impacted by changes in various factors (e.g., incubation time, temperature, sample preparation, buffer, or pH) or parameters that can be controlled and specified in the assay protocol.

Thus, timing is critical when determining whether to use a qualified or validated assay in the development of gene therapies, and sponsors must make thoughtful decisions so that data used to support development and subsequent BLA submission are reliable and utilize appropriate assays during a given stage of development.

If new analytical methods are implemented during development, older methods may be included in the comparability exercise. It is important to retain samples that can be retested when new and improved methods are implemented. Such samples will identify whether a newly identified item is actually new or whether it was present in the clinical material but not detectable by the old method. When the manufacturing experience contains multiple lots, trend analysis should be applied, including comparisons to historical lot releases. It is important to provide justification for how lots were chosen for the comparability exercise and to avoid "cherry picking" (i.e., using certain pre-change lots that are more comparable to your post-change lots).

SAMPLING PLAN AND STATISTICAL ANALYSIS

Manufacturers should provide sufficient justification for the proposed sampling plan, including the number of batches tested, types of batches used for manufacturing comparability, and sample collection methods for the comparability runs. The type of batches used to establish comparability should be a major point of discussion in the comparability study design. Due to a number of practical considerations, manufacturers should plan to establish comparability using a limited number of runs and batches that are manufactured using a process representative of the at-scale manufacturing (which may be performed under non-GMP conditions). This approach is not necessarily encouraged, but it could be potentially acceptable provided that appropriate scientific justification is provided.

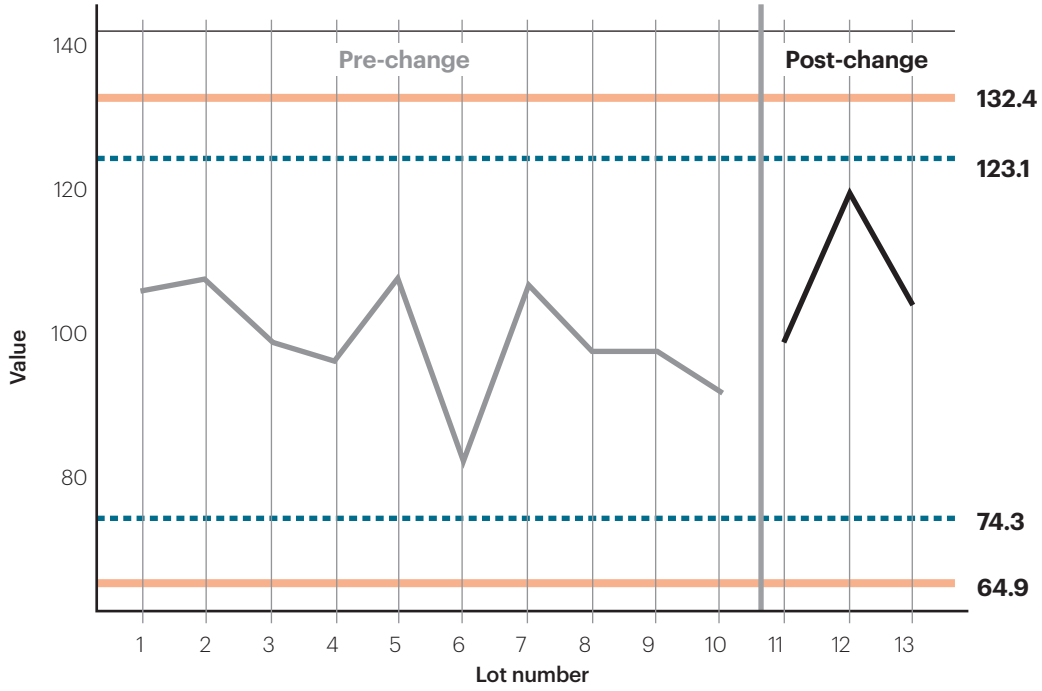
Selection of a statistical method to establish comparability is an important consideration when defining the required number of samples. It is extremely challenging to test a sufficient number of batches for cell and gene therapy products (e.g., due to lack of sufficient raw materials and starting materials, compressed timelines, and limited production capacity). Accordingly, the agency has shown a great deal of flexibility in accepting comparability based on a limited number of runs.

STATISTICAL STRATEGY FOR COMPARABILITY ASSESSMENT

Process comparability exercises for gene therapy pose the following challenges for the application of proper statistical tools, which are discussed in greater detail below.

- **Limited sample size:** Frequent process changes during early-stage development often results in only 1 or 2 batches per process. Also, depending on the indication and dose, few batches may be needed clinically, limiting the batches available for comparability.

Figure 8-2. *The side-by-side comparison of the pre-change and post-change lots against the mean+/-3SD (74.3, 123.1) and the SPI with 99% confidence level (64.9, 132.4)*



- **Unbalanced sample size:** Unbalanced sample sizes are common in process comparability data. The number of the pre-change batches could be large, but the number of post-change batches is usually no more than 4 at the time of comparison. This unique unbalanced data structure makes it challenging to properly apply commonly used statistical methods.

- **Lack of knowledge about the clinically meaningful difference between the pre- and post-change processes:** inability to assign a numerical value to represent the clinically meaningful difference has been a common challenge and poses a direct challenge to application of an equivalence test.

Overview of Commonly Used Statistical Approaches and Applicability to Process Comparability Assessment

Various statistical approaches have been developed and applied in comparability assessments. Commonly used statistical approaches include visual comparisons, minimum and maximum, confidence intervals, prediction intervals, tolerance intervals, and equivalence testing.[8] Although each of these statistical methods has an intended use, some may result in similar or identical limits by adjusting confidence and/or coverage levels. The choice of statistical methods depends on many factors, including 1) knowledge of the product CQA (e.g., is the CQA a numerical variable and amenable to statistical analysis), 2) relevance of the CQA to product efficacy and safety (e.g., CQA range considered to be safe and efficacious for the CQA), 3) associated analytical testing and process variability, 4) development stage of the product, and 5) the available number of batches for statistical assessment, which is generally very limited (e.g., <10).

Not all CQA are amenable to statistics. For instance, qualitative CQAs and quantitative CQAs with unreasonably large method variability are generally not suitable for statistical analysis. However, the stage of development may make a difference as the available knowledge and historical data are typically limited at the early stage but more plentiful in later stages. It could be misleading to blindly apply statistical analysis methods when data are limited.

Visual Assessment

Visual assessment is always helpful, regardless of the amount of data. Although visual assessment does not provide a direct answer of "pass/fail," it provides a basic understanding of the data and is generally recommended as the first step of data analysis. Visual assessment is probably the only assessment that is applicable with only 2 or 3 batches to compare per process. For instance, a side-by-side scatter plot (Figure 2) is a simple way to examine data trends.

Visual assessment generally does not follow explicit rules. However, when the number of batches is large, some of the trending rules commonly used in the typical statistical process control (SPC) field may apply. When data are limited, as they often are, it is difficult to identify trends and SPC trending rules are generally not suitable. Instead, it is tempting to use the data range of the pre-change batches to assess the performance of the post-change batches. However, when pre-change data are limited, the risk of post-change batches exceeding the pre-change data range is significant, even if the two processes are the same for the attribute of interest. Therefore, it is not appropriate to simply focus on the range of the pre-change and post-change batches. When data are limited, method variability can be used as an approximate gauge to assess the data spread. In other words, comparability may be concluded if post-change batches are within the expected method variability.

Minimum/Maximum

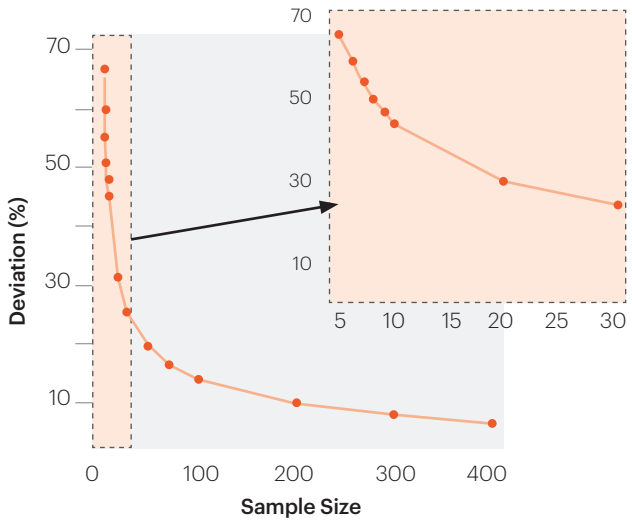
Minimum and maximum are two commonly used descriptive statistics that represent the range of current data. Although simple to use, minimum and maximum have no inference capability in that the range of the current data does not necessarily represent the range of the future data. Furthermore, a minimum/maximum based on a limited sample size is subject to large uncertainty and is often narrower than the true range of the data.[9] The EMA reflection paper on comparative assessment also criticizes it.[10]

Confidence Interval (CI)

CI is a widely used statistical measure defined as the interval that contains the true value of the population parameters, such as the mean or standard deviation. In a typical process comparability setting, the ideal outcome

Figure 1-3. *Plot of the deviation of SD estimated from the true SD*

Using methods from Burnett (1975). The inset box shows the sample size from 5 to 30



is that the CQA values of all post-change batches fall within some expected limits instead of, for example, the mean of post-change batches. Therefore, CI is generally not recommended for process comparability assessment unless the interest is in the population parameters such as mean or standard deviation, which can be reliably estimated from a decent number of historical batches.

Mean ± 3 Standard Deviations (SD)

Mean ± 3 SD is one of most well known statistical intervals probably because it is simple and easy to apply. For an attribute that follows normal distribution, the mean ± 3 SD represents the interval that 99.73% of the population (i.e., all values generated before and in the future under the same condition) will fall within if the true mean and the true SD are known. However, it is only recommended for the two following situations assuming normal distribution: 1) the true mean and the true SD of the pre-change process are known or 2) the pre-change process has a long history, thus permitting the true mean and the true SD to be estimated with high certainty based on a large number of pre-change batches (N). The SD requires a larger sample size than the mean to calculate a reliable estimate. The estimated SD can still differ from

the true SD by ~10%, even when N=200.[11] Figure 3 shows how much the SD estimate could deviate from the true value with increasing sample size. The deviation drops significantly when sample size increases from 5 to 10. The speed of the drop slows after N=10 and the deviation curve begins to flatten out after N=200. Therefore, mean ± 3 SD is often used for control chart setting for situations in which a large sample size is available. It is generally not suitable for limit or criterion setting, such as in process comparability, unless a very large sample size (N) is achieved.

Prediction Interval

Unlike the confidence interval, which is useful for population parameters, prediction interval is designed to predict the range of future individual values if the future values will come from the same population as the historical values.[12] It is usually called prediction interval if there is only one future value to predict and simultaneous prediction interval (SPI) if ≥2 future values need to be predicted.

In general, to predict m future values based on N historical data values, the interval can be calculated as the following: $\underline{x} \pm ks$ where $\underline{x}$ and s, respectively, represent the sample mean and sample standard deviation of the N pre-change batches, and k is the multiplier factor. The multiplier k is written as

$$k = t_{1-\frac{\alpha}{2m},\,(N-1)} * \sqrt{1+\frac{1}{N}}$$

for 2-sided prediction interval and

$$k = t_{1-\frac{\alpha}{m},\,(N-1)} * \sqrt{1+\frac{1}{N}}$$

for 1-sided prediction interval, where

$t_{1-\frac{\alpha}{m},\,(N-1)}$ is the $\left(1-\frac{\alpha}{m}\right)th$ percentile

of the central student t distribution with $N-1$ degrees of freedom, and

$t_{1-\frac{\alpha}{2m},\,(N-1)}$ is the $\left(1-\frac{\alpha}{2m}\right)th$ percentile

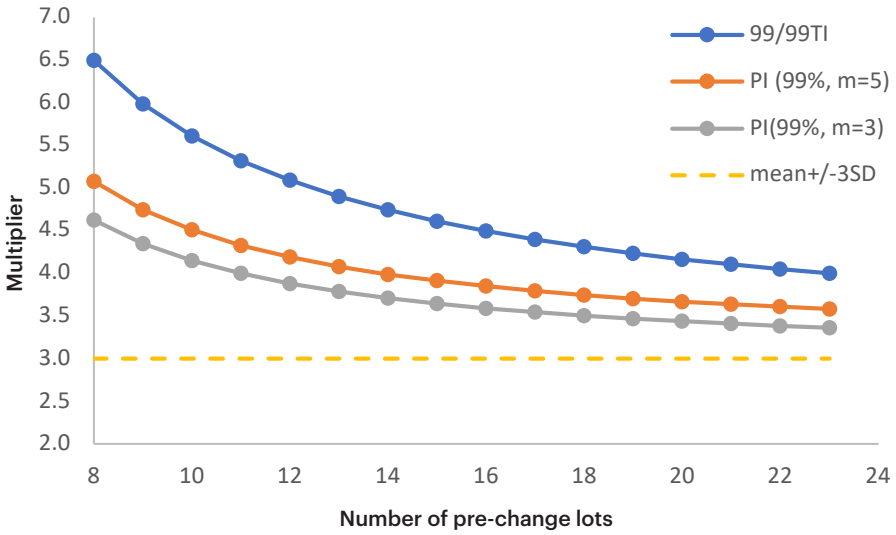
of the central student t distribution with $N-1$ degrees of freedom, and 1–α is called the confidence level of the interval, which represents the desired coverage probability to include all m post-change batches simultaneously.

This approach fits the process comparability data structure better in that 1) there is no requirement on the data balance (i.e., N can be much larger than m) and 2) it is the interval for individual future values and therefore can be used as pre-determined acceptance criteria for the assessment of the post-process changes once the pre-change data are available and the number of post-change batches to produce, m, is known. In most process comparability cases, $m>1$. Therefore, SPI is more often used than prediction interval. As shown in the equation above, both the number of pre-change batches (N) and the number of post-change batches (m) affect the width of the interval. Larger N leads to narrower SPI, while larger m leads to wider SPI. Having few batches and variable methods could result in very wide intervals that are not particularly informative for comparability.

Tolerance Interval (TI)

The tolerance interval (TI) is calculated as $\underline{x} \pm k * \sigma$ for normally distributed data and covers at least (100-α)% of the measurement population with (100-γ)% confidence. Here, $\underline{x}$ and σ are the sample mean and sample standard deviation, respectively. The multiplier k directly affects the width of the TI and it is determined by the prespecified confidence level (100-γ)%, the coverage level (100-α)%, and the sample size. α represents the specified target proportion of the population that is not covered in the interval, and γ represents the specified error rate in the calculated interval.[13] TI is generally calculated as the starting point for specification limit development because it covers almost all future data from the same population.[14] However, the typical TI with high confidence and high coverage level (e.g., >90%) tends to be too wide with a limited sample size.[15] Therefore, TI is not a recommended approach for biosimilar analytical similarity assessment.[16] It is generally not an appropriate approach for the typical process comparability setting for two reasons. First, similar to the situation with biosimilars, the TI tends to be too wide given that the number of pre-change batches is usually limited (e.g., <30). Figure 4 shows the multiplier for TI with 99% confidence and 99% coverage compared with the multiplier for SPI with 99% coverage for three or five post-change batches. The SPI

Figure 8-4. *Multiplier Comparison Between 95/99 TI and 99% SPI for 3 or 5 Post-Change Batches*



multiplier increases with the number of post-change batches. With the same number of pre-change batches, the 99/99 TI multiplier is larger than 99% SPI for 3 or 5 post-change batches. Second, the purpose of the comparability exercise is to determine whether the post-change batches generated at the time of comparability (usually no more than 4 or 5) are within the expected range based on the pre-change data trend. Therefore, it might be excessive to apply TI in the comparability setting with few post-change batches (sometimes only one).

Quality Range

The term "quality range" was first proposed by FDA statisticians to assess the analytical similarity between biosimilar and reference products for Tier 2 quality attributes.[17] It was adopted as the recommended statistical approach for both Tier 1 and Tier 2 quality attributes in 2019.[18] Quality range is defined as the mean ± k∗SD, which is the same form as the other statistical intervals described above. Conceptually speaking, quality range could be any of the above statistical intervals (CI, prediction interval, or TI). There is no detailed guidance on how to determine the multiplier *k* in practice, but mean ±3 SD is commonly used for biosimilar filings because of the relatively large sample size of the reference product.[19] However, SPI is more suitable for process comparability.

T-test

The T-test is a hypothesis test that is commonly used by researchers to establish whether data collected under two different conditions are significantly different. However, the T-test methodology sometimes is mistakenly used to establish comparability. In this situation, establishing similarity is based on not rejecting the null hypothesis that the two populations are the same. Although it seems like a logical approach, it is not. Failure to reject the hypothesis of sameness does not necessarily imply comparability. When the sample size (i.e., number of batches) is limited, there may be failure to reject the null hypothesis of sameness even with a relatively large difference. In contrast, the null hypothesis of sameness may be rejected with no clinically or practically meaningful difference if the sample size is large enough or the data variability happens to very small. Therefore, the T-test should not be used to demonstrate comparability of two populations.

Equivalence Test

In a situation in which additional batches and more information about the CQAs of interest are available, rigorous statistical methods can be applied. One of the most rigorous statistical methods is the equivalence test. Like the T-test, it is also a hypothesis test. However, unlike the T-test, the equivalence test is more suitable for comparability because it leads to a direct conclusion of "equivalent" or "not equivalent" based on a pre-set

acceptance criterion. A proper equivalence test requires a decent sample size and a quantitative scientific understanding of the clinical relevance. For instance, a minimum of 10 batches per group was suggested when this test was required for biosimilar analytical similarity in Tier 1 QA assessment.[20,21] In the process comparability setting, it can be used for testing several pre-change batches while there are few post-change batches available at the time of the comparability exercise. In addition, the acceptance criterion needs to be set prior to analyzing the data and ideally should be based on 1) the clinical relevance or clinically meaningful difference of the CQA and 2) the associated analytical and process variability. In practice, the first element is generally unknown or hard to quantify due to limited data or understanding. Without proper quantitative acceptance criterion, the equivalence test is practically meaningless, which is part of the reason that equivalence tests are no longer required for biosimilar similarity assessment by the FDA.[22] Generally, the equivalence test is not a practical approach for process comparability.

The appendix provides an example that illustrates the approaches described above.

Applying SPI as the Predetermined Acceptance Criteria

SPI has been proposed as a predetermined acceptance criterion to assess post-change batches. Although it is preferred to set $(1-\alpha)$ to a high number such as 99.7% (i.e., the same ideal coverage that mean ± 3 SD would provide with a large sample size), the width of SPI gets wider with higher $(1-\alpha)$ values. Due to the limited number of pre-change batches, $(1-\alpha)$=99% is recommended for N≥10 (i.e., at least 10 pre-change batches). Figure 2 provides an SPI with a 99% confidence level (99% SPI) compared with mean ± 3 SD based on 10 pre-change batches and 3 post-change batches. Although SPI can be calculated based on as few as 2 pre-change batches, it is recommended to apply SPI for N≥10. As shown in Figure 3, the SD estimate is subject to a much greater uncertainty for N<10.

As shown in Figure 2, a 99% SPI (orange line) is generally wider than mean ± 3 SD (i.e., the blue line) a realistic sample size (N). Table 4 lists the k values for both one-sided and two-sided limits for m=3. As the pre-change sample size (N) gets larger, the two-sided 99% SPI approaches the mean ± 3 SD. The multipliers shown in Table 4 are for two-sided 99% SPI. In some cases, only an upper or lower limit of the SPI is needed for certain CQAs, such as purity or impurity. In that situation, the user could choose to use one limit (either upper or lower limit) of the two-sided SPI, but the actual coverage of the limit is increased to 99.5%. Alternatively, the one-sided 99% SPI can be calculated using the one-sided k in Table 4. However, doing so could lead to multipliers <3 when N reaches 30, resulting in a narrower limit than mean ± 3 SD.

Note that the SPI here is used to represent the historical manufacturing range of the pre-change process given a decent number of pre-change batches. It does

Table 8-4. *The Multiplier k for Two-Sided and One-Sided SPI Based on N Pre-Change Batches for 3 Post-Change Batches*

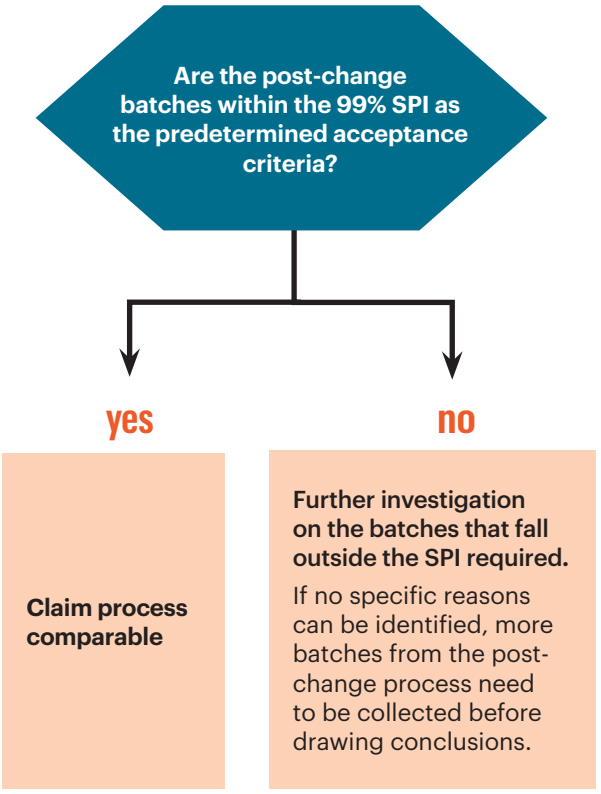
N	One-sided	Two-sided
3	14.07	19.95
4	7.54	9.59
5	5.66	6.85
6	4.81	5.67
7	4.34	5.02
8	4.04	4.62
9	3.83	4.35
10	3.68	4.15
11	3.56	4.00
12	3.47	3.88
13	3.40	3.79
14	3.34	3.71
15	3.29	3.65
16	3.24	3.59
17	3.21	3.54
18	3.17	3.50
19	3.14	3.47
20	3.12	3.44
30	2.97	3.25
50	2.86	3.12
100	2.79	3.02

not necessarily guarantee that SPI is always narrower than the release specification, which ideally reflects drug efficacy and safety. The post-change batches are expected to meet both specification and SPI criteria. Knowledge about the post-change process is often limited by the number of post-change batches, which sometimes can be as small as m=1. SPI with 99% confidence simply means that there is a 99% chance that the m post-change batches will fall within the SPI if there is no change between pre-change and post-change processes for the CQA of interest. However, the m post-change batches all falling within the SPI does not necessarily guarantee that there is absolutely no change between the pre-change and post-change processes. Rather, it indicates the lack of clear evidence or signal to claim that the post-change process is not comparable to the pre-change process. However, if one or more of the m post-change batches fall outside SPI, it is a signal to raise the alarm and consider an investigation. Although statistical assessment is an important input to decision making, the comparability decision should not be solely based on statistical analysis results. If one or more post-change batches fall outside the SPI, an investigation of the batches outside SPI is recommended. If no assignable cause is identified and there are few post-change batches (e.g., 2 or 3), more batches may be required prior to drawing conclusions (Figure 2).

Like most statistical methods, the SPI approach assumes that the CQA of interest follows a normal distribution. If it does not (e.g., some impurity measures), data may need to be transformed (a log transformation is often used to get data closer to normal distribution) prior to applying the SPI approach. Statisticians should be consulted in these situations.

The 99% SPI proposed here may be unrealistically wide if the number of pre-change batches is <10. However, it is very common to have frequent process changes and only 2 or 3 batches per process in the gene therapy field. One way to deal with this situation is to start with visual comparison for early process changes. If the first few processes (i.e., process 1 and process 2) are considered comparable, the data from these processes could be combined and treated together as from one historical process to meet the sample size requirement for the SPI calculation.

Figure 8-5. *Decision Process Using SPI to Assess the Comparability of Post-Change Batches*



It is also worth emphasizing that the SPI is proposed to deal with the imbalanced data structure in the typical process comparability exercise. If there are a decent number of pre-change batches and the number of future post-change batches is known, the SPI can be calculated as the pre-determined acceptance criteria. The data can be derived directly from the release test or from side-by-side testing. However, if the number of post-change batches is close to the number of pre-change batches or larger (e.g., N=10, m=10), the 99% SPI could become too wide due to the increase of the number of post-change batches. In such cases, there are two options to consider: lowering the confidence level of SPI; or using mean ± 3 SD if the number of pre-change batches is relatively large.

Summary of Statistical Test Approaches

As previously mentioned, not all quality attributes are relevant to the comparability assessment. Among relevant quality attributes, some may not be amenable for statistical assessment (e.g., qualitative quality attributes).

Assuming the quality attributes are relevant and amenable to statistical analysis, it is good practice to first apply visual assessment and descriptive statistics (e.g., mean, standard deviation, minimum, maximum, N) to the data. If ≥10 pre-batches are available, SPI is recommended to represent the historical range of the pre-change process. This approach assumes no mean shift and no variability change between pre-change and post-change processes. If there is a justifiable mean shift known in advance, the mean shift can be added to the sample average ($\underline{x}$) of the pre-change batches in the formula.

Like most comparability exercises, the available number of batches at the time of assessment remains one of the biggest challenges. However, as more information about the product, related process, and CQAs is acquired, the knowledge may be summarized quantitatively to form an "informative prior" as one of the key inputs for a Bayesian approach.[23] With reliable "informative prior" in place, the required number of pre-change batches decreases slightly without losing the reliability of the prediction interval. Although no such application has been observed, better and more use of historical data are becoming more feasible with the rapid development of digital solution transformations in data collection and accessibility.

In general, appropriately chosen statistical tools play a critical role in the process comparability assessment. However, the statistical conclusion can only be as good as the data at hand, and it should not serve as the only input for final regulatory decision-making; knowledge and information that are not contained in the data also need to be considered.

PROCESS VALIDATION FOR NEW PROCESSES

Manufacturers may argue that production of several batches at scale for the purpose of comparability should be sufficient evidence that the product can be manufactured with some degree of consistency. This argument is not necessarily adequate justification for not performing process validation studies after major manufacturing changes, particularly when the breadth of clinical data would be very limited.

For example, it may be sufficient to perform comparability studies for genetically modified cells using starting material collected from healthy donors with justification, but establishment of manufacturing consistency may require production of the final drug product from patient materials. For gene therapy products, one can argue that production of product consistently at full scale post-change is also required. This is particularly applicable if the product manufactured at full scale is sufficient to treat a small patient population. In this scenario, it is important for manufacturers to demonstrate not only comparability at scale that could be considered representative of full scale but also to show that the product at full scale can be manufactured consistently. The need for conducting process validation after a manufacturing change should be discussed with the agency prior to initiation of comparability studies because it could impact the design of the comparability study.

The 2011 *Guidance for Industry on Process Validation: General Principles and Practice*s provides the most updated guidance on the principles of process validation.[24] Process validation is documented evidence that a process will consistently produce product meeting pre-determined specifications. Validation takes time and can be expensive, but over the long-term it is good business because it diminishes the likelihood of batch failures.

Process validation = Consistent batches = Conforming batches

Process validation studies are generally not required for early-stage manufacturing, so most original IND submissions do not include a process performance qualification. It is recommended to use early-stage manufacturing experience to evaluate the need for process improvements and support future process validation studies. In addition, establishing process validation after making a manufacturing change is recommended. To show similarity between products pre- and post-change, three consecutive lots must be produced. All facility equipment qualification support systems, product specifications, and the process being validated must pass at all steps.

Gene therapy products may be produced in small quantities. Specific issues of concern in the validation of gene therapy viral vector manufacturing processes include the quality of raw materials, safety testing of cell and viral banks, production and purification of the vector, in-process and final-product testing, and

Table 8-5. *Stability Study Design*

Conditions	Stability-Indicating Assays					
	15 days	30 days	3 months	6 months	1 year	2 year
-80°C						
-60°C						
-20°C						

validation of analytical methods. Although risk can be reduced by proper facility and process design (e.g., use of single use systems), because most vectors are produced in multi-product facilities, cleaning validation may be a major concern due to potential product-to-product cross contamination. Viral clearance also presents a major validation challenge due to the nature of the product.

As development proceeds, analytical methodology and specifications should be evaluated and refined based on development data, engineering runs, and clinical batch analysis. Additional assays may also be added to monitor and control critical attributes of the product. ICH Q2R1 should be followed for late-phase assay validations. As the clinical program reaches pivotal trials, analytical methods and specifications should be based on a sufficient dataset to tighten the release and stability specifications in support of late-phase comparability and process validation activities. Health authority expectations increase considerably at this stage, and pharmaceutical quality and manufacturing control becomes a critical focal point of product development. This component is analogous with the term CMC used for small molecules.

For many gene therapy products, including AAV products, the application of principles for process validation outlined in the guidance document[25] is possible but may require innovative approaches such as rolling process validation (which is performed in very discrete steps) or unit operation or concurrent process validation (which is performed concurrently with the release of the final drug product).

STABILITY OF PRODUCTS MANUFACTURED USING A NEW PROCESS

Stability testing is a vital part of product development and is conducted throughout a product's life cycle. It is also a part of a biotherapeutic's quality target product profile (QTPP) and increases the understanding of how CQAs of DS and DP are influenced under specific conditions of temperature, relative humidity, light, storage, pH, and other factors. These studies are required to be conducted following the guidelines issued by the ICH, WHO, and/or other agencies.

Stability assessment for comparability has a specific purpose, which differs from the typical studies in the stability program. The product must be demonstrated to be stable for the period of time while stored at the storage site after manufacturing, and at the clinical site. For products formulated with carrier or support materials, the stability of the complex formed with the DS should be studied. Where relevant, the in-use stability of the DP (after reconstitution or after thawing) should be investigated, including its compatibility with any diluents used in reconstitution and if appropriate, devices used for administration. The recommended in-use time period should be justified. The impact of the transport conditions on the stability of DS or DP with a short-term shelf life should be considered. Stability protocols, stability data, justifications for the container-closure system used, and proposed shelf-lives and storage conditions, should be presented for the DS, DP, and DPI (i.e., intermediates for which a holding time is scheduled on the production process scheme). The need, extent, and type of stability studies depend on product development stage, product and process knowledge, extent of change, potential impact of the change on product CQAs, safety, and efficacy, and availability and capability of analytical methods (Table 5).

STABILITY DATA REQUIREMENTS ASSOCIATED WITH PROCESS CHANGES

The requirement for conducting stability studies requires careful analysis of how changes in manufacturing could impact short- and long-term stability of the DP. In general, for a gene therapy product with a long shelf life, batches manufactured using the new manufacturing

Table 8-6. *AAV Product Attributes and Predetermined Acceptance Criteria*

Attributes	Test	Method Procedure	Predetermined Acceptance Criteria	Priority
Purity (Vp1/Vp2/Vp3)	Measure capsid proteins	SDS-PAGE	X	High
Residual host DNA	Measure total DNA	qPCR	X	Medium
Replication-competent AAV	Measure replication-competent virus	qPCR	X	Medium
DNA identity	Transgene sequence	NGS	X	Medium
Vector concentration	Measure vg/mL	ddPCR	X	High
Vector infectivity	Measure infectivity	TCID50	X	Medium
Sterility	Measure of microbial contamination	Culture based		Mandatory
Endotoxin	Measure of endotoxin	LAL	X	Mandatory
Aggregation	Measure of aggregation	Drug Product	X	High
Full/empty capsid ratio	Measure of percent full	IEX-HPLC	X	High
Potency	Measure RNA, protein expression, or biological activity	Cell-based assay or preclinical model	X	High

process should be evaluated in short- and long-term stability studies sufficient to initiate the clinical study. The shelf life of batches released post change may be further extended. Stability-indicating assays include vector concentration, vector infectivity, potency, and measurement of other characteristics that could potentially be impacted during storage conditions.

The stability studies can also be performed in real-time, accelerated, or under stress conditions. Accelerated and stressed stability studies are not always possible and/or recommended. However, accelerated stability studies (e.g., at elevated temperatures or under other stress conditions relevant for the product of interest) may provide complementary supporting evidence for the stability of the product and help to establish the stability profile during temperature excursions. They are often useful tools to establish degradation rates and/or pathways, identify stability-indicating tests, and provide a direct comparison of pre-change and post-change product.

COMPARABILITY STUDY CONCLUSION

The conclusion of a comparability study should contain a detailed summary of the results collected with a bulleted conclusion. The conclusion drawn by the manufacturers should be articulated in a succinct and comprehensive format. In cases for which the data are not complete or the information collected is not supportive of establishment of comparability based on the predetermined acceptance criteria, the manufacturers are encouraged to include a section on risk assessment to better define the risk to product quality and define next steps/future plans.

Case Study

Let's consider a real-life case of an AAV product for which a significant manufacturing change is introduced. For the purpose of this case study, the AAV manufacturing platform will be changed from an adherent to a suspension culture to be conducted in a new facility. This change is being introduced very late during the product development cycle such that the IND holder cannot generate sufficient clinical data to assess the product effectiveness prior to licensure.

First, the most relevant attributes to the study should be determined, as well as the methods and procedures that should be used to measure these attributes. A

Table 8-7. *AAV Product Attributes, Number of Batches Tested, Predetermined Acceptance Criteria*

Attributes	Method Procedure	Predetermined Acceptance Criteria	Batch* Before Change	Batch* Before Change	Batch 1	Batch 2	Batch 3	Results (pass or fail)
Purity (VP1/Vp2/Vp3)	SDS-PAGE	Min-max range						
Residual host DNA/ protein	qPCR	X						
Replication-competent AAV	qPCR	X						
DNA identity	NGS	X						
Vector concentration	dd-PCR	X						
Vector infectivity	TCID50	X						
Sterility	Culture-based							
Endotoxin	LAL	X						
Aggregation	Drug Product	X						
Full/empty capsid ratio	IEX-HPLC	X						
Potency	Cell-based assay	X						

*Batches represent product used in clinical studies prior to implementing major manufacturing changes. The testing of different attributes should be performed if possible, using sample retained from old batches and compared to the new batches using the same method under identical conditions.

risk- and science-based approach allows prioritization of the relevant attributes, which may include biological activity, potency, identity, and purity. These attributes are key elements of comparability studies along with standard safety tests such as sterility and endotoxin. Based on the risk assessment and assay considerations, it is possible to select a subset of release tests to evaluate comparability with the highest relevance to product quality and effectiveness. These tests must be performed on the final DS/DP, but it is also important to monitor the process by conducting additional in-process testing (which could be a subset of the tests described here). Table 6 summarizes an example of a prioritized list of attributes to be measured, testing methods, procedures, and predetermined acceptance criteria. Table 7 provides a summary of the results collected for each attribute for several batches and indicates whether the results met the predetermined acceptance criteria.

If the pre- and post-change products exhibit characteristics that are similar based on the pre-determined acceptance criteria and statistical methods, the manufacturer could argue that the product quality is not affected by the process change. However, if the product is not shown to be similar in one or two out of 10 parameters measured, for example, manufacturers may consider conducting a risk assessment to determine the impact of such excursions on product quality. The risk assessment must take into account several factors, for example, attribute criticality and product efficacy, when predetermined acceptance criteria are not met. Further, it is important to determine the frequency failure observed in the test and whether there is a known reason for the observed failure that could be related to operator training or excursions in test methods and procedures.

POSSIBLE SCENARIOS

If the comparability study results are not sufficient to establish comparability based on the *in vitro* results, it is possible that the agency would ask for additional information. Because a major change is introduced late during

Table 8-8. *Possible Outcomes for Introducing Major Manufacturing Changes After Completion of Pivotal Study*

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
***In vitro* analytical similarity**	X	X	X	X	X	X
Preclinical analytical similarity		X	X	X	X	X
Bridging clinical study before licensure			X		X	
Post-marketing clinical requirement				X		
Additional pivotal study before licensure						X

the product life cycle in this hypothetical case, the agency may ask for additional *in vitro* studies, establishment of comparability in relevant animal models, and establishment of comparability based on additional clinical studies prior to or after licensure. Agreement with the agency on the comparability approach and study design does not bind the agency to accept the results as adequate if the results do not meet the quality standards. For gene therapy products, the use of animal models to establish product comparability is generally highly recommended if available and should be included in the proposed study. In some cases, the preclinical establishment of comparability may not be sufficient, and the agency may require additional clinical data to establish product comparability (e.g., small bridging study). In general, additional small bridging clinical studies are not meant to demonstrate efficacy using the newly manufactured commercial product but are focused on establishing safety and perhaps limited effectiveness based on patient outcome and/or surrogate biomarkers. It should be noted that if the treatment effects are high and easily detectable, then it may be possible to demonstrate effectiveness by treating a small number of patients. However, if the treatment effects are not as apparent, then additional clinical studies may need to be performed prior to or after licensure as part of post-marketing requirements to verify the final drug product effectiveness using the newly manufactured commercial product. In some scenarios, a small bridging study prior to licensure or post-marketing studies may not be sufficient or informative to establish clinical comparability, thus requiring a stand-alone clinical study prior to licensure. The regulatory scenarios discussed here are purely hypothetical, and manufacturers are encouraged to have early discussions with the agency and obtain clarification from the agency regarding their expectations of comparability results and possible scenarios (see Table 8 for possible scenarios).

Table 8 summarizes possible outcomes of introducing major manufacturing changes using worst-case scenarios. The requirement for establishing comparability is shown for a hypothetical gene therapy product. In this scenario, the product is undergoing a major change after the pivotal or licensing trial has been completed.

Conclusion

To date, a significant amount of critical insights into AAV vectors as tools for gene delivery have been gathered through preclinical and clinical studies. We now know that the AAV therapeutic platform has the ability to express a given transgene at therapeutic levels for multiple years, potentially representing a cure for chronic genetic diseases. Although safety must remain the overarching goal for the field, achieving therapeutic efficacy in a consistent manner in adults and pediatric patients will likely be essential for gene therapies to become competitive with other approaches that are emerging as treatment modalities for genetic diseases. To meet the challenge, many aspects of AAV biological properties in the context of the human host, such as AAV vector immunogenicity, therapeutic potency, persistence, and potential genotoxicity, will have to be

further elucidated. Even though preclinical animal models cannot be used to accurately predict the outcome of gene transfer in humans, they will continue to be essential for the development of highly optimized gene therapy drugs.

In this chapter, we have discussed that major changes in the manufacturing of gene therapy products are an inevitable part of process improvement. As such, manufacturers should have a well-defined plan to introduce manufacturing changes and establish product comparability. A comparability plan should be developed as early as possible in product development, preferably before a phase 1 trial. The acceptability of comparability depends on a large number of factors, including but not limited to knowledge of the product, availability of well-defined CQAs that are informative for product safety and efficacy, and timing of when the major change is introduced in the product development cycle. If new methods are implemented during development, retaining samples for retesting is important for identifying any important changes to product quality or efficacy, as well as to determine whether new analytical methods will be required to detect changes not accounted for by old analytical methods. Ultimately, the more predictive or informative analytical parameters that are included when measuring product quality and efficacy, the less likely it is that bridging clinical studies will be required. For this reason, it is strongly recommended that manufacturers introduce major manufacturing changes early during the development cycle. If this is not possible, manufactures should anticipate the need for well-qualified assays and highly relevant *in vitro* and preclinical measurement tools to establish product comparability during later phases of clinical study.

Appendix

ILLUSTRATION OF THE DIFFERENT STATISTICAL APPROACHES FOR COMPARABILITY

Process changes often occur during gene therapy product development. In the following hypothetical example, the process has changed four times and results in five processes from P1 to P5 (Table 9). The number of batches per process ranges from 1 to 4. The potency is not expected to be affected by these process changes. Therefore, all the potency values are randomly generated by computer from the same normal distribution with mean 100% and standard deviation of 10%. Therefore, 99.73% of the potency values are expected to fall within (70%, 130%).

Table 8-9. *Simulated Potency Data from Five Processes*

Batch	Process	Potency (%)	Average
1	1	97	97
2	2	120	104
3	2	88	
4	2	103	
5	3	98	97
6	3	96	
7	4	102	103
8	4	93	
9	4	107	
10	4	108	
11	5	83	96
12	5	111	
13	5	95	

Visual Assessment

The potency values from 5 processes that overlap with each other quite well (Figure 6) indicate that these processes appear to be comparable in potency. Note that if the comparison were made prior to Process 3, the highest value from Process 2 (120) would likely cause unnecessary concern. This type of situation often occurs when the sample size (i.e., number of batches per process) is limited.

Statistical Assessments

As discussed previously, formal statistical approaches should be applied only when the number of batches reaches ≥10. In this case, the total number of the first 4 processes is 10. Therefore, the first 10 batches can be treated as pre-change batches to compare with the batches from Process 5, the post-change process. Two types of statistical approaches were introduced in the

statistical section: 1) statistical intervals (e.g., min/max, confidence interval [CI], mean ± 3 SD, simultaneous prediction interval (SPI), and tolerance interval (TI)) and 2) hypothesis testing (e.g., T-test and equivalence test).

Min/max, mean ± 3 SD, SPI, and TI are calculated based on the first 10 batches and the number of post-change batches (3) and are presented in Table 10 and Figure 7. Note that 99% represents the coverage level. If these intervals are used as the comparability criteria, all batch values from Process 5 are expected to fall within these intervals. However, Batch #11 from Process 5 fails the min/max criteria. It is worth noting that even though Process 5 passes the mean ± 3 SD criteria, there is a fair chance that it could also fail the mean ± 3 SD criteria because this interval is narrower than the true mean ± 3 SD, which is (70%, 130%). Even though it is wider than the true mean ± 3 SD, SPI is a good compromise between the often overly wide TI and the often overly narrow min/max and mean ± 3 SD based on the limited sample size.

The focus of the CI approach is based on the comparison between the means of the pre- and post-change process. In this case, the CI of the mean difference between the pre- and post-change processes can be calculated using the following formula assuming that variability is not affected by the process change:

$$\left(m_{post} - m_{pre}\right) \pm t \times s \times \sqrt{\frac{1}{n_{pre}} + \frac{1}{n_{post}}}$$

where m_{post} and m_{pre} are means of the post- and pre-change process and n_{pre} and n_{post} are the corresponding sample sizes. In this case, n_{pre}=10 and n_{post}=3. t is the multiplier based on central t-distribution and sample size and corresponding confidence level. s is the pooled standard deviation of the two processes. The 99% CI of the mean difference is (-26%, 16%), which indicates the range that the true mean difference between the processes is very likely fall within. It is up to the expert to defend or justify whether the potential difference is small enough to claim comparability.

The T-test and equivalence tests are both hypothesis tests that focus on mean comparison, but have opposite goals. The T-test evaluates whether pre- and

Figure 8-6. *The Potency (%) Value for Batches from Different Processes*

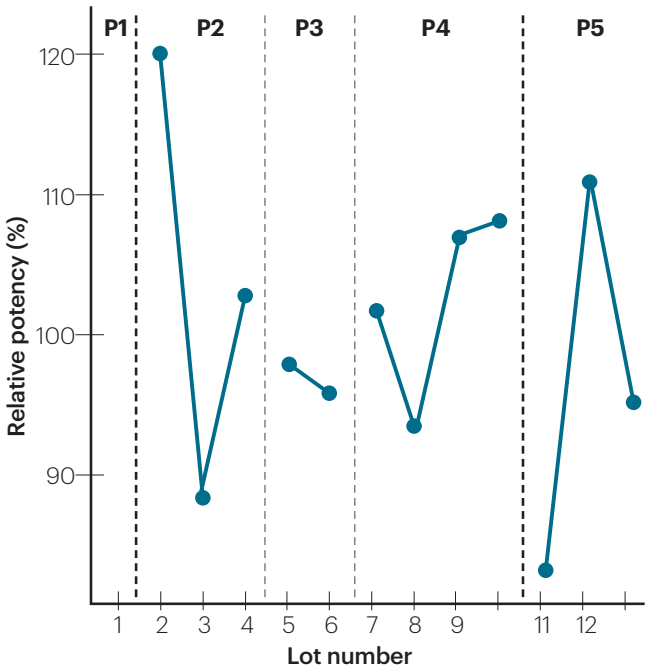


Figure 8-7. *Potency for Each Batch with the Different Statistical Intervals*

Based on the Pre-change Processes (P1 to P4) and the Number of Batches in the Post-change Process (P5)

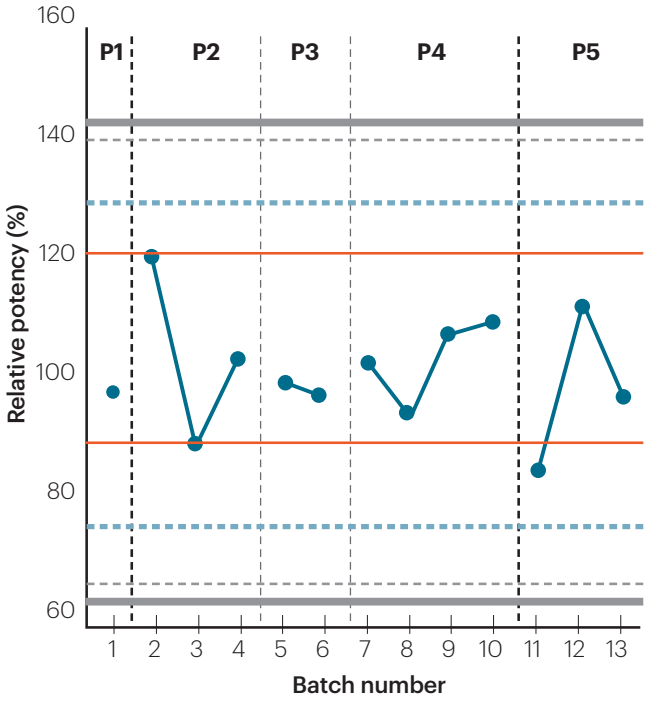


Table 8-10. *Statistical Intervals Based on the First 10 Batches and Number of Post-Change Batches*

Statistical Intervals	Result (%)	Do Process 5 Batches Fall Within the Interval?
Min/max	(88, 120)	No
Mean ± 3 SD	(74, 128)	Yes
99% SPI	(64, 139)	Yes
TI with 99% coverage and 95% confidence	(61, 142)	Yes

post-change processes are significantly different. The equivalence test evaluates whether the difference between the two processes is within a preset acceptable limit, often called equivalence margin. The equivalence margin must be set prior to data collection. Assuming equal variance, the p-value of the T-test is 0.4807, indicating that the difference is not statistically significant.

The equivalence test is equivalent to comparing the CI of the mean difference with the equivalence margin. If the confidence interval (usually with 90% confidence level) is fully within the equivalence margin, then a claim of "equivalence" can be made. If the equivalence margin is set as ±10%, then the 90% CI of the mean difference is (-17%, 7%) and it falls outside the equivalence margin. Therefore, Process 5 does not pass this equivalence criterion, which conflicts from the truth that all the batches are from the same distribution.

Abbreviations

AAV	Adeno Associated Virus
BLA	Biologics License Application
CBE	Changes Being Effected
CBER	Center for Biologics Evaluation and Research
CFR	Code of Federal Regulations
cGMP	Current GMP
CI	Confidence Interval
CMC	Chemistry Manufacturing and Controls
CMO	Contract Manufacturing Organization
CPP	Critical Process Parameters
CQA	Critical Quality Attributes
ddPCR	Droplet Digital PCR
DNA	Deoxyribonucleic Acid
DP	Drug Product
DPI	Drug Product Intermediate
DS	Drug Substance
DSI	Drug Substance Intermediate
EMA	European Medicines Agency
FDA	Food and Drug Administration
GDUFA	Generic Drug User Fee Act
GMP	Good Manufacturing Practice
ICH	International Council for Harmonisation
IND	Investigational New Drug
KPP	Key Process Parameters
MOI	Multiplicity of Infection
PAS	Prior Approval Supplement
PCR	Polymerase Chain Reaction
QA	Quality Assurance
QbD	Quality by Design
QTPP	Quality Target Product Profile
RNA	Ribonucleic Acid
SD	Standard Deviation
SPC	Statistical Process Control
SPI	Simultaneous Prediction Interval
TI	Tolerance Interval
USP	United States Pharmacopeia
WHO	World Health Organization

Endnotes

1. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). ANDA submissions – prior approval supplements under GDUFA: guidance for industry. U.S. Food and Drug Administration website. https://www.fda.gov/media/89263/download. Published October 2017. Accessed September 18, 2020.*

2. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Chemistry, manufacturing, and controls changes to an approved application: certain biological products: draft guidance for industry. U.S. Food and Drug Administration website. https://www.fda.gov/media/109615/download. Published December 2017. Accessed September 18, 2020.*

3. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Chemistry, manufacturing, and controls changes to an approved application: certain biological products: draft guidance for industry. U.S. Food and Drug Administration website. https://www.fda.gov/media/109615/download. Published December 2017. Accessed September 18, 2020.*

4. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs). U.S. Food and Drug Administration website. https://www.fda.gov/media/109615/download. Published January 2020. Accessed September 18, 2020.*

5. *Alliance for Regenerative Medicine. ARM-USP Workshop Comparability in Cell & Gene Therapies Final Report & Summary. Rockville, MD: May 31, 2019.*

6. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for industry Q5E comparability of biotechnological/biological products subject to changes in their manufacturing process. U.S. Food and Drug Administration website. https://www.fda.gov/media/71489/download. Published June 2005. Accessed September 22, 2020.*

7. *Gavin DK. Advanced topics: successful development of quality cell and gene therapy products. U.S. Food and Drug Administration website. https://www.fda.gov/media/80404/download. Accessed September 22, 2020.*

8. *Burdick RK, LeBlond DJ, Pfahler LB, et al. Statistical Applications for Chemistry, Manufacturing and Controls (CMC) in the Pharmaceutical Industry. Basel, Switzerland: Springer International Publishing AG; 2017.*

9. *Dong X, Tsong Y, Shen M. Statistical considerations in setting product specifications. J Biopharm Stat. 2015;25(2):280-294.*

10. *European Medicines Agency. Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development. EMA website. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-reflection-paper-statistical-methodology-comparative-assessment-quality-attributes-drug_en.pdf. Published March 23, 2017. Accessed September 22, 2020.*

11. *Burnett R. Accurate estimation of standard deviations for quantitative methods used in clinical chemistry. Clin Chem. 1975;21(13):1935-1938.*

12. *Hahn GJ, Meeker WQ. Statistical Intervals: A Guide for Practitioners. New York, New York: John Wiley & Sons; 1991.*

13. *Hahn GJ, Meeker WQ. Statistical Intervals: A Guide for Practitioners. New York, New York: John Wiley & Sons; 1991.*

14. *Burdick RK, O'Neill JC. The Goldilocks challenge–controlling uncertainty when setting product specifications. PDA J Pharm Sci Technol. 2020;74(4):439-445.*

15. *Dong X, Tsong Y, Shen M. Statistical considerations in setting product specifications. J Biopharm Stat. 2015;25(2):280-294.*

16. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Development of therapeutic biosimilars: comparative analytical assessment and other quality-related concerns: guidance for industry. U.S. Food and Drug Administration website. https://www.fda.gov/media/125484/download. Published May 2019. Accessed September 22, 2020.*

Endnotes, continued

17. *Tsong Y, Shen M, Dong X. Development of statistical approaches for analytical biosimilarity evaluation. Presented at DIA/FDA Statistics Forum 2015. North Bethesda, MD: April 20-22, 2015.*

18. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Development of therapeutic biosimilars: comparative analytical assessment and other quality-related concerns: guidance for industry. U.S. Food and Drug Administration website. https://www.fda.gov/media/125484/download. Published May 2019. Accessed September 22, 2020.*

19. *Gutka HJ, Yang , Shefali K. Statistical considerations for demonstration of analytical similarity. In: Biosimilars: regulatory clinical and biopharmaceutical development. Berlin, Germany; Springer: 2018.*

20. *Tsong Y, Shen M, Dong X. Development of statistical approaches for analytical biosimilarity evaluation. Presented at DIA/FDA Statistics Forum 2015. North Bethesda, MD: April 20-22, 2015.*

21. *Tsong Y, Shen M, Dong X. Equivalence margin determination for analytical biosimilarity assessment. Presented at IABS Workshop. Rockville, MD: June 2015.*

22. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Development of therapeutic biosimilars: comparative analytical assessment and other quality-related concerns: guidance for industry. U.S. Food and Drug Administration website. https://www.fda.gov/media/125484/download. Published May 2019. Accessed September 22, 2020.*

23. *Cheng A, Wang K. Process comparability: setting appropriate acceptance criteria at late stage/post approval for biological products. Presented at: Midwest Biopharmaceutical Statistics Workshop; May 20-22, 2019: Carmel, IN.*

24. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Veterinary Medicine (CVM). Guidance for industry – process validation: general principles and practices. U.S. Food and Drug Administration website. https://www.fda.gov/media/71021/download. Published January 2011. Accessed September 22, 2020.*

25. *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Veterinary Medicine (CVM). Guidance for industry – process validation: general principles and practices. U.S. Food and Drug Administration website. https://www.fda.gov/media/71021/download. Published January 2011. Accessed September 22, 2020.*