November 16-17, 2023

Workshop on Gene Therapy Product Activity & Comparability & the Evaluation of T-Cells Slides

STANDARDS COORDINATING BODY

REGENERATIVE MEDICINE

Standards Recognition Program for Regenerative Medicine Therapies

Judith Arcidiacono M.S.

International Regulatory Expert

Lead for Regenerative Medicine Therapies Standards Program

FDA/CBER/Office of Therapeutic Products

Workshop for the Identification and Standardization of Methods for Assessing Gene Therapy Product Activity and Comparability, and the Evaluation of T Cell Therapies

November 16 – 17, 2023

Benefits of Standards Use for Regenerative Medicine Therapies

FDA

- Help facilitate consistent and predictable product manufacturing and assessment, field testing, clinical trial data exchange, and product labeling
- Foster innovation and support R&D
- Reduce R&D costs by building on existing standardized technologies
- Eliminate redundancy, minimizing errors and reducing time to market
- Improve quality, lead-time, factory flexibility, and supply chain management
- Can streamline premarket review by FDA

Standards Basics

Definition of a Standard

U.S. National Technology Transfer and Advancement Act of 1995

https://www.nist.gov/standardsgov/national-technology-transfer-and-advancement-act-1995

Common and repeated use of rules, conditions, guidelines or characteristics for products or related processes and production methods, and related management systems practices

Written/Documentary Standards

- Documents that set forth:
  - Performance characteristics
  - Testing methodology
  - Manufacturing practices
  - Scientific protocols
  - Ingredient specifications
  - Data standards
  - Terminology/Nomenclature
  - Others

Physical Standards/Reference Materials

Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

ISO REMCO Committee on Reference Materials

Voluntary Consensus Standards Body (VCSB)

Develop voluntary consensus standards

Characteristics of VCSB Processes

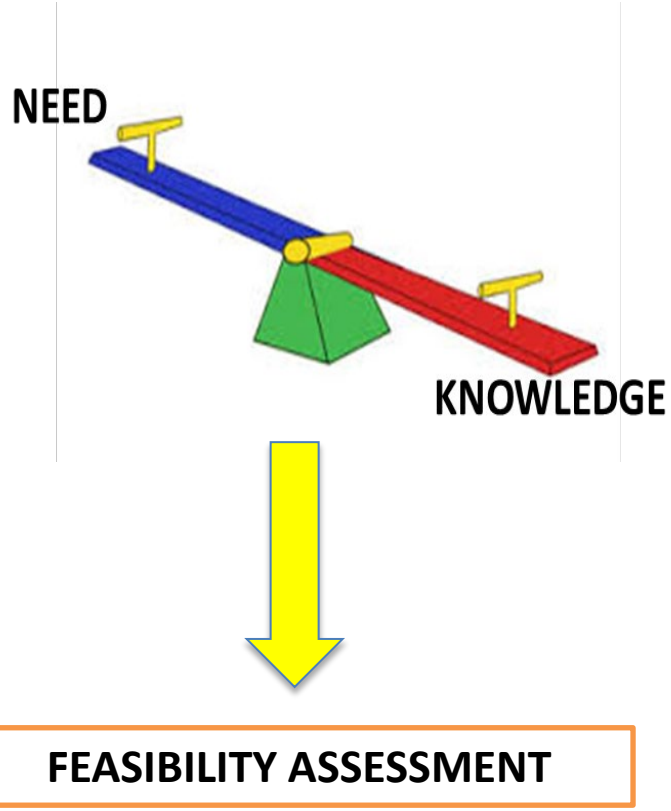
1. Openness
2. Balance
3. Due Process
4. Appeals Process
5. Consensus

OMB Circular A-119

7

Balancing the Need for a Standard with the State of Science

Does the base of scientific knowledge on the subject support the development of standardized approaches to methods, testing, etc. ?



Is there consensus among the scientific community that the approaches proposed are appropriate to address the need for standardization?

Feasibility Assessment

Considerations

1. What are the possible intended and unintended consequences?
2. How does the proposed standard effect existing work?
3. Are there other efforts to develop a specific standard in other standards venues?
4. Are there experts available to draft the standard?
5. How would the standard be implemented?

Standards Recognition Program for Regenerative Medicine Therapies (SRP-RMT)

Voluntary Consensus Standards Recognition Program for Regenerative Medicine Therapies

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
October 2023

OMB control number: 0910-0338
Current expiration date available at https://www.reginfo.gov.
See additional PRA statement in Section VIII of this guidance.

Table of Contents

What is the SRP-RMT?

- A program designed to identify Voluntary Consensus Standards (VCS) that facilitate the development and assessment of regenerative medicine therapy products regulated in the FDA Center for Biologics Evaluation and Research (CBER). https://www.fda.gov/media/159237/download
- The program fulfills requirements outlined in Section 3036 of the 21st Century Cures Act of 2016 where FDA, the National Institute of Standards and Technology (NIST), and RMT stakeholders coordinate and prioritize the development of standards that promote the development of RMTs, promote regulatory predictability and enhance regulatory review of submissions for RMTs.
- Consistent with US policy for standards (OMB Circular A-119[1] and NTTAA[2]) for promoting the use of VCS by the Federal Government.

[1] https://obamawhitehouse.archives.gov/sites/default/files/omb/inforeg/revised_circular_a-119_as_of_1_22.pdf.
[2] https://www.nist.gov/standardsgov/national-technology-transfer-and-advancement-act-1995.

Voluntary Consensus Standards Body (VCSB)

- Qualities of VCSB
  - Processes the follow openness, balance, consensus, and due process
- American National Standards Institute (ANSI)
  - Accredits Standards Development Organizations that adhere to these principles
  - Examples:
    - ATCC- American Type Culture Collection,
    - ASTM- American Society for Testing Materials
    - IEEE- Institute of Electrical and Electronics Engineers
    - PDA- Parenteral Drug Association
    - CLSI- Clinical and Laboratory Standards Institute

Non-Voluntary Consensus Standards

Pharmacopeial standards

- Examples: US Pharmacopeia, Japanese Pharmacopeia, European Pharmacopeia

Accreditation standards

- Standards set forth by accreditation organizations to ensure that certain criteria are met for a specified process or system.
- Examples: Foundation for the Accreditation of Cellular Therapy (FACT), Association for the Advancement of Blood & Biotherapies (AABB)

Standards created by institutions or societies

- Examples: International Society for Stem Cell Research (ISSCR), International Society for Cell & Gene Therapy (ISCT)

Benefits of the SRP-RMT

Promote	Identify	Assist
Promote the development of standards that can streamline the review of RMT products	Assist product developers in identifying standards that have been reviewed by FDA for scientific soundness and consistency with FDA regulations and policies.	Assist FDA reviewers in evaluating the proper use of a standard (fit-for-purpose) in a regulatory submission

How will standards be identified for consideration in the recognition program?

FDA staff serving as liaisons to SDOs can nominate standards to be reviewed for recognition

Stakeholders may request recognition by emailing the SRP-RMT at: SRP-RMT@fda.hhs.gov

- Stakeholders should provide the name of the SDO, standard designation, title, version and year published and a short rationale for recognition

Who will evaluate the VCS for Recognition?

FDA subject matter experts will evaluate standards for:

- Complete Recognition- the entire contents of the standard is recognized
- Partial Recognition- only portions of the standard are recognized
  - FDA will identify the section(s) of the standard that are recognized.
- Standards that do not meet the criteria for recognition will not be recognized.
- Recognition is NOT required to use a standard in a regulatory submission.

Criteria for Evaluating Standards for Recognition

- The standard was developed by a VCSB
- The standard does not conflict with current FDA statute, regulations, or policy
- The standard is scientifically sound
- The standard may facilitate the ability of a sponsor to meet regulatory expectations
- The standard can assist FDA in the assessment of a regulatory submission for RMT products

How will stakeholders know if a standard has been recognized?

- Recognized standards will be posted on the Standards Development for Regenerative Medicine Therapies page of the FDA website twice/year https://www.fda.gov/vaccines-blood-biologics/standards-development-regenerative-medicine-therapies
- Recognized standards will be accompanied by a Standards Recognition Sheet (SRS) that defines the terms of recognition
  - Components of the SRS:
    - CBER Assigned Recognition Number
    - Designation/title/scope of the standard
    - Extent of recognition (complete or partial)
    - Rationale for recognition
    - Name of the Standards Development Organization

Sample Standards Recognition Summary

CBER Recognized Standards for Regenerative Medicine Therapies
Standards Recognition Summary (SRS)

Recognition Number (CBER Assigned) **Date of Recognition:**

Standard Information
ISO XXXX, Edition YEAR

Scope/Abstract

Extent of Recognition (Complete or Partial)
(For partial recognition CBER intends to identify the parts of the standard that are not recognized)

Rational for Recognition
(Ex. This standard is relevant to the characterization of CAR T cells and supports existing regulatory policy.)

Standards Development Organization
(Ex. ISO International Organization for Standardization https://www.iso.org)

Use of a Standard in a Regulatory Submission

- Standards use is NOT required.
- Non-recognized standards may be used if fit-for-purpose.
- Use of a standard does not preclude FDA from asking for additional information to support the regulatory evaluation of a product.
- Standards that do not meet the definition of voluntary consensus standard may be used.
- When citing a standard, the following is required:
  - Name of the SDO
  - Designation and title
  - Version and date published
  - Statement of conformity
    - Used standard as written
    - Modified the standard- description of deviations from the standard and rational for deviation

Example of Citation of a Standard in a Gene Therapy Regulatory Submission

Standard*: GTX 1234:Quantification of Nucleic Acids in a Biological Sample; 2021**

Possible sponsor statements in a submission:

Methods used to quantify nucleic acids in the final product were conducted according to GTX 1234; 2021 without deviation.

OR

Standard GTX 1234; 2021 was utilized for the quantification of nucleic acids except that the method of sample preparation was modified to be more suitable for our manufacturing conditions.

*The standard referred to in this example is hypothetical

Take Home Message

- Standards use is not required
- Non-recognized standards can be used in a regulatory submission
- When used, a standard must be fit for purpose
- Stakeholders may request a standard be reviewed for recognition by emailing SRP-RMT@fda.hhs.gov
- List of Recognized Standards https://www.fda.gov/vaccines-blood-biologics/standards-development-regenerative-medicine-therapies

THANK YOU
FOR
YOUR
ATTENTION
ANY QUESTION?

Judith Arcidiacono, M.S.
International Regulatory Expert
US Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Judith.Arcidiacono@FDA.HHS.GOV

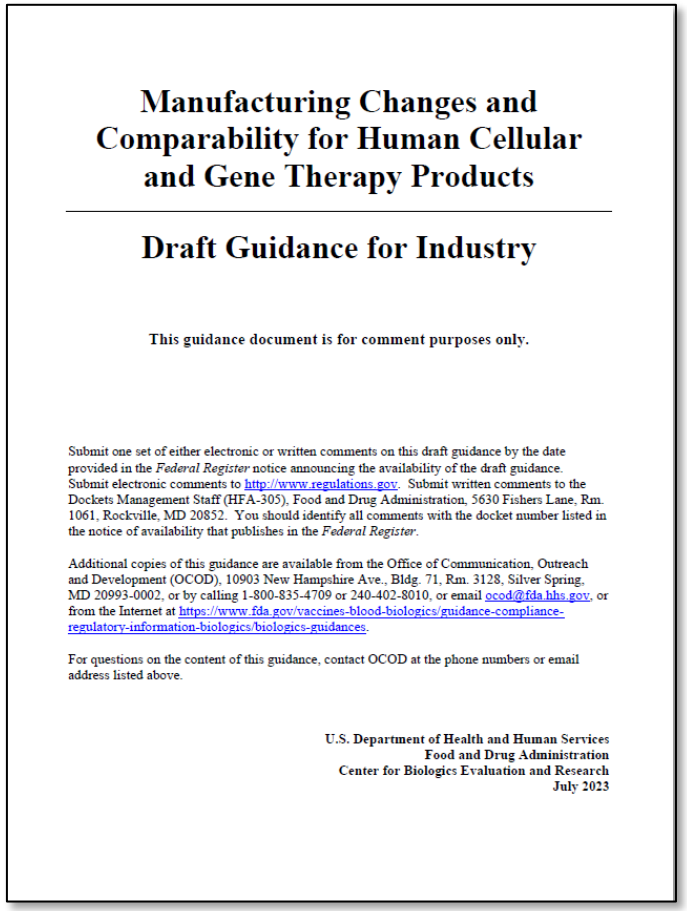
U.S. FOOD & DRUG ADMINISTRATION

Comparability and the management of manufacturing changes for cellular and gene therapy products

Anurag Sharma, Ph.D.
Acting Team Lead, Gene Therapy CMC
Division of Gene Therapy 1
Office of Therapeutic Products
FDA Center for Biologics Evaluation and Research

Nov 16, 2023

2023 Draft Guidance
Manufacturing changes and comparability



Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
July 2023

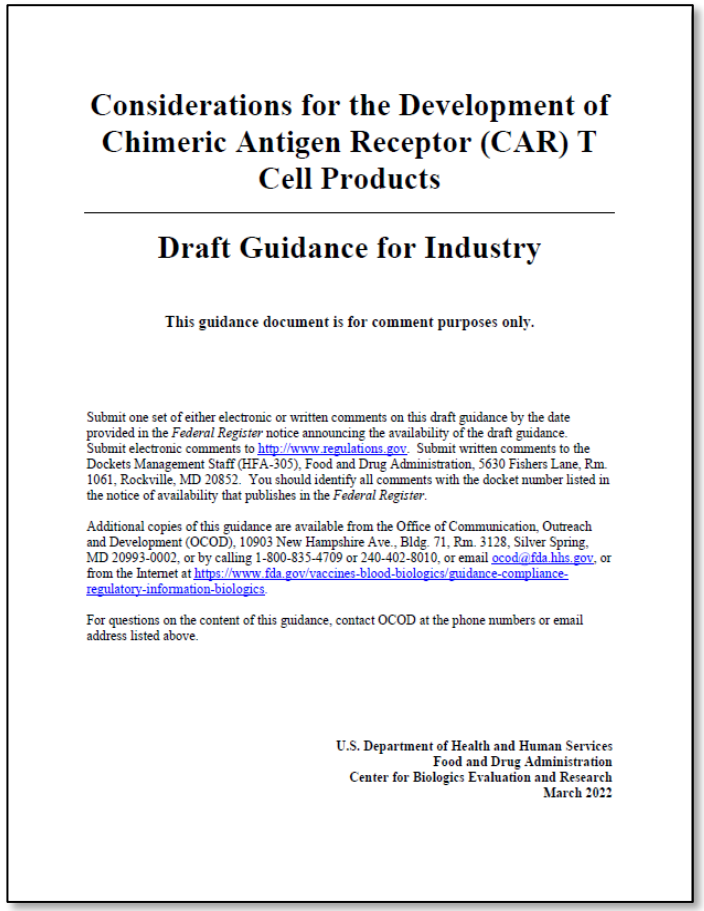
This draft guidance document is issued for comment purposes only

You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations

Provide advice for manufacturers of human cellular and gene therapy products regarding:

- Managing manufacturing changes and reporting the changes to FDA
  - For both investigational and licensed products
- Analytical comparability studies
  - Special considerations for cellular and gene therapies
  - Comparability study design and statistical approaches

2022 Draft Guidance
Chimeric Antigen Receptor T cell products



Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products

Draft Guidance for Industry

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
March 2022

Provide advice for manufacturers of CAR T cell products regarding:

- Manufacturing of vectors and CAR T cells
  - Including advice on change management and comparability
  - Manufacturing a CAR T cell product at multiple different facilities
- Preclinical recommendations
- Clinical recommendations

This draft guidance document is issued for comment purposes only

You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations

Broad themes and highlights

Risk management

Planning for future changes

Phase-dependent expectations

Comparability studies

Obtaining advice and feedback from FDA

OUTLINE

Managing manufacturing changes

Reporting manufacturing changes to an IND or BLA

Assessing comparability

MANAGING MANUFACTURING CHANGES

Common reasons for manufacturing changes

Improving product quality

Improving efficiency or reducing costs

Adjusting to changes in the availability of materials

Expanding product supply

- Scale up
- Scale out
- New facility

Risk management

The risk of a significant manufacturing change can be high for cellular and gene therapies

- These are complex and novel products
- Risk management relies on a clear understanding of critical aspects of the product and manufacturing process

Use a formal risk management strategy

- Will enable you to evaluate manufacturing changes effectively and efficiently
- Will aid when deciding whether a comparability study is needed
  - And will guide how to design the comparability study

We recommend *Q9(R1) Quality Risk Management* for advice on how to systematically manage risk

Manufacturing changes can pose risks to product quality

It is critical that manufacturing changes do not adversely affect product quality

Changes cannot compromise the safety or effectiveness of the product

Perform a risk assessment before making manufacturing changes

Risk assessment plays a central role in quality risk management

If a change has a potential to adversely affect product quality, determine the impact of the change

Perform comparability studies to evaluate any adverse effects of the change on product quality

Phase-dependent considerations when making manufacturing changes

The extent of comparability data needed is highly dependent on:

- The stage of clinical development
- The severity and likelihood that the change might adversely affect product quality

Comparability studies and statistical approaches should typically be more rigorous later in the product lifecycle

- Changes in the middle of a pivotal study
- Changes right before a BLA submission
- Changes post-licensure

Best practices

- Develop a thorough understanding of the product's quality attributes and how the manufacturing steps affect these attributes
- When possible, implement any extensive changes before initiating pivotal studies

Challenges in managing manufacturing changes for cellular and gene therapy products

Challenges when assessing risks

- Limited characterization of the product and the manufacturing process
- Uncertain mechanisms of action and difficulty measuring potency

Challenges for comparability studies

- Variable cellular source material
- Limited cellular source material
- Limited number of lots
- Small lot size or limited sample volume
- Changes in assays

Planning ahead for changes

Plan ahead to reduce risks and disruptions from future manufacturing changes

- Develop a scalable process
- Retain sufficient samples of all lots
- Manufacture a sufficient number of lots to support future comparability studies
- Understand how changes to assays might affect your ability to evaluate comparability

REPORTING MANUFACTURING CHANGES TO AN IND OR BLA

Reporting manufacturing changes to INDs

Submit manufacturing changes that may affect product quality

- Update CMC information using IND amendments
- Submit CMC information well in advance of implementing the change
  - To allow sufficient time for FDA review and feedback
- Also summarize any significant manufacturing changes in your IND's annual report

An IND may be placed on clinical hold if:

- You make a manufacturing change with a potential adverse impact on safety or effectiveness, but you do not adequately evaluate the impact of the change

Some changes may yield a different product

Some changes fundamentally alter the design or nature of a product

- A fundamentally new product should be submitted in a new IND
- These types of changes are not amenable to comparability studies
- Please ask us if you are unsure

Examples:

- For a T cell therapy:
  - Change from CD4+ T cells to a mixture of CD4+ and CD8+ T cells
- For a gene therapy vector:
  - Change to the vector capsid that alters vector tropism
- For a genome editing product:
  - Different target gene

Reporting manufacturing changes to BLAs

Assess the potential impact of all manufacturing changes

Report the change in a supplement:

For manufacturing changes that have a substantial or moderate potential to have an adverse effect on product quality

Annual report:

For manufacturing changes that have a minimal potential to have an adverse effect on product quality

Include data to evaluate the effect of the change on product quality

An approved comparability protocol may ease implementation of a change

Submit your comparability protocol in a supplement, and we will review

ASSESSING COMPARABILITY

Obtaining advice on comparability studies

Involve a statistician when you design a comparability study

Obtaining feedback from FDA

- Prospectively discuss significant manufacturing changes
- Provide a detailed comparability protocol
- If a change needs to be made but the product is not comparable, discuss proposed clinical studies with the post-change product
- Mechanisms for obtaining feedback:
  - Formal meeting request
  - IND amendment
  - BLA product correspondence

Comparability studies, protocols and reports

Before conducting a comparability study, prospectively write a comparability protocol:

- Describe the manufacturing change
- Assess the risk of the change
- Describe the study design in detail, including which lots will be used
- List test methods and acceptance criteria
- Describe how the data will be analyzed

Submit the comparability report to your IND or BLA

Goals of an analytical comparability study

Ensure that the safety and effectiveness of the product will not be compromised by the manufacturing change

- It is not necessary for product quality to be identical after a manufacturing change
- Demonstrate that the change has no adverse effect on the safety or effectiveness of the product

Evaluate all attributes of the product that might be adversely affected by the change

- Changes to the manufacturing process can have higher risk than routine manufacturing
  - Lot release assays may not always be sufficient to evaluate comparability
  - Additional product characterization is often appropriate

CAR T cell products

Changes to vector manufacturing

- Compare quality attributes of the pre- and post-change vector
- Compare quality attributes of CAR T cells manufactured with the pre- and post-change vector, using the same cellular starting material

Manufacturing at multiple facilities

- If the same product is made at more than one facility, it is important that these products are comparable
  - It is also important that assays give reproducible results even if performed at different sites

Changes to CAR T cell manufacturing

- Some CAR T cell attributes are strongly linked to the cellular starting material
  - Variation among donors → variation among CAR T cell lots
- We recommend split-source studies, to decrease the impact of this variability
  - Use the same cellular starting material in both the old and new manufacturing process

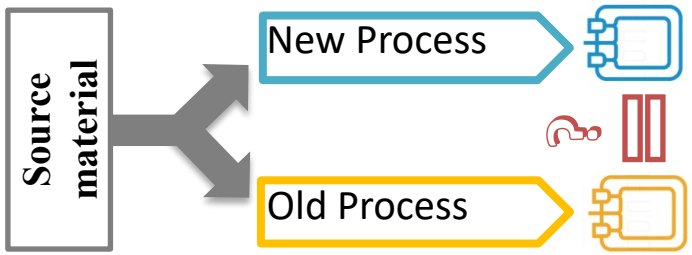
Products derived from a variable cellular starting material

Donor-derived cells are highly variable

This results in variable product attributes, which can make it difficult to evaluate how manufacturing changes affect product quality

Split-source material study design and paired statistical analysis may help

This study design can minimize the effect of source material variability



When manufacturing product for comparability studies:

Best to use the same type of source material as the clinical product

But may be able to use other material if justified
(for example, cells from healthy donors)

The comparability report

Introduction

- Description of the manufacturing changes
- Rationale and justification for the changes
- Justification of the comparability study design
- Timeline for implementing the changes

Risk Assessment

- Determine quality attributes that are at risk from the change
- Select product attributes and process parameters to be evaluated

The comparability report

Comparability Study Design

- List the lots included in the study, and sources of historical product data
- Describe the test methods
- List the acceptance criteria for comparability of each attribute
  - These should be based on understanding the relationship of the attribute to safety or effectiveness
- Refer to the draft guidance for some advice on specific situations

Results and conclusions

- Include data in tabular format, along with summary statistics
- Describe the conclusions from the study
- Explain any changes or deviations from the comparability protocol

Reaching a conclusion about comparability

A comparability study should reach a definitive conclusion

- Is the post-change product comparable to the pre-change product?

Failing to detect differences is *not* the same as demonstrating equivalent product quality

- Some comparability studies are inconclusive because of:
  - Lack of statistical power
  - Imprecise assays
  - Poor understanding of a product's quality attributes
  - Lack of assays to measure a product attribute that may be affected by the change
- A two-sample *t*-test is usually not an appropriate method

If a product is not analytically comparable after a change (or if the comparability study is inconclusive), then:

- Nonclinical or clinical studies may be needed to demonstrate the safety and/or effectiveness of the post-change product

Statistical considerations

Lots for the study should be representative and selected in an unbiased manner

Describe the statistical methods

Justify the assumptions of the statistical approach

For example, many parametric tests assume that data are normally distributed

Different statistical methods may be used to analyze different attributes

Studies should have adequate power to show that the change has no biologically-relevant adverse effects on product quality

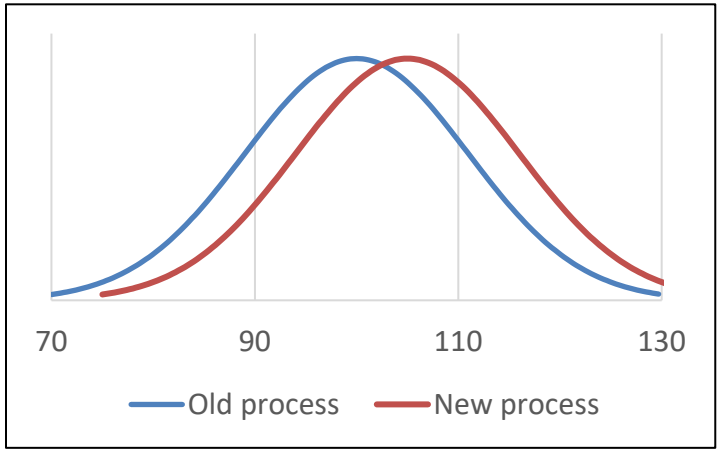
Use an adequate number of lots

Assays need sufficient precision to detect biologically-meaningful differences

Two fundamentally different statistical approaches to evaluating comparability

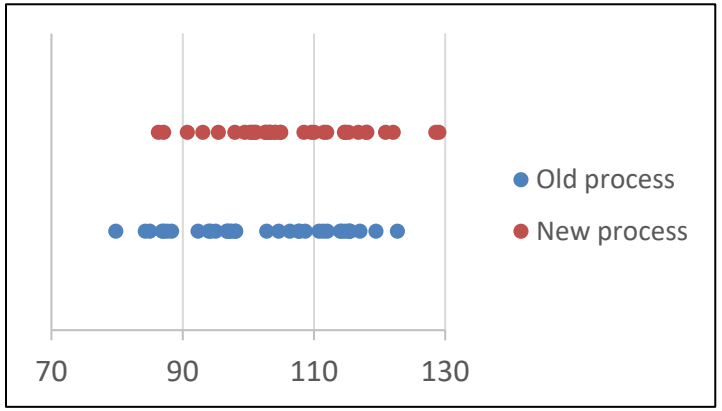
Equivalence

- Evaluate whether two populations are similar enough
  - Set acceptance criteria for the confidence interval of the difference in means
  - A rigorous approach, suitable for high-risk attributes



Quality range

- Evaluate whether individual lots fall within an acceptable range
  - Post-change values should not fall outside a certain range



Conclusions

Risk management should play a central role in managing manufacturing changes and designing comparability studies

Plan ahead for future changes

FDA can provide advice through your IND or BLA

Contact Information

- **Anurag Sharma, PhD**

  Anurag.Sharma@fda.hhs.gov
- **Regulatory Questions:**

  OTP Main Line – 240 402 8190

  Email: OTPRPMS@fda.hhs.gov

- **OTP (OTAT) Learn Webinar Series:**

  http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm

- **CBER website:** www.fda.gov/BiologicsBloodVaccines/default.htm
- **Phone:** 1-800-835-4709 or 240-402-8010
- **Consumer Affairs Branch**: ocod@fda.hhs.gov
- **Manufacturers Assistance and Technical Training Branch**: industry.biologics@fda.gov
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Opportunities for Standardization in Comparability of Cell and Gene Therapies

Workshop for the Identification and Standardization of Methods for Assessing Gene Therapy Product Activity and Comparability and the Evaluation of T-Cell Therapies

Tal Salz, PhD | Practice Expert
tsalz@darkhorseconsultinggroup.com
Dark Horse Consulting Group

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What is a standard?

"The term "standard" (or "technical standard") includes all of the following:

(1) common and repeated use of rules, conditions, guidelines or characteristics for products or related processes and production methods, and related management systems practices;

(2) the definition of terms; classification of components; delineation of procedures; specification of dimensions, materials, performance, designs, or operations; measurement of quality and quantity in describing materials, processes, products, systems, services, or practices; test methods and sampling procedures; formats for information and communication exchange; or descriptions of fit and measurements of size or strength; and

(3) terminology, symbols, packaging, marking or labeling requirements as they apply to a product, process, or production method."

FDA Guidance for Industry: Standards Development and the Use of Standards in Regulatory Submissions Reviewed in the Center for Biologics Evaluation and Research (March 2019)

CGT Comparability is Complex

Ethical

Scale

Analytical methods

Number of lots

Potency

Variability

Stability

Relationship between CQA/clinical outcomes

Unknown PK/PD profile

Process

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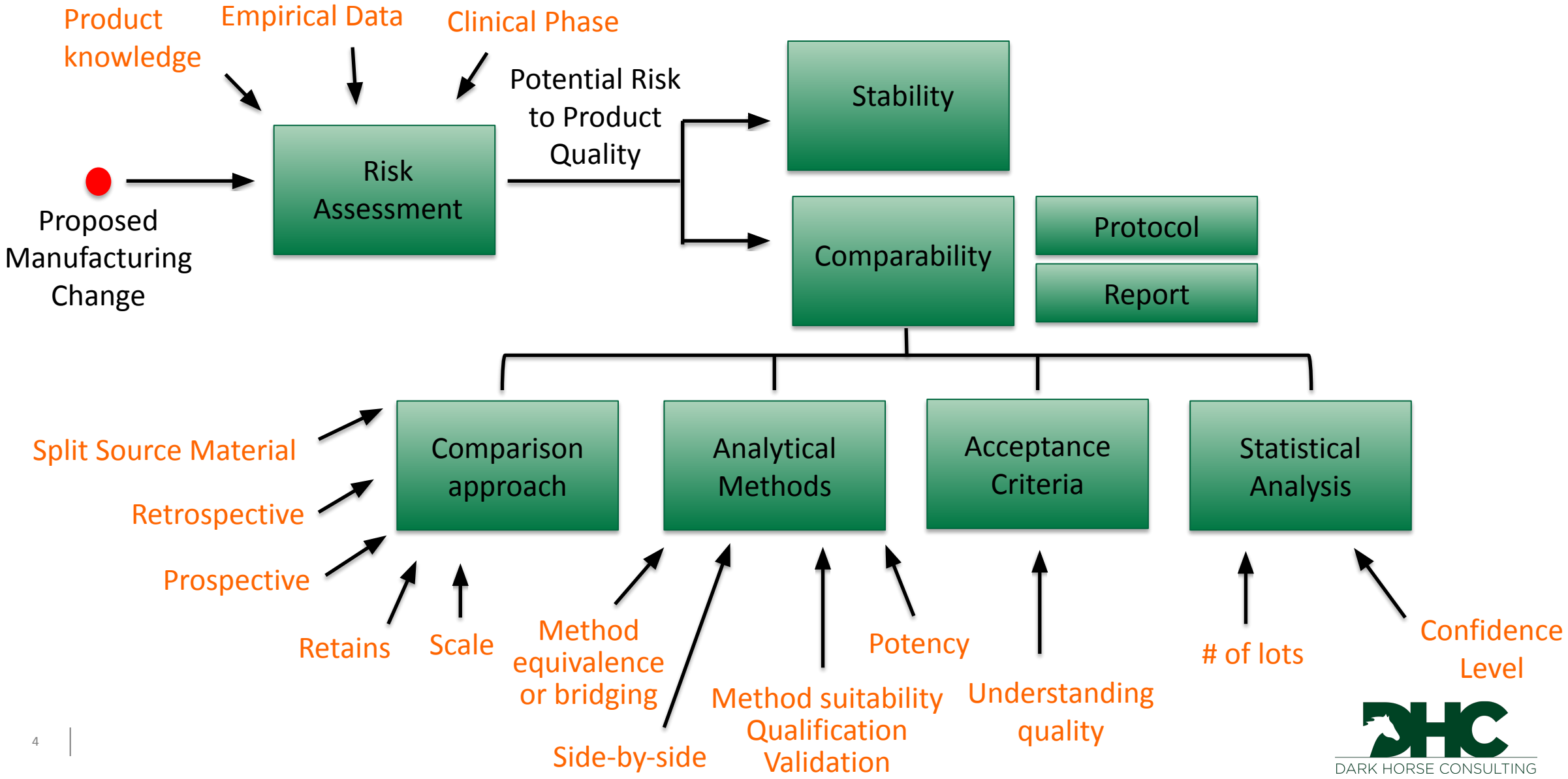
Considerations in Comparability



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The Questions We need to Ask Ourselves

- Is comparability standardization desired by industry and stakeholders?
- Is comparability standardization desired by FDA?
- Can we find common ground and opportunities for comparability standardization
- Will comparability standardization effectively reduce burden for industry, stakeholders and FDA?

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Can We Standardize Comparability?

- Demonstrating comparability is a complex exercise, particularly in the cell and gene therapy space and there is no one size fit all approach.
- However, there could be an opportunity for standardizing some elements associated with comparability evaluation:
  - Risk Assessment
  - Study Procedures (e.g., split-source material)
  - Method equivalence
  - Side-by-side testing
  - Statistical approaches
  - Qualification of Scale-down model
  - Qualification of retains
  - Study report/study protocol
  - Terminology

Risk Assessment

- Risk analysis is the estimation of the potential risk posed to product quality by a manufacturing change.
- ICH Q9(R1): “achieving a shared understanding of the application of risk management among diverse stakeholders is difficult because each stakeholder might perceive different potential harms, place a different probability on each harm occurring and attribute different severities to each harm”
- It is important that subjectivity inherent in performing a risk assessment is managed and minimized

Can Manufacturing Change Risk Assessment Be Standardized?

- The output of a risk assessment is either a quantitative estimate of risk or a qualitative description of a range of risk.
- Risk can be expressed using qualitative descriptors, such as “high”, “medium”, or “low”, which should be defined in as much detail as possible.
- Risk can be expressed as a "risk score" to further define descriptors in risk ranking.

Evaluated elements:

Severity (S)	If a failure were to occur, what effect would that failure have on the product quality and on the patient (if any)?
Probability (P)	How likely is it for a particular failure to occur (probability of occurrence)
Detectability (D)	What mechanisms are in place (if any) to detect a failure if it were to occur?

Risk Priority Number (RPN)

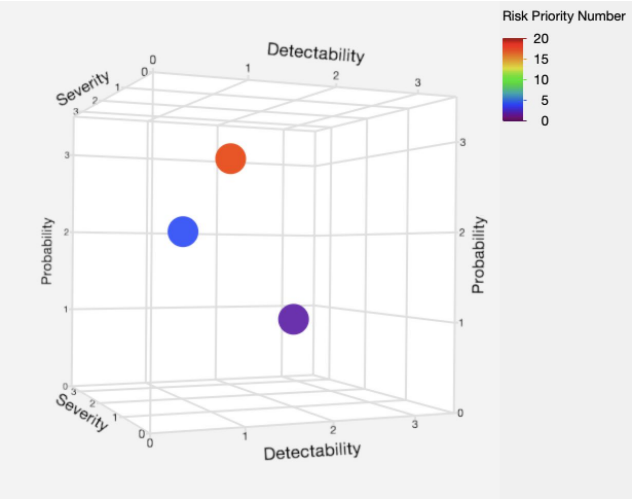
- RPN = S × P × D
- The higher the RPN, the greater the risk

Knowledge Data Science controls →

Severity, Probability, Detectability

score	Risk to CQA
1	Low
2	Medium
3	High

→



RPN Ranges	Risk to CQA
X > 20	High
15 < X ≤ 20	Medium-High
10 < X ≤ 15	Medium
5 < X ≤ 10	Low-Medium
X ≤ 5	Low

→ Tier approach

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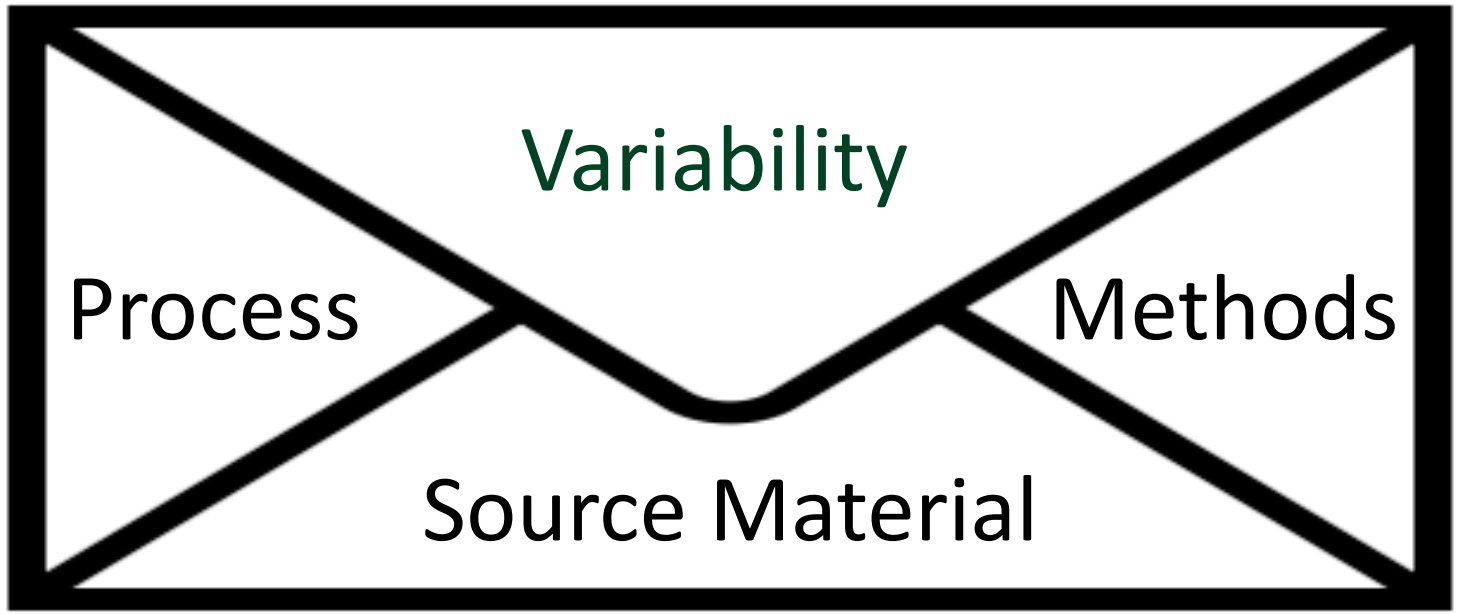
Variability is a Confounding Challenge for Comparability

Scale-down
Model



Qualification
Equivalence
Side-by-side testing

Representative
Split

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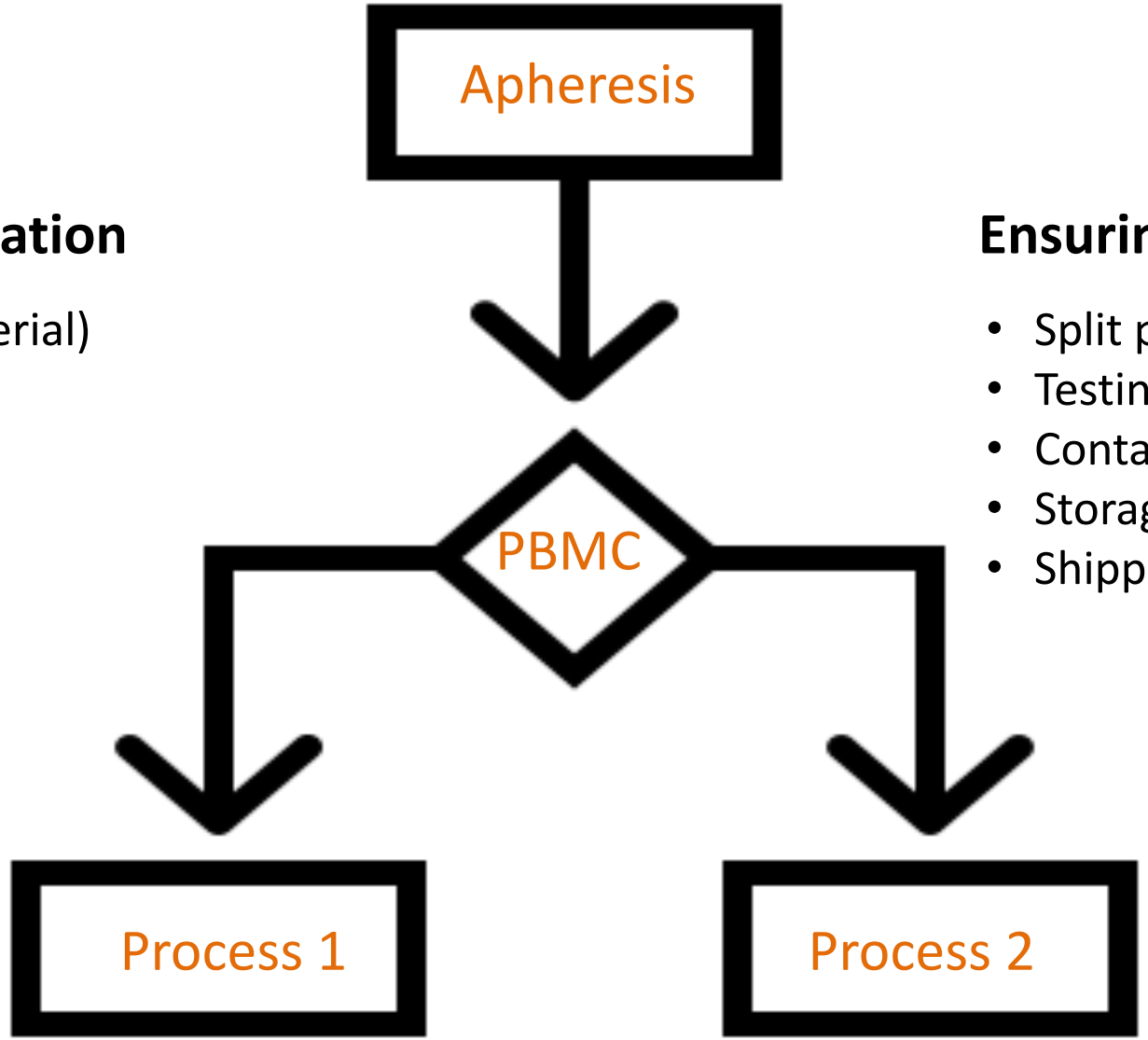
Split Source Material Comparability Approach



Ensuring representation

- Donor (starting material)
- Processing
- Scale
- Stability

Ensuring comparable portions:

- Split procedures
- Testing
- Container
- Storage/hold conditions
- Shipping

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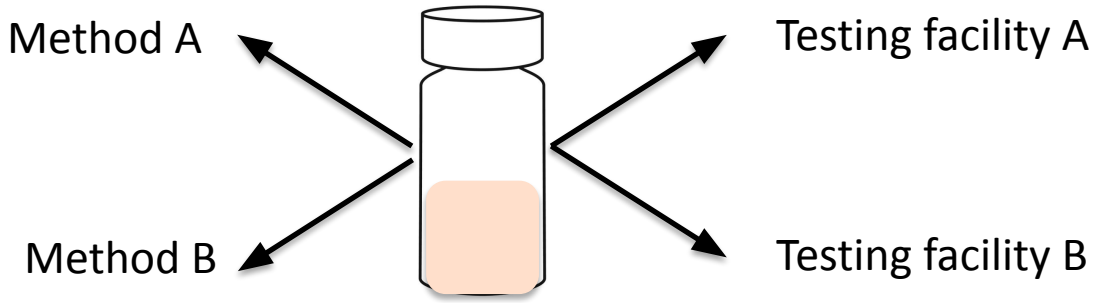
Method Equivalence

- Methods and testing facilities change overtime
- Method equivalence is a working assumption in comparing the pre- and post-change test results when they were generated in different testing facilities or with different methods.
- Method equivalence is typically demonstrated through split sample(s) study

  - How to determine the number of samples?
  - Should samples with known range of results be included?
  - What is an acceptable %CV?
  - Sample stability
  - Number of operators



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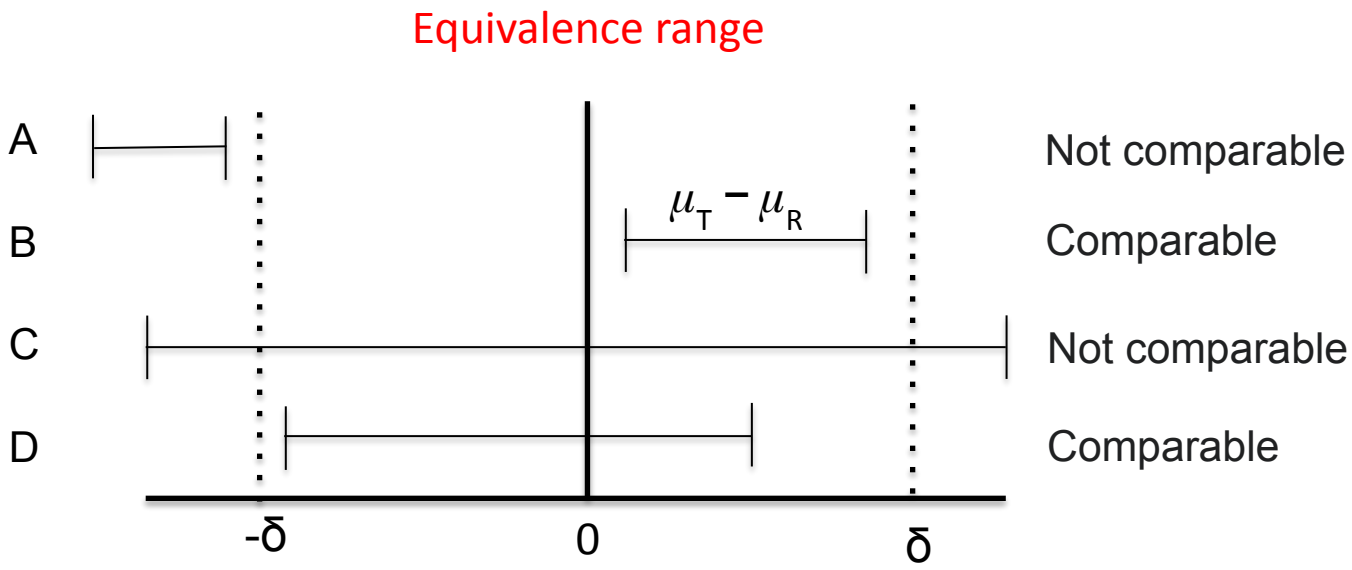
Side-by-side Testing

- Method variability can impact the results of comparability
- To reduce method variability, the pre- and post-change product samples could be tested side-by-side
- Side-by-side testing is defined differently by different people and includes the following considerations:
    - Same testing facility?
    - Same reagent lots?
    - Timing (you can get better at performing a method over time; trending)?
    - Simultaneous processing?
    - Same run?
    - Same instrument?
    - Same operator?

Statistical Methods

- Two-One-Sided Tests procedure (TOST) is a statistical method for comparability which has gained popularity in recent years
- Equivalence range is the largest acceptable difference between the pre-change and post-change attribute
- Working assumptions and statistics could be standardized
- The equivalence range should be determined on a case-by-case basis



$H_0: \mu_T - \mu_R \leq -\delta$ or $\mu_T - \mu_R \geq \delta$

$H_1: -\delta < \mu_T - \mu_R > \delta$

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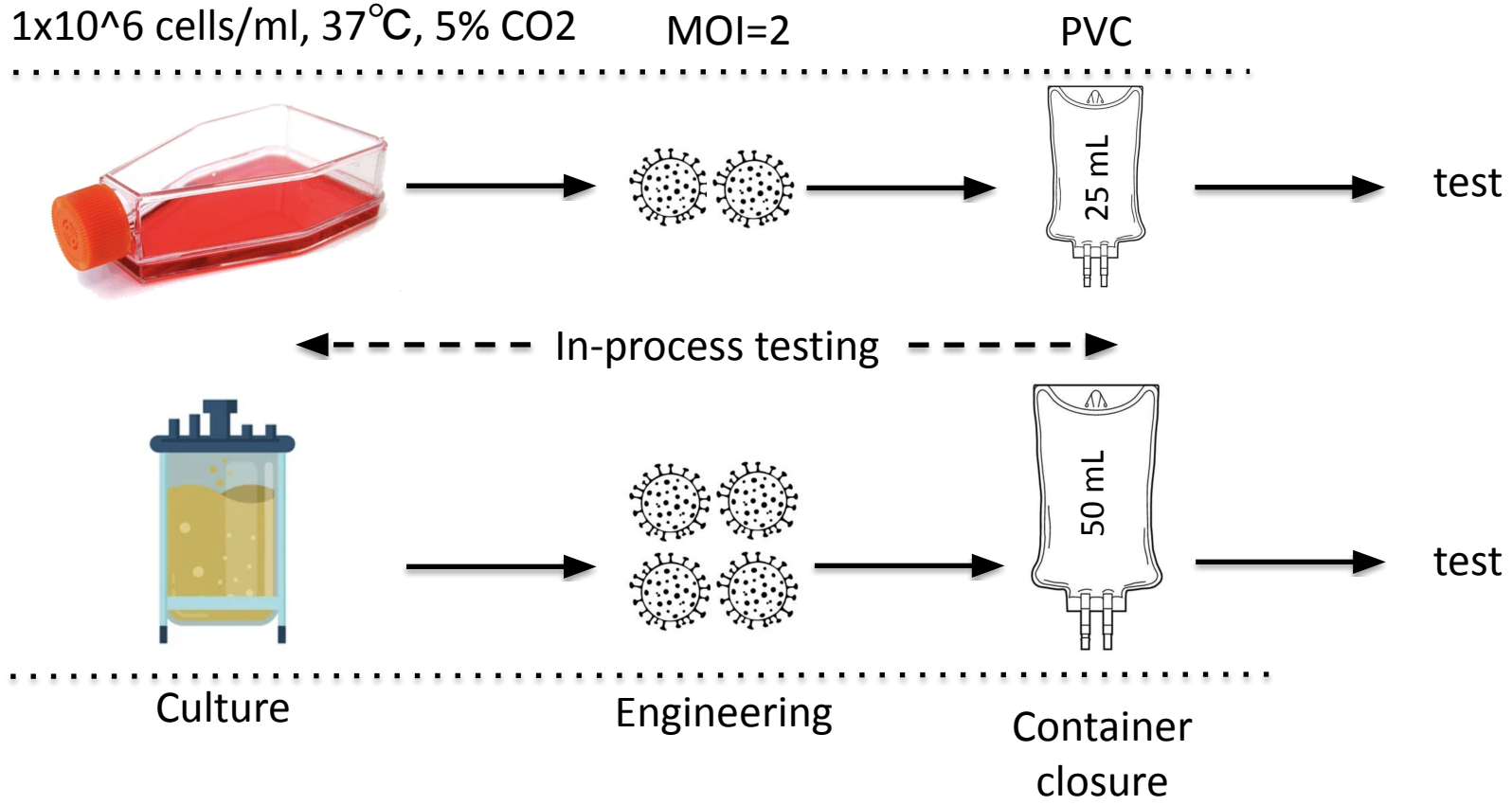
Qualification of a Scale-down Model

- CGT processes are costly
- Manufacturing lots strictly designated for comparability at the CDMO is often not possible
- Scale-Down process models can be used for demonstrating CGT comparability
- The Scale-Down process should be shown to be representative of the full-scale process and generate a product with similar quality
- What parameters should be kept constant?
- What attributes should be evaluated?
- What acceptance criteria should be satisfied?

Qualification of a Scale-down Model

- Is there an opportunity to define standards for qualifying a Scale-Down model?
  - Proportion
  - Unit operations
  - Materials used
  - In-process sampling
  - Comparability?



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Retains

- Retains are often used to test pre-change lots
- The storage and age of retain samples can impact their quality
- How do we ensure that the retain sample used is representative of the pre-change product?
    - Stability
    - Storage conditions
    - Control test

Study report/protocol

- Comparability protocol and comparability report submissions are notably different and often are missing important information
- Standardization of the structure and/or information that should be included in a comparability protocol/report will streamline submissions and facilitate FDA review

Table of Contents

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Terminology

- Analytical Comparability
- Side-by-side testing
- Prospective analysis
- Retrospective analysis
- Method equivalence
- Method bridging
- Split source material (same donor? Same apheresis collection? Same PBMCs?)
- Equivalence Range
- Quality Range

Summary Thoughts

- Comparability comes in many shapes and forms
- However, there are opportunities for standardizing elements relevant to comparability of CGTs which could benefit both FDA and industry
- However, the most difficult task of establishing comparability acceptance criteria is unique to the product
- Limited number of lots available for comparability analysis remain a significant challenge which could be salvaged using a scale-down model.

Thank you!

tsalz@darkhorseconsultinggroup.com

DARK HORSE CONSULTING

Evolving Standards and Tools to Meet Industry Needs in Cell and Gene Therapy

Diane McCarthy
November 16, 2023

Agenda

- Value of Standards for Cell and Gene Therapy
- Raw and Starting Materials
- Product Quality Attributes
- Impurities

Partnerships through our Shared Challenges

Partnering with our expert volunteers

USP's public quality standards, developed by volunteer experts, including government liaisons, enable transparent processes that ensure quality methods, APIs, and education

Access to quality medicines

Partnering with industry

Manufacturers must be able to bring quality pharmaceuticals to market and depend on quality-assured methods, materials, and resources to reduce risk to market entry

Partnering with regulators, including the FDA

Regulators must be able to ensure pharmaceuticals are approved for market regardless of technology used. They depend on a quality-assured scientific basis for decision-making in regulatory review, manufacturing practices, and enforcement

Partnering with strategic collaborators

Strategic collaborations are critical for identifying and pursuing key growth objectives, deepening and broadening quality systems, and enabling the overall medicine value chain, end to end

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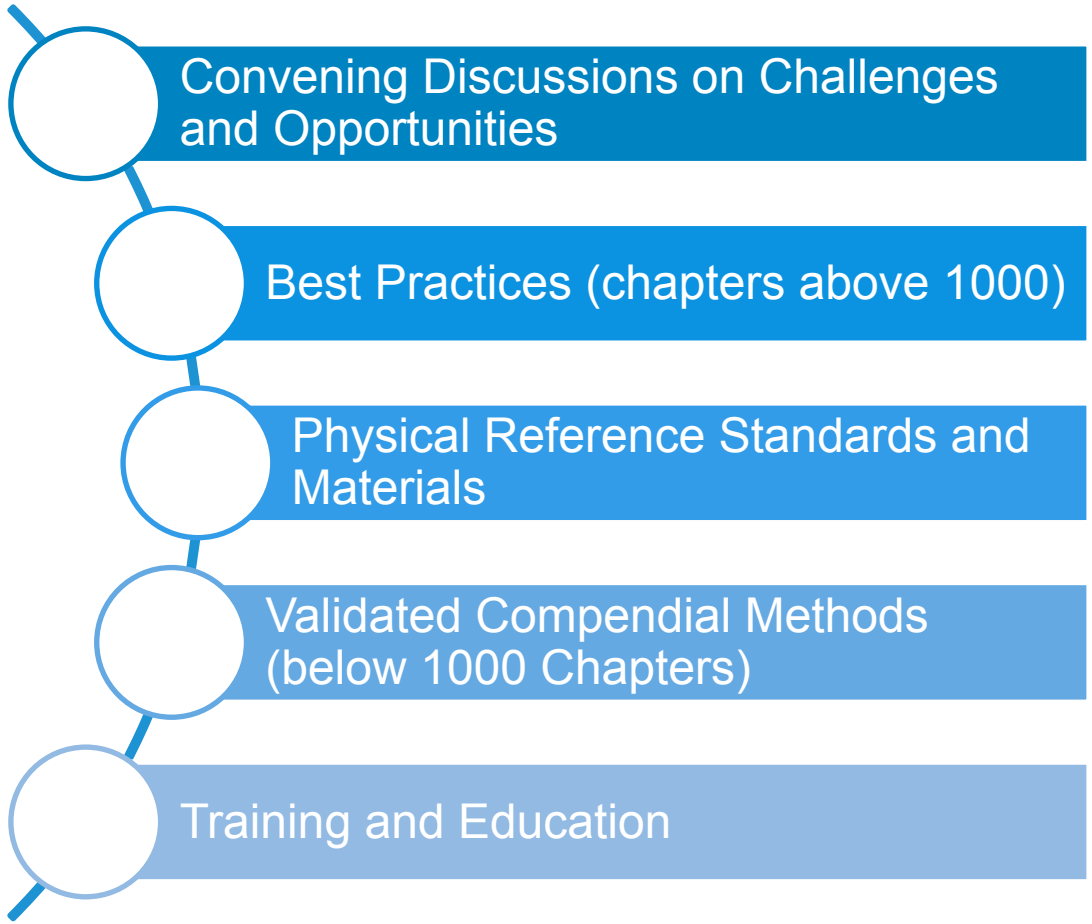
Need for Standards and Best Practices

- "The use of standards can **facilitate product development** and **reduce the amount of documentation** needed in a regulatory submission, thus contributing to a more efficient submission evaluation and, ultimately, **improving time to market**."
  - FDA 2019 Guidance for Industry: Standards Development and the Use of Standards in Regulatory Submissions Reviewed in the Center for Biologics Evaluation and Research
- Validated **compendial assays** reduce the burden of method development and technology transfer
- **Reference materials** benchmark measurements and validation criteria across batches, manufacturing sites, and product developers
- Phase-appropriate **best practices** balance risk and benefit

Supporting Quality and Consistency of Emerging Modalities

- Benefits of Standards include:
  - **Consistency** Help facilitate consistent and predictable manufacturing processes, product testing throughout lifecycle
  - **Innovation** Foster innovation and adoption of new technologies, lower R&D costs by building on existing standards
  - **Supports** meeting regulatory expectations, and facilitate market entry for safe and effective products, including products from emerging technologies
- Remains challenging to defining a standard that suits every developer's needs
  - Diverse range of product types
  - Unique requirements for raw materials
  - Lack of alignment on PQAs and test methods



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1 Raw and Starting Materials

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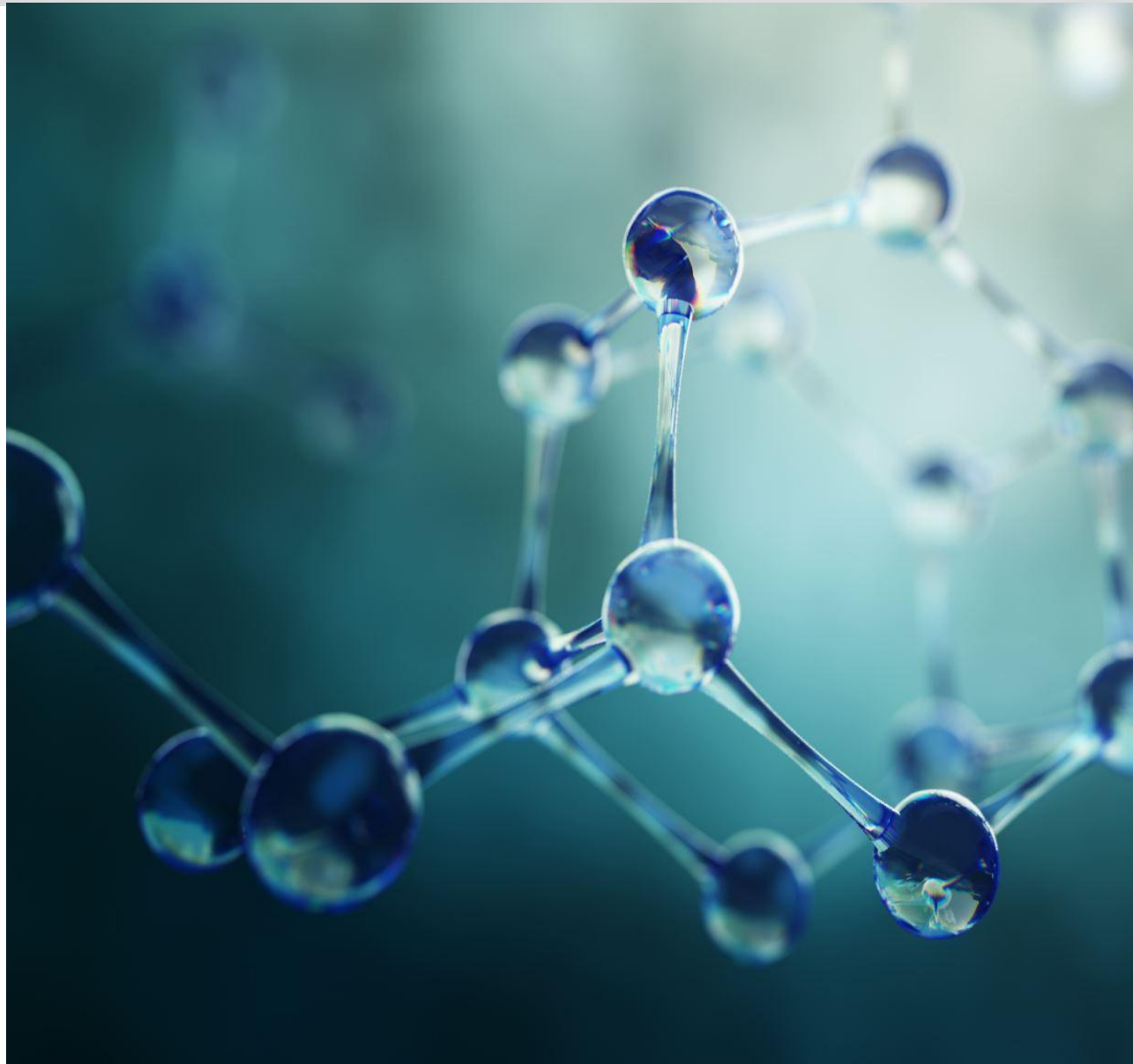
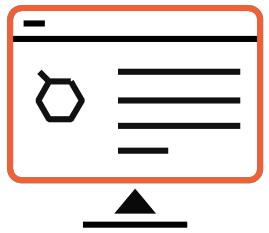
Challenges in Ensuring Quality of Raw Materials

- Some products are not amenable to extensive purification, filtration, or terminal sterilization
- Compendial testing may not be possible
  - No appropriate compendial methods
  - Limited or expensive material
  - Short shelf-life
- Raw material produced using GMP principles may not be available
- Vendor testing insufficient to assure raw material functionality
- Lot-to-lot variability in material quality and function

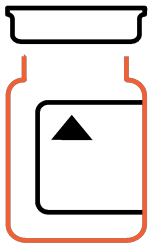


Existing USP Public Standards for Raw & Starting Materials



Documentary standards–General chapters

- *<1044> Cryopreservation of Cells*
- *<1043> Ancillary Materials for Cell, Gene, and Tissue Engineered Products*
- *<1042> Cell Banking Practices for Recombinant Biologics* **NEW**
- *<1027> Flow Cytometry*
- *<1024> Bovine Serum*
- *<1040> Quality Considerations of Plasmid DNA as a Starting Material for Cell and Gene Therapies published* ***in PF 49(6)***
- *<90> Fetal Bovine Serum--Quality Attributes and Functionality Tests*
- *<89> Enzymes Used as Ancillary Materials in Pharmaceutical Manufacturing*
- *<92> Growth Factors and Cytokines Used in Cell Therapy Manufacturing*
- *<127> Flow Cytometric Enumeration of CD34+ Cells*



Reference Standards

- CD34+ Enumeration System Suitability (freeze-dried cells)
- Fetal Bovine Serum
- Albumin (recombinant and bovine)
- Trypsin
- Collagenase I and II

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Development of Chapter on Best Practices for Plasmid DNA

- Stakeholder feedback indicated there was insufficient regulatory guidance for plasmid DNA used in the manufacturing of cell and gene therapy
- USP has recognized this gap and initiated efforts to define plasmid DNA best practices
  - USP Expert Panel for plasmid DNA was established to provide guidance
    - Recruited expert volunteers through an open Call for Candidates
  - General Chapter was published in Pharmacopeial Forum on Nov 1, 2023
    - Open for public comments until Jan 31, 2024
    - https://www.uspnf.com/pharmacopeial-forum

CHAPTER OUTLINE

- Manufacturing Considerations
  - Master Cell Bank
  - Facility Design
- Quality Management
  - Phase Appropriate Quality Systems and Facilities
- DNA Starting Material Quality
  - Quality Attributes
  - Stability Testing
  - Performance Testing
  - Plasmid to Plasmid Cross-Contamination
  - Receipt Testing
  - General Acceptance Criteria and Manufacturing Considerations

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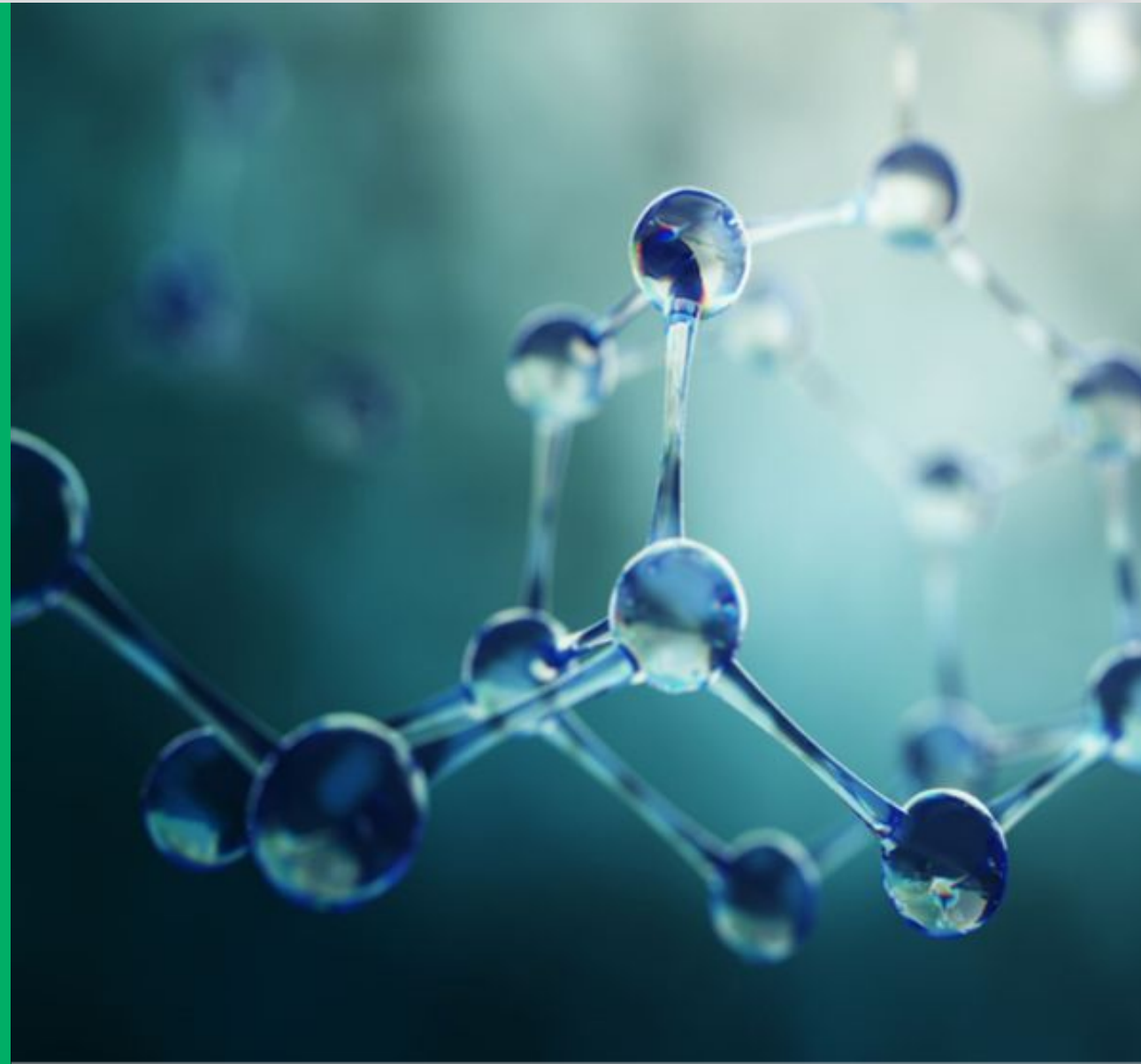
Standards and Tools in Development

- General Chapters (<1000) with validated methods and associated Reference Standards for
  - Enzymes used in CGT processing
  - Cytokines and growth factors used in cell culture

- Reference Materials:
  - Plasmid DNA for quality assessment of ancillary materials



Product Quality Attributes

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Existing USP Chapters Supporting Manufacturing and Quality Control of Cell, Gene and Tissue Products

<1046> *Cell-based Advanced Therapies and Tissue-Based Products*

- Qualification of source cells/tissues
- Manufacturing process considerations
- Final product release testing
- General considerations of CQAs for potency, purity, identity, stability, sterility, packaging and administration
- Fundamental steps for tech transfer

<1047> *Gene Therapy Products*

(under revision)

- Addresses both commercial and clinical trial materials
- Manufacturing and process development considerations
- Vector design, manufacturing and purification
- Analytical tests for CG products

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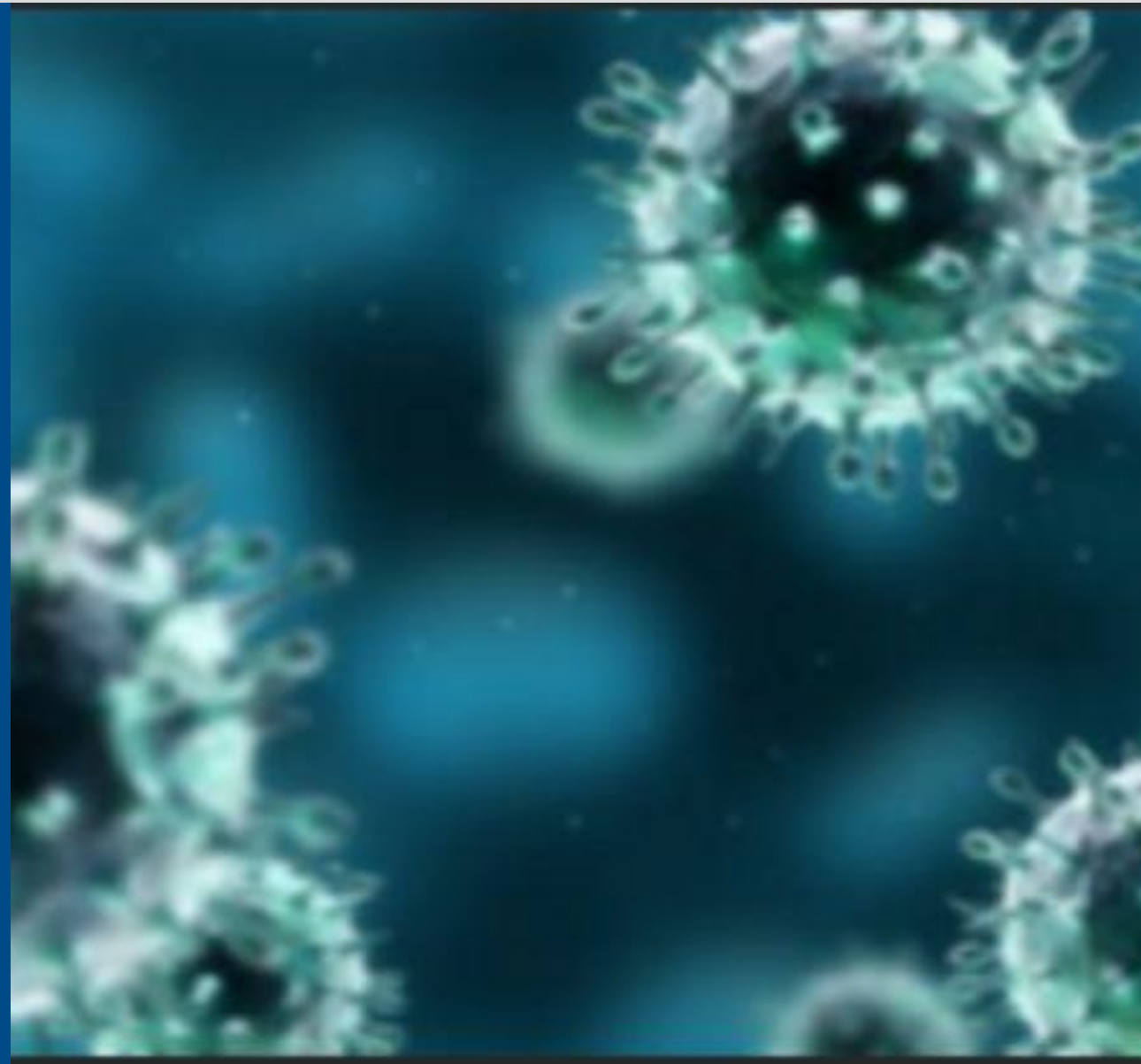
Challenges of AAV Gene Therapies Manufacturing

- Challenges
  - Manufacturing viral vectors requires several discrete manufacturing activities, each with requirements for production, purification, release and stability testing
  - A variety of analytical methodologies are in use for assessing PQAs and there is limited harmonization or use of common standards used across products/developers
- Solutions
  - Convened industry experts through USP sponsored roundtables and events to identify challenges and potential solutions
  - Build alignment on key considerations and best practices
  - Identify opportunities for standards to alleviate bottlenecks and improve efficiency of development



Aligning on Best Practices for AAV Products

- Stakeholders expressed need for harmonization of CGT methods
  - Feedback from Expert Committee, industry stakeholders, and USP roundtable in December 2021
- USP established an AAV Gene Therapy Expert Panel
- Panel initiated work in June 2022

CHAPTER OUTLINE *(as of Nov 2023)*

- **Vector Characteristics & Design**
  - Safety, transgene cassette, capsid
- **Materials**
  - Raw and critical starting materials
- **Manufacturing**
  - Drug Substance (Seed train to purification)
  - Drug Product
- **Formulation & Final Presentation**
- **Control Strategy**
  - Microbial and viral testing
  - Reference Standards, Assay Controls, In-Process Controls
  - Drug Substance/Drug Product Quality
- **Stability**
  - Starting Materials, DS, DP, Stability studies
- **Comparability**
  - Phase Appropriate Comparability Strategies

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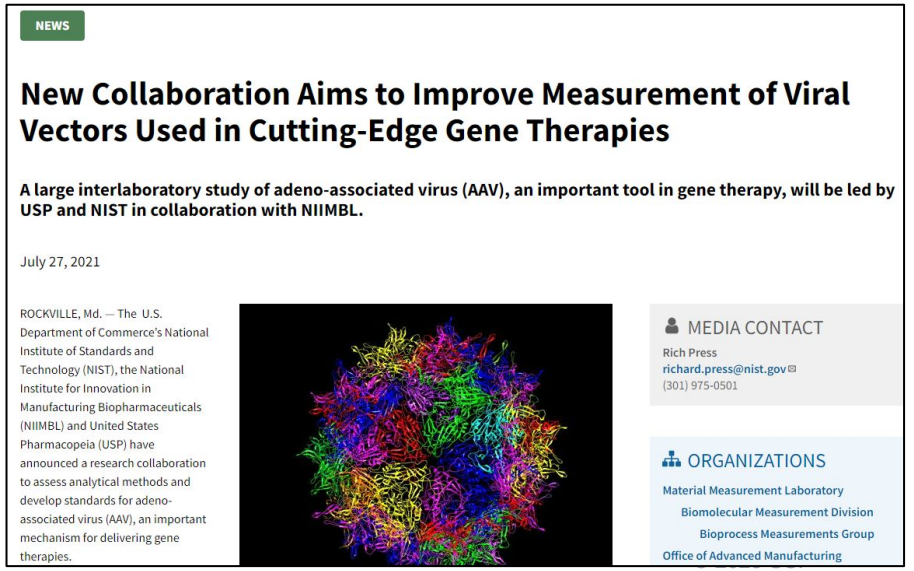
Full-to-empty AAV Vector Characterization

- November 2019 NIIMBL Technology Workshop identified an interlaboratory study on measuring the **ratio of full-to-empty viral capsids as a high impact activity**

- USP, NIST and NIIMBL collaborated on a study to assess and harmonize (where applicable) analytical methods for **analysis of full-to-empty ratio**
  - Interlaboratory studies to measure full-to-empty ratio of AAV
    - AAV serotype 5 and 8 were evaluated with different full to empty ratio
    - 7 organizations participated to the study
  - Analyze and share data to help standardize methods
    - Manuscript in preparation



NEWS

New Collaboration Aims to Improve Measurement of Viral Vectors Used in Cutting-Edge Gene Therapies

A large interlaboratory study of adeno-associated virus (AAV), an important tool in gene therapy, will be led by USP and NIST in collaboration with NIIMBL.

July 27, 2021

ROCKVILLE, Md. — The U.S. Department of Commerce's National Institute of Standards and Technology (NIST), the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) and United States Pharmacopeia (USP) have announced a research collaboration to assess analytical methods and develop standards for adeno-associated virus (AAV), an important mechanism for delivering gene therapies.

MEDIA CONTACT

Rich Press
richard.press@nist.gov
(301) 975-0501

ORGANIZATIONS

Material Measurement Laboratory
Biomolecular Measurement Division
Bioprocess Measurements Group
Office of Advanced Manufacturing

Physical Reference Materials in Development

- Vector genome titer for AAV
- Vector genome titer for LVV
- LVV integration copy number
- AAV Capsids
  - Empty: full ratio
  - Capsid protein analysis
  - Aggregation

3 Impurities

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Process Related Impurities

Impurities are process-specific, not an exhaustive list!

- Cell substrate-derived process impurities
  - Residual DNA (host cell DNA)
  - Host Cell Proteins
- Cell culture derived impurities
  - Growth factors, FBS
  - Process additives including surfactants, antifoam agents, residual solvents
- Residual Raw/ Starting Materials
  - Plasmids
  - Transfection reagents

- Quantities in final product need to be controlled and monitored
- WHO and FDA guidelines recommend a limit of 10 ng/dose residual DNA in a final product dose
- Physical Materials needed to support quantitation

- Residual HCPs can impact product quality, safety, and efficacy
- HCP levels should be measured in preclinical toxicology lots, clinical development and process validation
- Due to complex assay development, best practices can improve consistency

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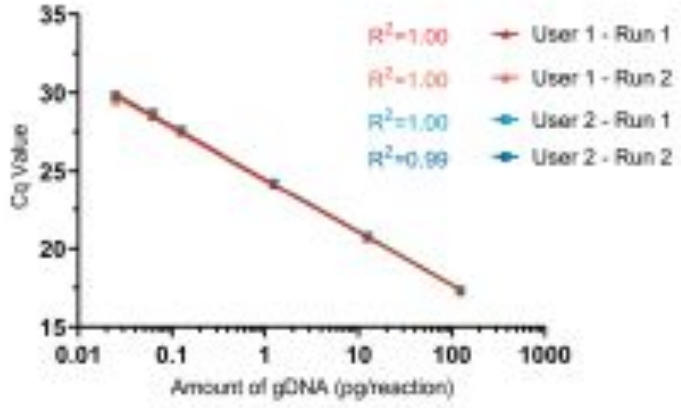
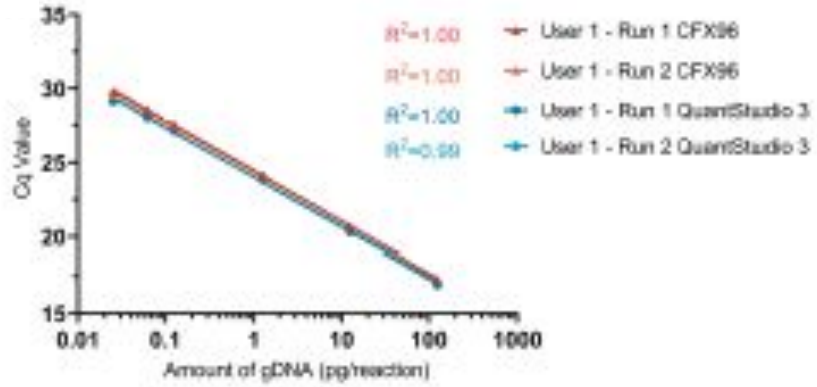
New Reference Materials for Residual DNA

- Residual Host Cell DNA
  - USP-ATCC Genomic DNA products
  - Support quantitation of residual DNA by qPCR for common CGT cell lines
    - Residual HEK293 DNA
    - Residual Sf9 DNA

https://www.usp.org/biologics/atcc-usp-genomic-dnas





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Resources to Support Host Cell Protein Measurement

- **Immunoassays** are routinely used to monitor total HCP content
  - Reagent development and qualification can be complex
  - <1132> *Residual Host Cell Protein Measurement in Biopharmaceuticals*
    - Outlines best practices for total HCP measurement
    - Describes Immunoassay Methods, Reagents, Method Development, Qualification, and Validation

- **Mass spectrometry** is increasingly to identify individual HCPs
  - Supports more detailed risk assessment and enables detection of HCPs that may be under-represented in immunoassay results
  - *<1132.1> Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry*
    - Published in PF in May 2023
    - Covers Sample Preparation, Standards, Acquisition Methods, Data Processing and Reporting

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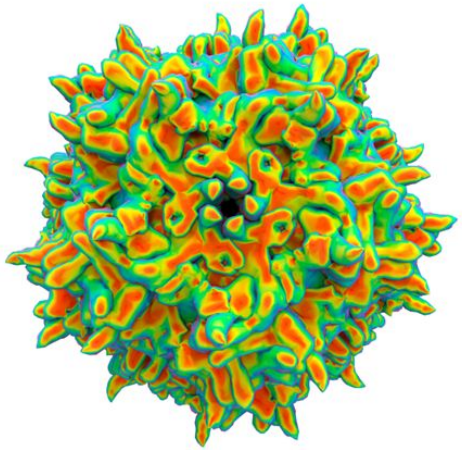
Standards and Tools in Development for Other Residual Impurities

- General Chapters (<1000) with validated methods and associated Reference Standards for
  - Replication competent testing for AAV
  - Residual testing of PEI



- Reference Materials:
  - Plasmid DNA for residual analysis (Ampicillin and Kanamycin)

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Continued Collaboration

- The complexity and diversity of cell and gene therapies present challenges in standardization of methods and assays
- USP is committed to working with stakeholders to streamline and expedite development of safe and effective therapies to patients
- USP will continue to support the standardization of CGT products through the development of standards and tools

Opportunities for Engagement and Collaboration

- Roundtables and working groups
- Donate methods and/or material to support standard development
- Round Robin studies and Collaborative Testing
- Review of Chapters and Stimuli Articles on Pharmacopeial Forum
  - https://www.uspnf.com/pharmacopeial-forum
- Stakeholder Forum: *Nuanced Analytical Approaches to Cell and Gene Therapy* - February 22, 2024

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Thank You

diane.mccarthy@usp.org

The standard of trust

NIST Genome Editing Consortium Overview

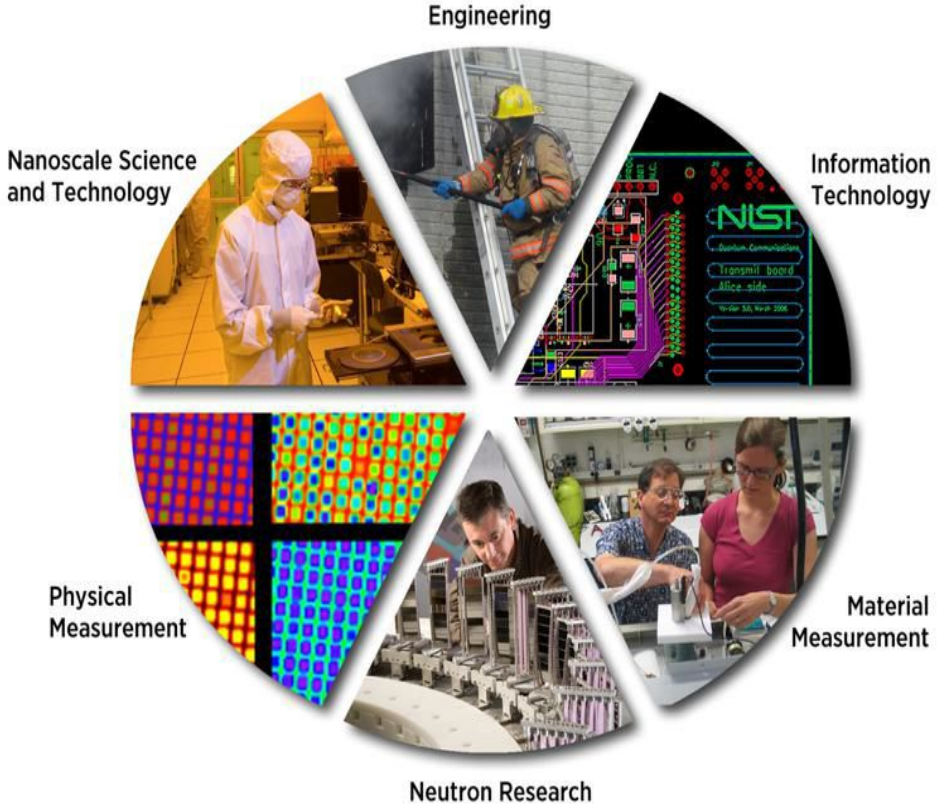
National Institute of Standards and Technology
U.S. Department of Commerce

Samantha Maragh
Leader, NIST Genome Editing Program

NIST – National Institute of Standards & Technology

MISSION

To promote U.S. innovation and industrial competitiveness by advancing **measurement science, standards,** and **technology** in ways that enhance economic security and improve our quality of life



The U.S. National Metrology Institute

Global harmonization of measurement and traceability to the SI

"Industry's National Laboratory"

Non-regulatory agency partnering/serving industry to help maintain US leadership in science and technology products

Department of Commerce

Developing standards to support international trade and commerce

How does NIST work with communities to meet needs?

- NIST one-on-one collaborations: Academic, Other gov., Industry
- NIST led consortia
- NIST coordination with international measurement institutes and organizations

Technology Development
Data/Measurement Quality
Standards

NIST

The Joint Initiative for Metrology in Biology
biofabusa
U.S. FOOD & DRUG ADMINISTRATION
DARPA
NIMBL
The National Institute for Innovation in Manufacturing Biopharmaceuticals
ISO International Organization for Standardization
World Health Organization
NIH National Institutes of Health
usp
CGE Somatic Cell Genome Editing
STANDARDS COORDINATING BODY REGENERATIVE MEDICINE
ERM European Reference Materials
PTB
LGC
SP your Science Partner
ANSI American National Standards Institute
ASTM INTERNATIONAL

NIST-FDA Collaborations on Standards

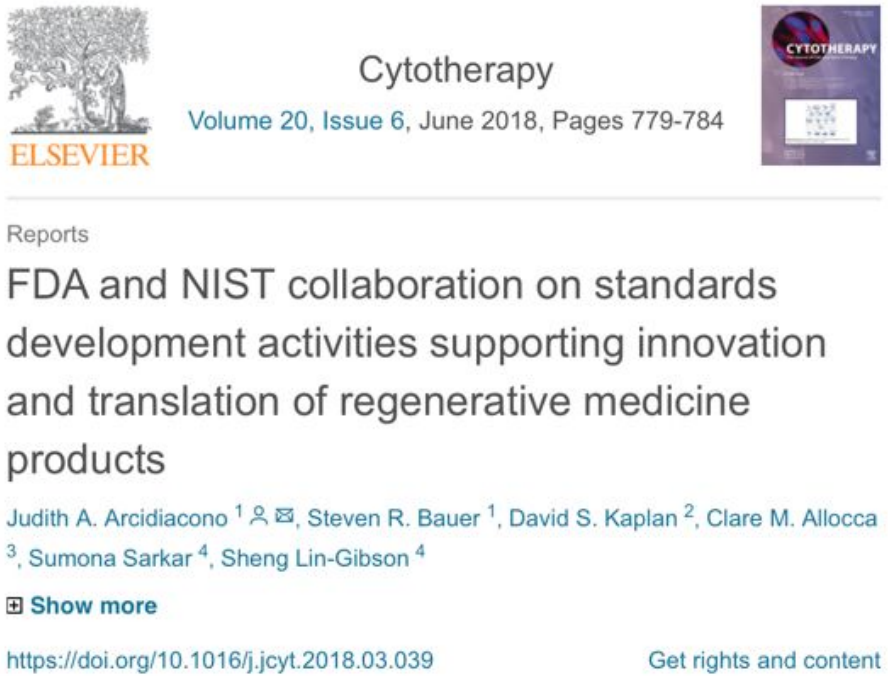
Leveraging unique expertise

NIST engages in discussions and collaborates with industry and others on pre-competitive technologies

NIST expertise in measurement sciences address specific analytical challengesA

FDA scientific and regulatory expertise ensure that standards:

- do not conflict with **FDA** regulation and policy
- address significant regulatory challenges that recur across the field



ELSEVIER

Cytotherapy

Volume 20, Issue 6, June 2018, Pages 779-784

Reports

FDA and NIST collaboration on standards development activities supporting innovation and translation of regenerative medicine products

Judith A. Arcidiacono [1], Steven R. Bauer [1], David S. Kaplan [2], Clare M. Allocca [3], Sumona Sarkar [4], Sheng Lin-Gibson [4]

Show more

https://doi.org/10.1016/j.jcyt.2018.03.039 Get rights and content

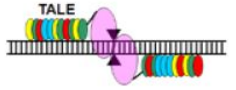
- **Standards Development**
- Workshops and Public Meetings
- **Research Collaborations**

Genome Editing Overview

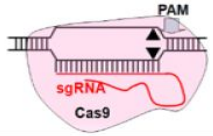
Viral vectors (various types)

Meganuclease

Zinc Finger

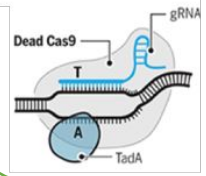


TALEN

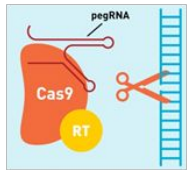


CRISPR-Cas

Base Editor



Prime Editor



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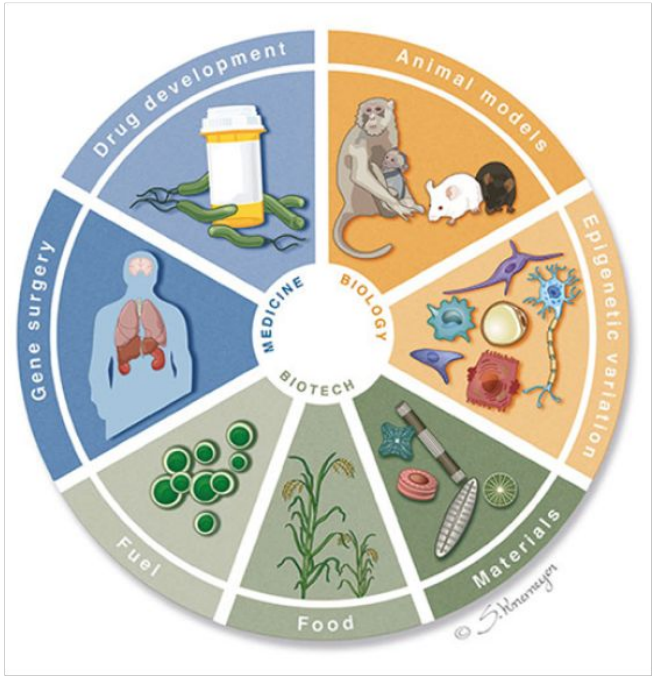
DNA sequence change



Genome Editing/Gene Therapy Molecules

+

Cells of interest (*in vivo* or *ex vivo*)

=

Engineered Cells

Fit for purpose?

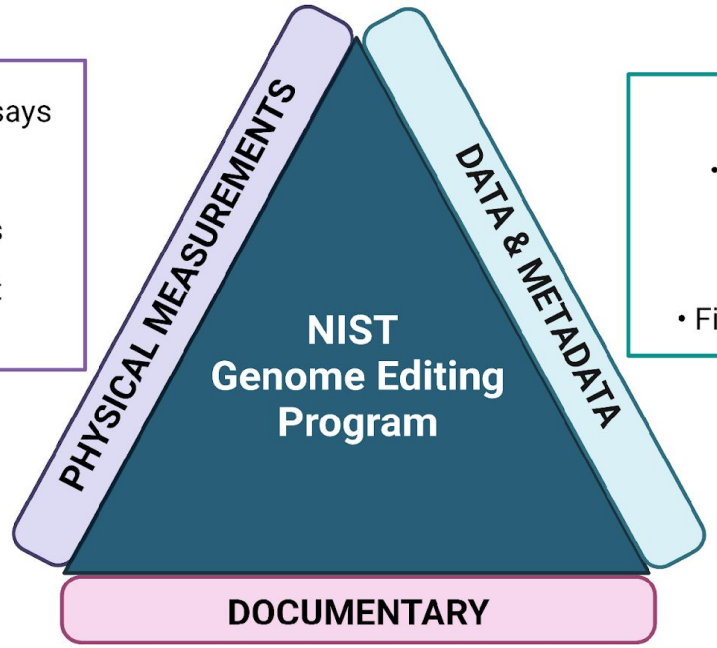
Deliver engineering molecules into cells

NIST Genome Editing Program

Vision: Support quality in measurements for translating genome edited product to market

Goal: Develop measurement tools standards to increase the confidence of utilizing genome editing technologies in research and commercial products.

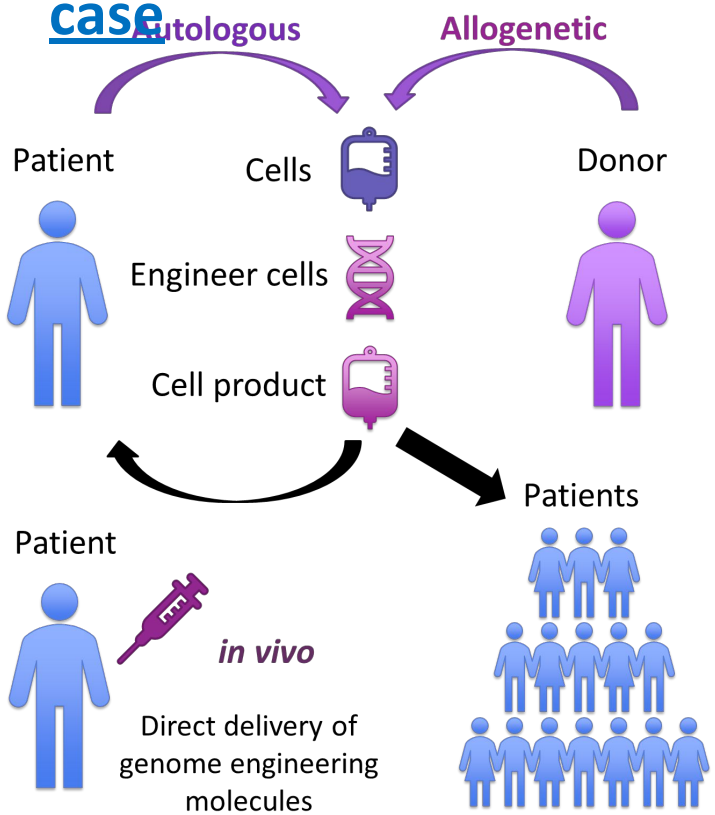
- Confidence in existing assays
- Control materials
- Interlaboratory studies
- New assays for unmet measurement needs



- *in silico* data sets
- Experimental data from well-designed studies
- Standard datasets
- File format / Metdata norms

- Metadata minimum information standards
- Well documented protocols / recommended practices
- Guidelines for comparing informatics pipelines
- Standard lexicon

Gene Therapy use case



FDA requires reporting including:

- off-target genomic positions,
- on-target and off-target sequence change
- relative frequency of variant occurrence

Define the problem

Where in your process is it very important to know if the answer/data is reliable?

Where of theses processes is it currently difficult to understand if these answers/data are reliable?

What can be done to give more confidence in these types of assays/data?

Standards needs identified by the Genome Editing Community

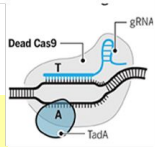
Genome editing components considered critical for manufacturing!

- cGMPs should be followed during manufacturing
- Components should be tested (identity, purity, activity)
- Specifications and controls needed for qualifying these starting materials

Design Genome Editing molecules

Targeted Viral vectors

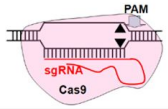
Base Editor



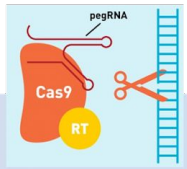
Meganuclease

Zinc Finger

TALEN



CRISPR-Cas



Prime Editor

Mechanism of Action

DNA double strand break

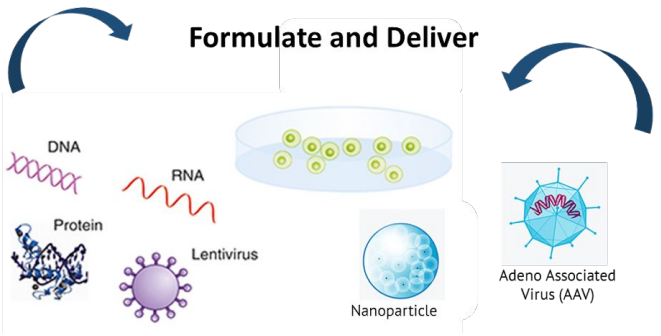
Deamination + DNA single strand break/nick

Confirm DNA alteration

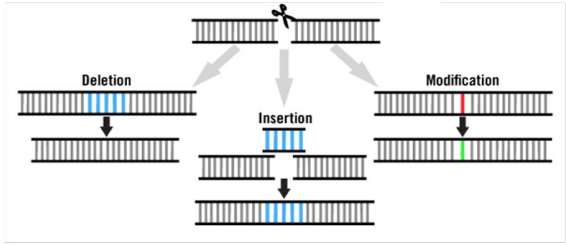
Determine fit for purpose

Durability of edit(s), cell characterization, assessment of immune response

Formulate and Deliver



Possible Edit Generated



Where may there be off-target activity?

- Assays to detect where editing molecules cut/nick DNA
- Demonstration these assays are reliable to report nominated off-target genomic positions

What genome variants were generated?

- Report off-target edits locations
- Report edits generated on-target & off-target
- Report relative frequency of edits
- Demonstrate these assays are reliable and can detect variant types/sizes.

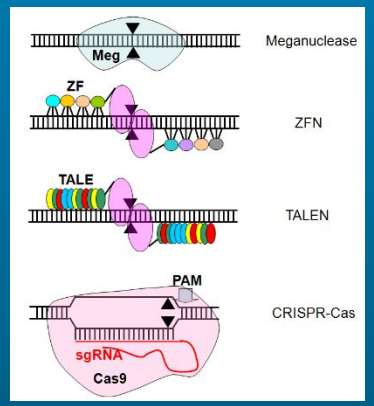
How do we evaluate and compare delivery systems?

- Delivery systems are varied and expanding, may be part of an *ex vivo* cell engineering process or your final product, but there are no norms on how to evaluate them and compare

What are resources or practices to get most use our of data, understand if data is comparable, and understand bioinformatics performance?

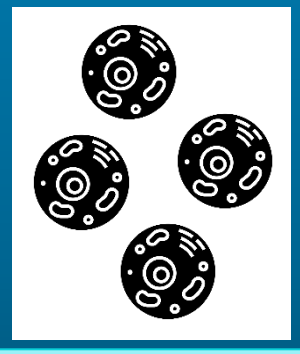
- Metadata norms and infrastructure to capture and share metadata
- Standard datasets and interlab comparisons

Genome Editing Process



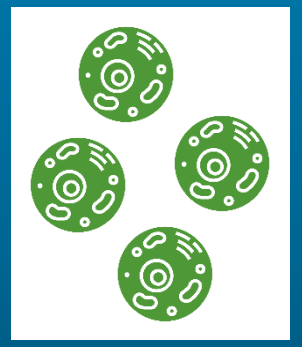
Genome Editing Molecules

+



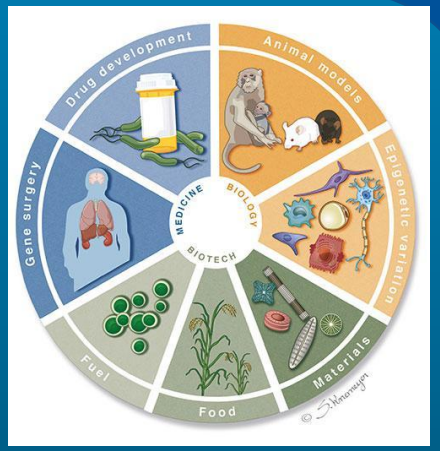
Cells of interest (*in vivo* or *ex vivo*)

DNA sequence change



Edited Cells

=



Fit for purpose?

Measurements + control samples

Data + control datasets

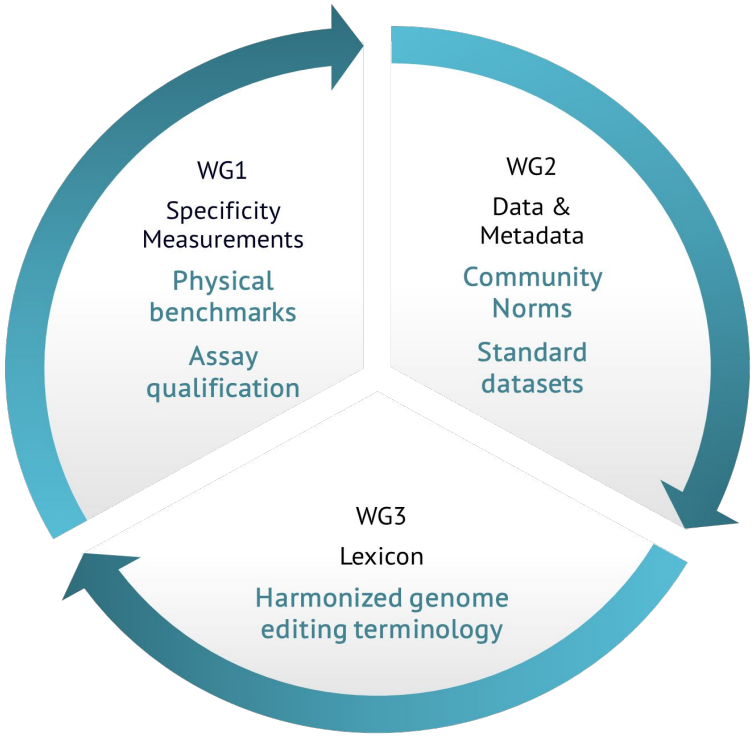
Metadata

NIST Genome Editing Consortium (launched October 2018, still accepting members)

MISSION

Convene experts across academia, industry, non-profit & government to addresses the measurements and standards needed to increase confidence of utilizing genome editing technologies in research and commercial products

ORGANIZATION



Cost sharing model. All members contribute $20,000 annually or *in-kind*

MEMBERS

- Abigail Wexner Research Institute at Nationwide Children's Hospital
- Agilent*
- Aldevron
- Applied StemCell
- AstraZeneca
- Bionano Genomics
- Bio-Rad*
- BioSkryb Genomics
- Bluebird bio*
- Broken String Biosciences
- CRISPR QC
- Caribou Biosciences
- Catalytic Data Science
- Cergentis
- COBO Technologies
- **College of American Pathologists (CAP)**
- CRISPR Therapeutics
- DARPA
- DowDuPont Agroscience (Corteva)*
- Editas Medicine
- EMBL-EBI
- Emerzene Inc
- **FDA CBER**
- Genomic Vision
- Revvity (Horizon Discovery)
- Illumina
- Inscripta *
- Integrated DNA Technologies
- Intellia Therapeutics
- KromaTiD
- Lonza*
- Psomagen (Macrogen)
- Mass General Hospital
- Metagenomi
- Mission Bio
- Novartis
- New England Biolabs
- NIH/NINDS
- NIH SCGE
- Pfizer
- Precision Biosciences
- Resiliance US, Inc.
- Sangamo Therapeutics
- Scribe Therapeutics
- SeQure Dx
- St. Jude Children's Research Hospital
- Synthego
- ThermoFisher Scientific
- Twinstrand Biosciences
- UCSC
- Verve Therapeutics
- WhiteLab Genomics

* Former members

NIST coordinates with FDA Center for Veterinary Medicine (CVM)

NIST Genome Editing Consortium Progress

WG1: Specificity Measurements

Develop cell and DNA based control materials and test via interlab analysis (Genome in a Bottle and Human iPSCs)

Documented process, baseline data, mixture studies, interlab studies, benchmark data

DNA and cells representing a variety of DNA sequence benchmarks "looks like a genome editing output" Deeply characterized at benchmark locations

WG2: Data & Metadata

- Community norms for data formats and tools for benchmarking data analysis (*in silico* and experimental data sets)
- Identify metadata that would be needed to be shared, housed, and interrogated from genome editing experiments and develop tools to accelerate metadata sharing

✓ **Transfer of Knowledge**
✓ **Reproducibility**

What are the data we are generating?
When do we need to collect the metadata?
How do we store data and metadata?
Where do we store data and metadata

metadata from consortium studies and benchmark datasets as shared resources for the community

WG3: Lexicon

Identify terms and related definitions to form a common genome editing community lexicon

WG1 – Progress:

- A set of Phase 1 DNA and cell based control materials have been generated and an interlab study is completed with final data analysis in progress
- Additional engineered cell controls are in progress with some clonal cell lines completed

WG2 – Progress:

- Phase 1 metadata entries and template completed
- Metadata schema in progress with test database integration
- Testing use cases and user interfaces, and interoperability of a metadata standard format and database(s) to house records.

WG3 – Progress:

- ISO Standard for *Genome Editing Vocabulary* released with update July 2022

Physical Measurement samples & data

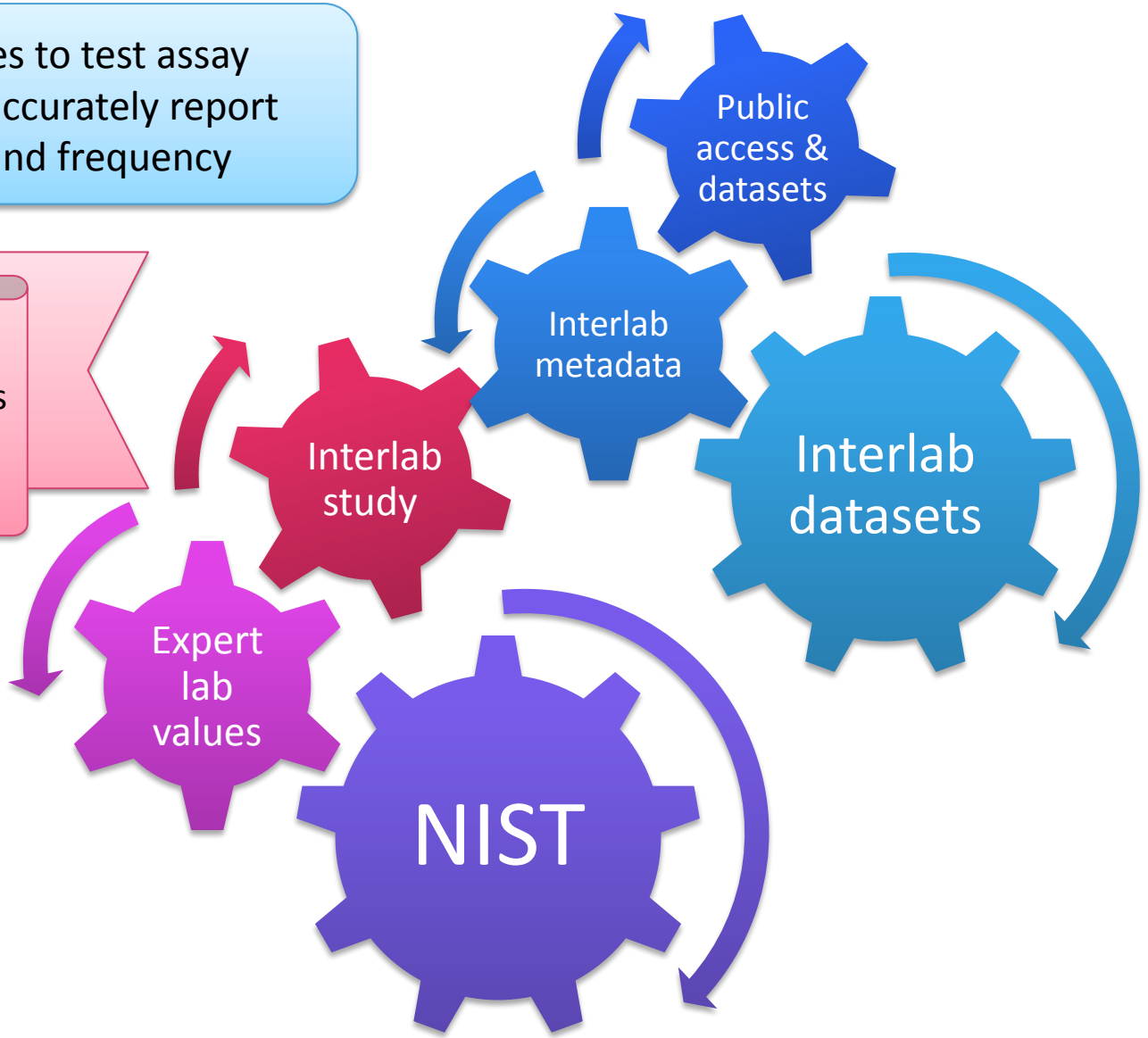
WG1: Specificity Measurements

Goal:
Develop DNA/cell based control materials and test analytical methods via interlab analysis

DNA and cells representing a variety of DNA sequence benchmarks "looks like a genome editing output" Deeply characterized at benchmark locations

Blinded studies to test assay capabilities to accurately report variant size and frequency

Interlab #1 Data analysis and release in process



Interlab Study design using unedited Genome in a Bottle samples

Blinded study to test assay capabilities to accurately report variant size and frequency

DNA mixtures

Cell mixtures

- Participants provided a **list of Positions of interest**, but **are blinded to the genomes uses, the variant(s), and variant frequencies**
- Samples made and qualified by NIST and expert control labs for sequence and variant frequency
- Samples bottled and shipped by NIST to interlab participants in relevant formulations for their technology (purified DNA mixtures or cell mixtures)
- Interlab participants perform DNA detection as they would normally or to test any technology of interest to verify DNA variants after editing

Variant frequency

5 required samples with benchmark variant frequencies:

- ✔ 0 %
- ✔ 0.1 – 0.25 %
- ✔ 0.5 – 2 %
- ✔ 5 – 10 %
- ✔ > 30 % (high variant control)

2 optional samples

- ➢ 0.01 – 0.025 %
- ➢ 0.001 – 0.0025 %

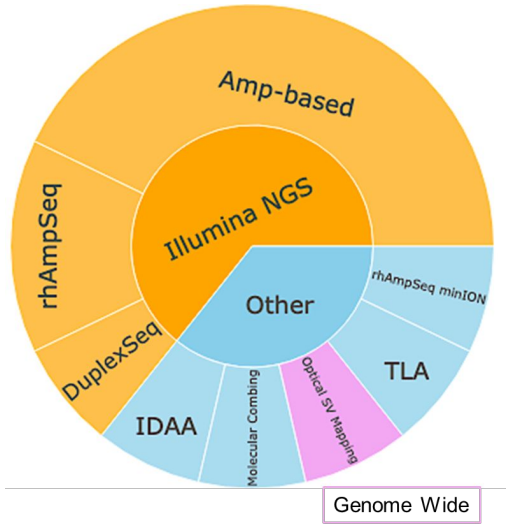
Variant sequence identity (length and/or sequence)

Substitutions	< 15 bp del	< 50 bp del	~ 300 – 500 bp del	> 2 kb ins
Small complex	< 15 bp ins	< 50 bp ins	~ 300 – 500 bp ins	~ 10 kb del
1 bp del	< 25 bp del	~150 bp del	~ 1 – 2 kb del	~ 10 kb ins
1 bp ins	< 25 bp ins	~ 150 bp ins	~ 1 – 2 kb ins	> 50 bp del

Justin Zook

Nate Olson

Overview of interlab participation



14 total participants
(2 of which were unblinded)

	6 Technology Users	8 Technology Makers
Blinded:	6	6
Unblinded:	0	2
Cells:	0	5
DNA:	6	3
Illumina NGS-based:	6	5
DNA electropherogram:	0	1
DNA imaging/microscopy:	0	2
# Replicates:	1-4	1-4
# Workflows per submitter:	1-2	1-2

NOT IDENTIFIED with their data

IDENTIFIED with their data

TECHNOLOGY MAKERS:
Bionano Genomics, Inc.
BioSkryb Genomics
Cergentis
COBO Technologies
Genomic Vision
Illumina
MissionBio
TwinStrand Biosciences

TECHNOLOGY USERS:
CRISPR Therapeutics
Editas Medicine
IDT
Intellia Therapeutics
St. Jude Children's Reseach Hospital

[additional participants not listed]

Metadata & Data Return

Structured Experimental Metadata

- ❑ Date
- ❑ Sequencing Assay Type
- ❑ Sequencing Reagents or Kit
- ❑ DNA Concentration per reaction (ng)
- ❑ # Library Replicates
- ❑ UMI - used/index or in-line/length
- ❑ Multiplex PCR process
- ❑ Sequencing Platform
- ❑ Flowcell kit or Capillary type
- ❑ # Libraries pooled onto flowcell
- ❑ # Libraries pooled per lane of flowcell
- ❑ Read depth per panel
- ❑ Re-Quanitifcation instrument/kit
- ❑ Re-Quantification concentration (ng/uL)

Structured Results Reporting

- ❑ Coordinates
- ❑ Reference Sequence
- ❑ Variant Sequence
- ❑ Variant Type (substitution, deletion, insertion, complex)
- ❑ Total Reads for Signal or Site
- ❑ Variant size (bp)
- ❑ Reads or Signal for Variant
- ❑ Variant Frequency (%)
- ❑ Confidence or Significance of Variant Call (%)
- ❑ Describe how confidence is determined

Structured Bioinformatics Metadata

- ❑ Average read length used to call variant
- ❑ Amplicon/ Average fragment length of DNA sequenced
- ❑ Median observed read length
- ❑ Software – name, version
- ❑ Parameters
- ❑ Code

Requested Raw & Additional Data

Raw

- ❑ .fsa
- ❑ .fastq
- ❑ .bnx

Processed

- ❑ .bed
- ❑ .smap
- ❑ .vcf
- ❑ .bam

Other

- ❑ QC Reports
- ❑ Images
- ❑ READMEs
- ❑ Protocols

Total Data Size:

~ 43Tb

Sierra Miller

GIAB Interlab Mixture Study: Overview & Progress

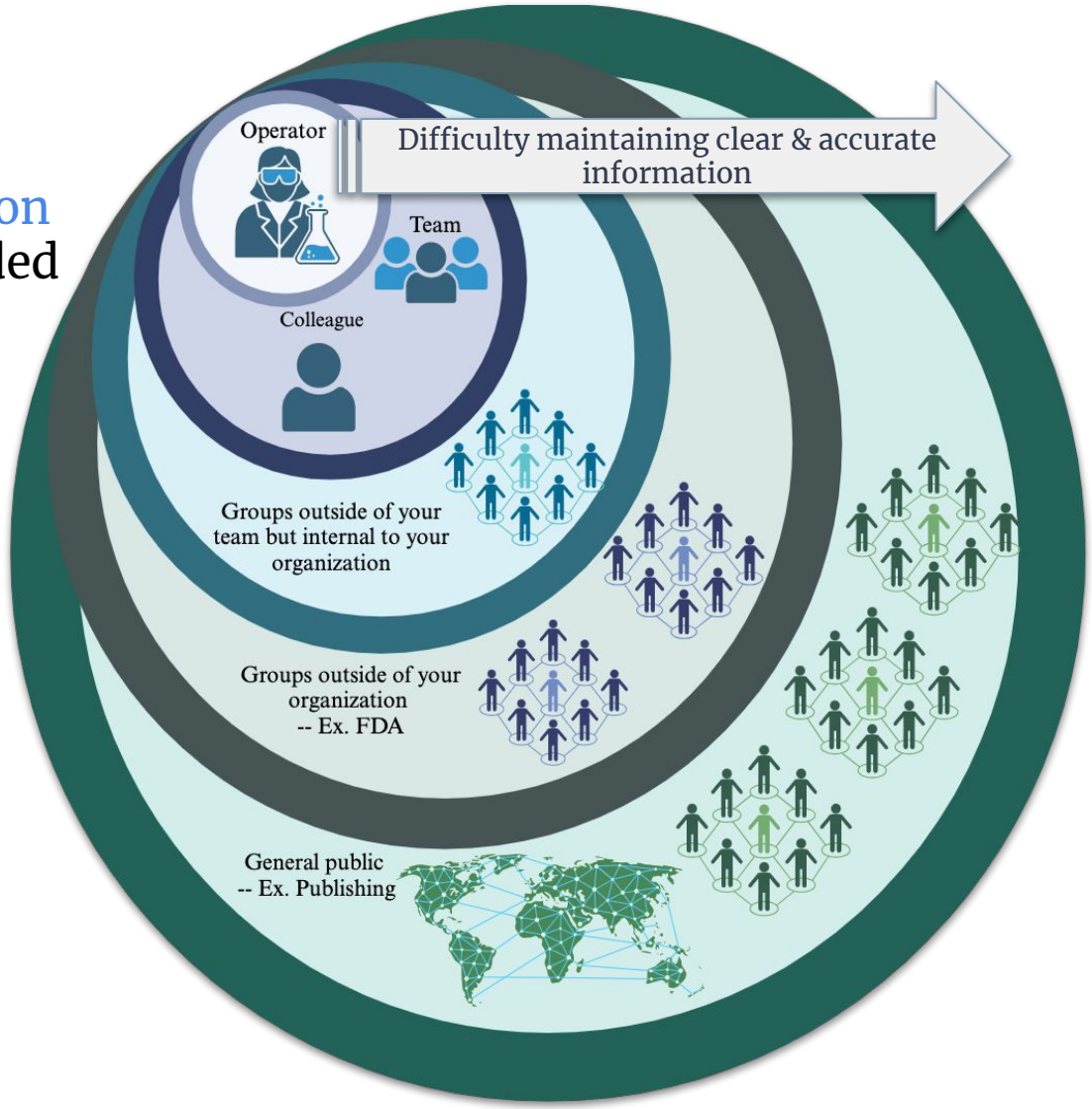
- Study design completed ✓
- List of genomic coordinates for study sent to participants to make assays ✓
- Study samples generated and qualified: WGS + ddPCR & targeted NGS ✓
- Study samples shipped to participants with data/metadata collection template ✓
- Participants perform analysis and return results, with metadata & data files ✓
- NIST compilation of metadata, data, & results and share with members ✓
- Draft paper for publication, and public release of metadata, data & results

Data & Metadata: A Need for Standards NIST

Knowledge Transfer - comprehending what that information is or is not telling you - only as good as the metadata provided

There is a **need** for **accessible**, **organized**, & **structured metadata** with **understood terms** to promote:

- scientific integrity
- reproducibility
- efficiency
- sharing
- cooperation
- knowledge transfer
- scientific advancement
- positive public perception
- Findable Accessible Interoperable Reusable (FAIR) Data Principles



Created with BioRender.com

Data & Metadata norms and tools for Genome Editing

NIST

✔ **Transfer of Knowledge**
✔ **Reproducibility**

What are the data we are generating?

When do we need to collect the metadata?

How do we store data and metadata?

Where do we store data and metadata

metadata from consortium studies & benchmark datasets as shared resources for the community

1. Metadata entry curation:

(how can this be normalized and low burden for a user)

EDITOR-PROTEIN	
metadata_field	**example**
Editor Type	(Cas, TALEN, Zinc Finger)
Reagent Type	(mRNA, plasmid, protein)
PAM Preference	(3' NGG)
Target Sequence	((i.e. for TALEN or ZFN))
Editor Cleavage Type	(blunt)
Editor Activity	(Target strand nickase)
Editor Protein Variant	(i.e., D10A)
Annotated Map	(annotated genbank sequence file, filename here)
Editor Substrate	(double stranded DNA)
Editor Alias	(NA)
Source	(IDT)
Editor Subtype	(Cas9)
Editor Species	(S. pyogenes)

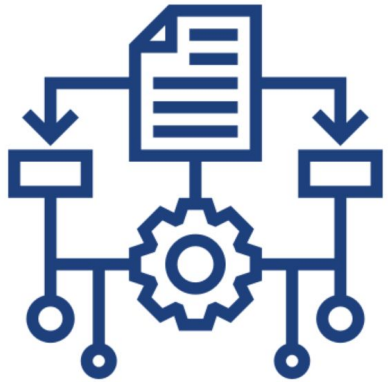
2. Metadata file format (GEM)

JSON format

1. Human readable
2. Database ready
3. Can be validated (JSON Schema)
4. Already used by NIST
5. Easy to extend

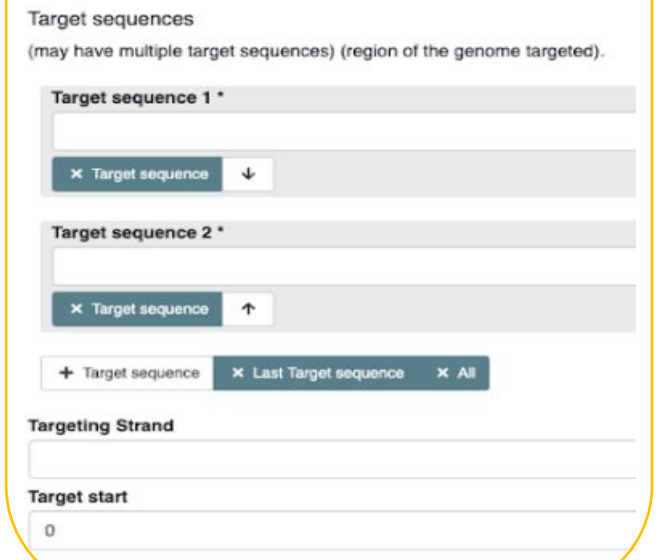
METADATA

4. Datasets as control data linked to metadata

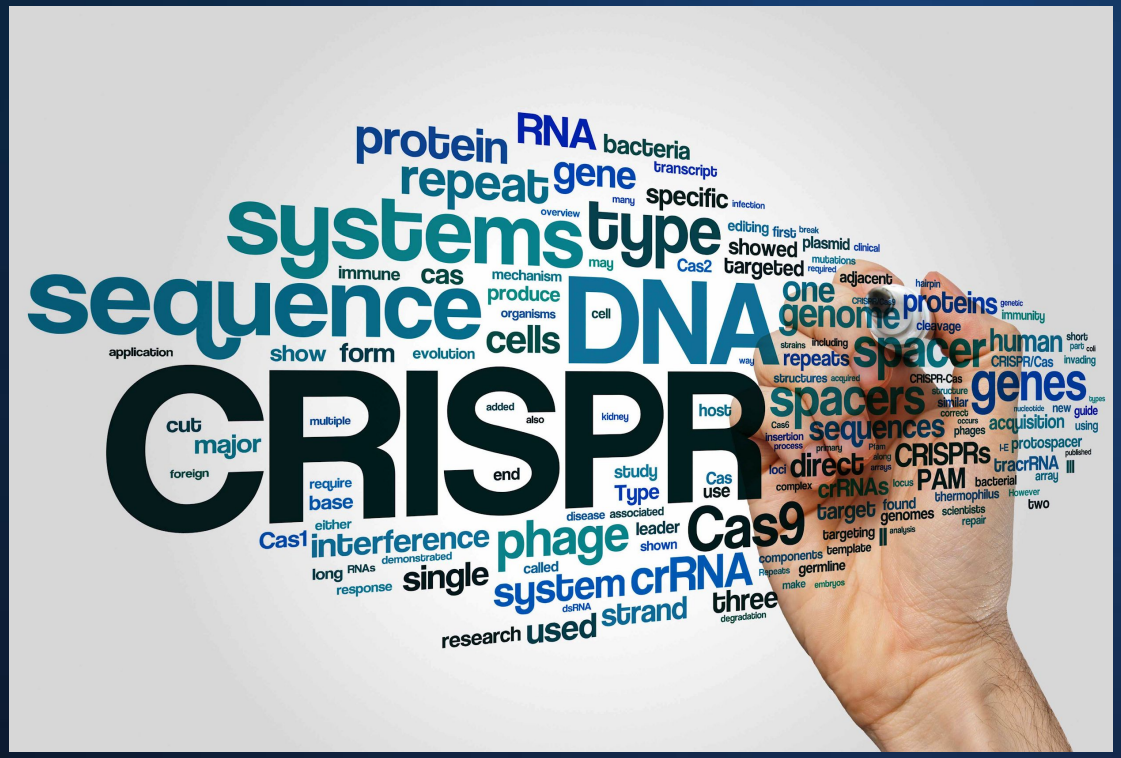


3. Metadata access

Design and feasibility of a database with easy user interface



Lexicon working group



Identify terms and related definitions to form a common genome editing community lexicon

- enable clear communication of scientific results
- facilitate effective communication with regulators (e.g., FDA, USDA)
- have the potential for international acceptance

Terms v1

Genome Editing Concepts

Gene editing
Genome editing
Genome engineering
Specificity
Target
Off-target

Genome Editing Tools

General
Site-directed nuclease
Site-directed DNA modification enzyme
Repair template

CRISPR-Specific
Guide RNA (gRNA)
CRISPR RNA (crRNA)
tracrRNA (trans-activating CRISPR RNA)
sgRNA (single-guide RNA)
PAM (protospacer adjacent motif)
RNP (ribonucleoprotein)
Cas nuclease target site
Target strand

Genome Editing Tools

Meganuclease - Specific
Meganuclease
Meganuclease single chain
Meganuclease linker
Meganuclease target site

TALEN - Specific
TALEN
TALEN linker
Repeat variable diresidue (RVDs)
TALEN target site

megaTAL - Specific
megaTAL
megaTAL linker
megaTAL target site

ZFN - Specific
Zinc Finger Nuclease (ZFN)
ZFN linker
Zinc Finger Protein (ZFP)
Zinc Finger
Recognition helix
ZFN target site

Genome Editing Outcomes

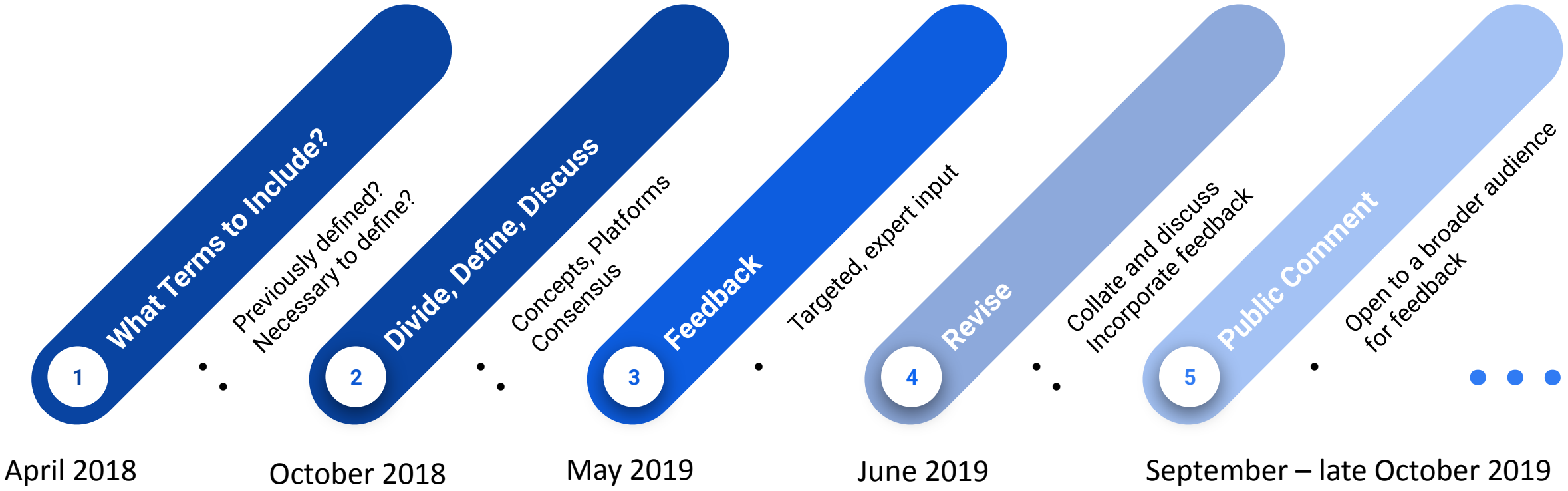
Edit
Unintended edit
Intended edit
HDR (homology-directed repair)
NHEJ (non-homologous end-joining)
Microhomology-Mediated End Joining Repair (MMEJ)
InDel mutation

NIST National Institute of Standards and Technology
U.S. Department of Commerce

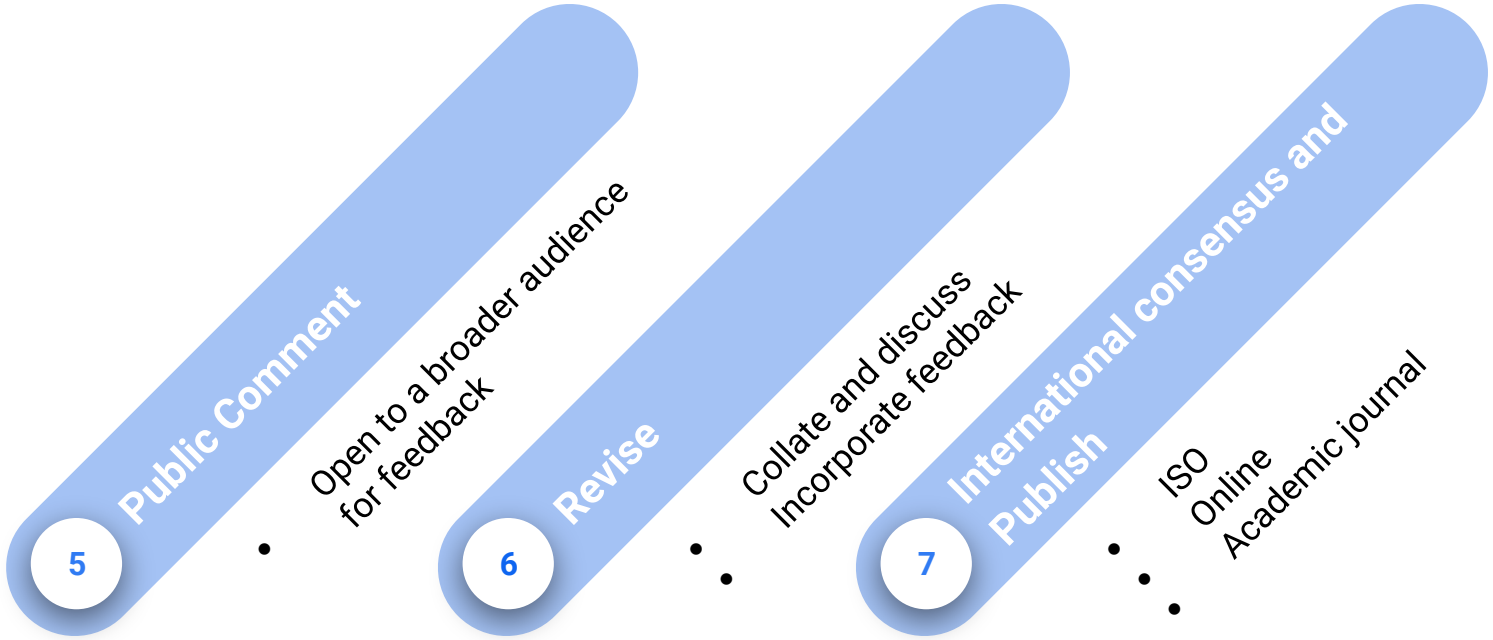
Lexicon contributing drafting organizations and expert commenters

Industry and commerce – large industry	Industry and commerce – SMEs	Government	Academic and research bodies	Non-governmental organizations
• Novartis • AstraZeneca • Thermo Fisher Scientific • New England Biolabs • Illumina • Lonza • Johnson & Johnson	• Bluebird bio • Caribou Biosciences • Cortevea Agroscience • CRISPR Therapeutics • Editas Medicine • Horizon Discovery • Integrated DNA Technologies • Intellia Therapeutics • Precision Biosciences • Sangamo Therapeutics • Synthego • Casebia Bio	• FDA • USDA • NIH	• Harvard University • St Jude Children's research hospital • The Broad Institute • MIT • Stanford University • University of California Berkeley • The Jackson Labs • The University of Copenhagen • The CRISPR Journal • NC State University • The University of Massachusetts Medical School • UCSF • UCSC	• EMBL-EBI • The World Health Organization • Alliance for Regenerative Medicine • USP

Process prior to ISO submission



Process prior to ISO submission continued



September – late October 2019

Dec 2019 – proposed as new project at ISO meeting
May 2020 – ISO project began…

ISO 5058-1:2021 Genome editing – Part 1:Vocabulary

https://www.iso.org/standard/80679.html

ICS › 07 › 07.080

ISO 5058-1

Biotechnology —

ABSTRACT PREVIEW

This document defines terms related to gen

This document is applicable to general use

GENERAL INFORMATION

Status : Published

Edition : 1

Technical Committee : ISO/TC 276 Biotechn

ICS : 07.080 Biology. Botany. Zoology | 01.((Vocabularies)

https://www.i

Online Brows

Searc

ISO 5058-1:2021(en) Biote

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3.1 Genome editing concepts
3.2 Genome editing tools
3.3 Genome editing outcomes
4 Abbreviated terms
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https://www.iso.org/obp/ui/#iso:std:iso:5058:-1:ed-1:v1:en

Online Browsing Platform (OBP)

Search ISO 5058-1:2021(en) ×

ISO 5058-1:2021(en) Biotechnology — Genome editing — Part 1: Vocabulary

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3 Terms and definitions

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1 Genome editing concepts

3.1.1

gene editing

techniques for **genome engineering** (3.1.3) that involve nucleic acid damage, repair mechanisms, replication and/or recombination for incorporating site-specific modification(s) into a gene or genes

Note 1 to entry: Gene editing is a subclass of **genome editing** (3.1.2).

Note 2 to entry: There are various genome editing tools (see 3.2 and Figure 1).

3.1.2

genome editing

techniques for **genome engineering** (3.1.3) that involve nucleic acid damage, repair mechanisms, replication and/or recombination for incorporating site-specific modification(s) into a genomic DNA

Note 1 to entry: **Gene editing** (3.1.1) is a subclass of genome editing.

Note 2 to entry: There are various genome editing tools (see 3.2 and Figure 1).

Genome editing technology is a fast-growing and rapidly advancing global bioscience field with applications in many biotechnology sectors. Genome editing is used to modify the nucleic acids of a genetic code, which can be composed of DNA or RNA, in a site-specific manner. Modifications can include insertion, deletion or alteration of nucleic acids. The technology operates by biochemical

Ontology for Genome Editing Lexicon now in BioPortal

BioPortal Ontologies Search Annotator Recommender Mappings sdmiller Support

NIST Genome Editing Lexicon

Last uploaded: December 8, 2021

Summary | Classes | Properties | Notes | Mappings | Widgets

Details

Acronym	NIST_GEL
Visibility	Public
Description	Genome editing technology is a fast-growing and rapidly advancing global bioscience field with applications in many biotechnology sectors. Genome editing is used to modify the nucleic acids of a genetic code, which can be composed of DNA or RNA, in a site-specific manner. Modifications can include insertion, deletion or alteration of nucleic acids. The technology operates by biochemical principles generally applicable to every kind of cell. Examples of genome editing technology applications with global significance include human cell-based therapeutics, agriculture, microbial based therapeutics, synthetic biology and biomanufacturing. While genome editing technology is being actively utilized, there is a need for international standardization in terms and definitions for this field, so as to enhance interpretation and communication of concepts, data and results. This document has been developed to provide a unified standard set of terms and definitions that serve the needs of biotechnology stakeholders and act as a reference for genome editing technology. Standards in the field of genome editing are intended to harmonize and accelerate effective communication, technology development, qualification and evaluation of genome editing products. This document is expected to improve confidence in and clarity of scientific communication, data reporting and data interpretation in the genome editing field. Specific requirements for the application of genome editing technologies in agriculture and food are not included. For specific requirements, users can consult standards developed by appropriate ISO Technical Committees, e.g. ISO/TC 34/SC 16 Horizontal methods for molecular biomarker analysis, or ISO/TC 215 Health informatics.
Status	Production
Format	OWL
Contact	Sierra D. Miller, sierra.miller@nist.gov
Categories	Gene Product, Genomic and Proteomic, Molecule

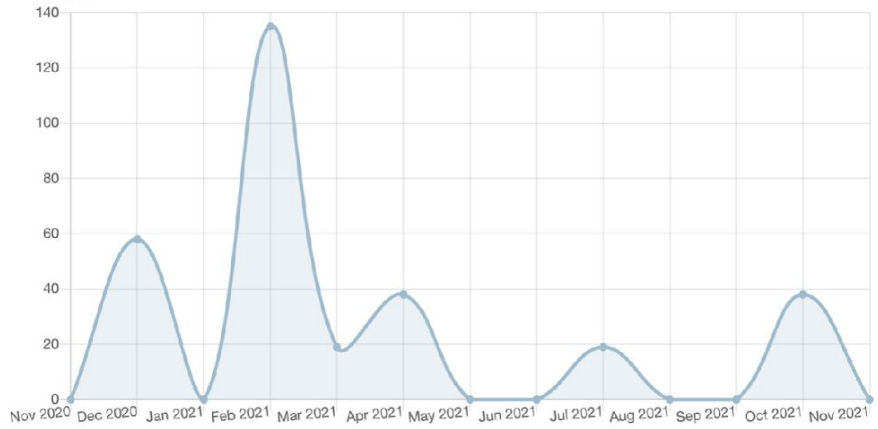
Submissions

Version	Released	Uploaded	Downloads
2.1 (Uploaded)	11/23/2020	12/08/2021	OWL
2.0 (Parsed, Metrics, Annotator, Error Indexed)	11/23/2020	12/01/2021	OWL | CSV | RDF/XML | Diff
1.3_relationship_test (Archived)	11/23/2020	02/11/2021	OWL | Diff
1.2 (Archived)	11/23/2020	01/21/2021	OWL | Diff
1.1 (Archived)	11/23/2020	11/30/2020	OWL | Diff

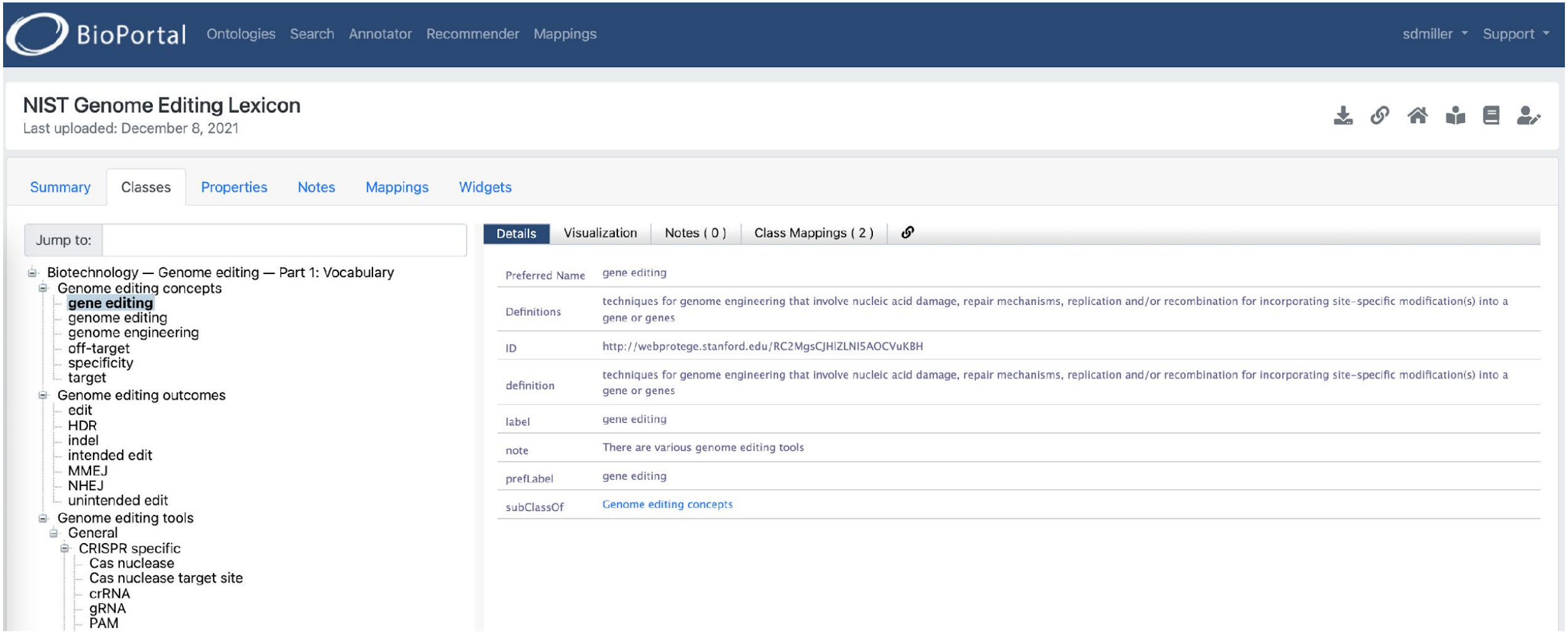
Metrics

Classes	52
Individuals	0
Properties	9
Maximum depth	4
Maximum number of children	9
Average number of children	4
Classes with a single child	2
Classes with more than 25 children	0
Classes with no definition	10

Visits



Ontology for Genome Editing Lexicon now in BioPortal



NIST Consortia

GENOME IN A BOTTLE (GIAB) CONSORTIUM

Provides authoritative characterization of benchmark human genomes

POC: Justin Zook

GENOME EDITING CONSORTIUM

Addresses the measurements and standards needed to increase confidence and lower the risk

POC: Samantha Maragh

FLOW CYTOMETRY STANDARDS CONSORTIUM

Addresses the measurements and standards needed for flow cytometry applications

POC: Lili Wang

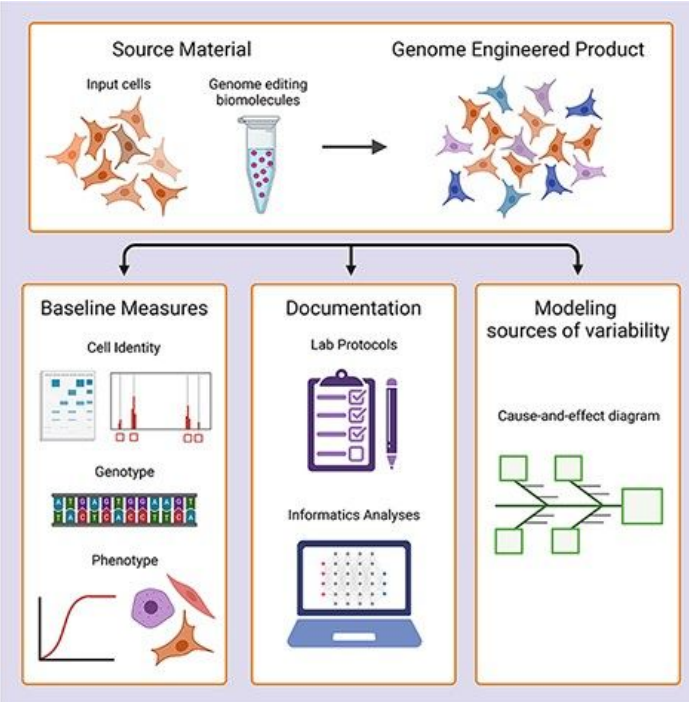
RAPID MICROBIAL TESTING METHODS CONSORTIUM

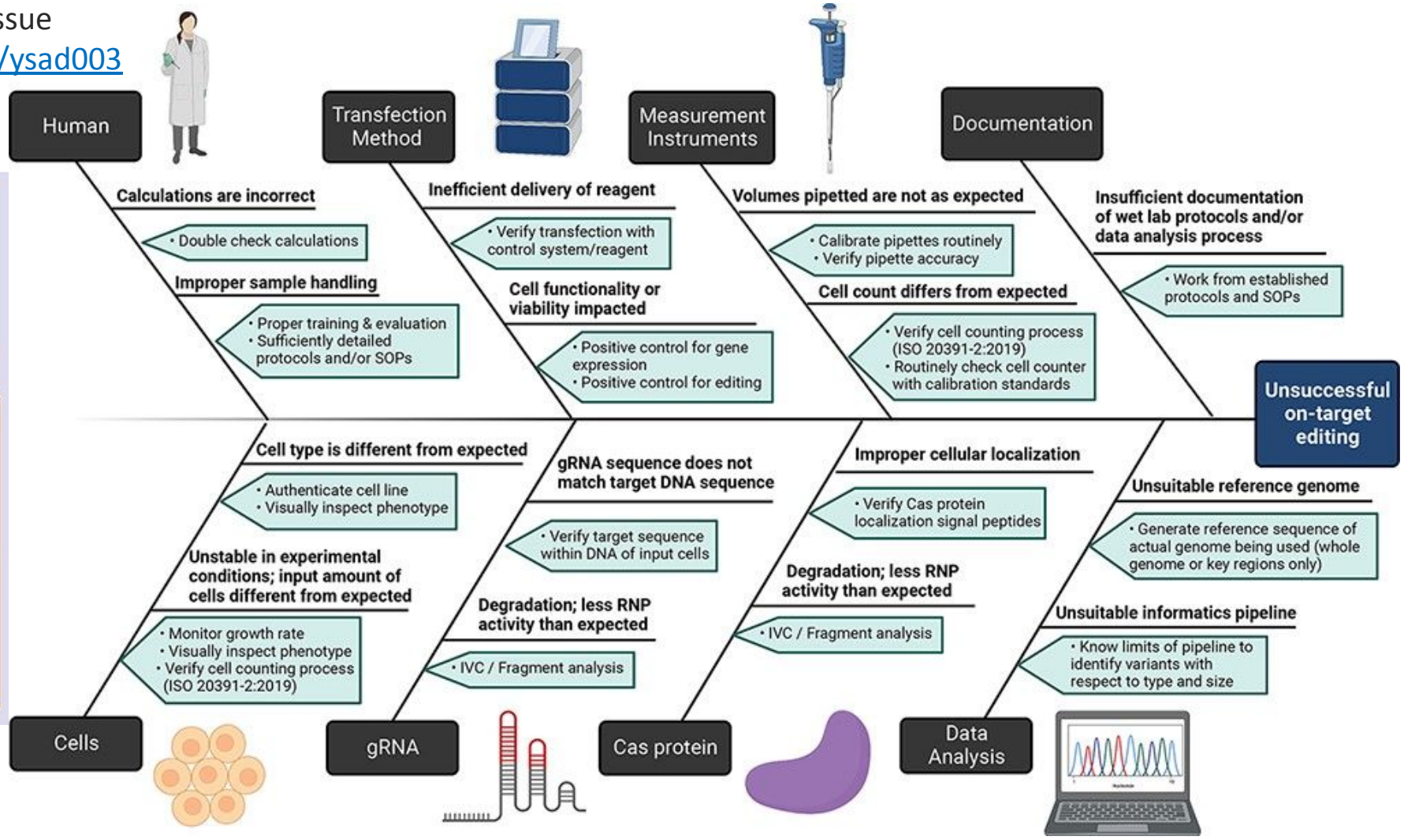
Addresses the measurements and standards needed to increase confidence and lower the risk

POC: Nancy Lin

Variability in genome-engineering source materials: consider your starting point

Simona Patange, Sierra Miller, Samantha Maragh, *Synthetic Biology*, Volume 8, Issue 1,2023 https://doi.org/10.1093/synbio/ysad003





Fire Burn and Cauldron Bubble: What Is in Your Genome Editing Brew?

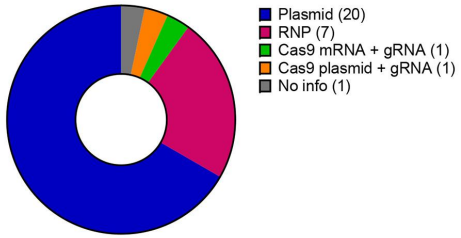
Simona Patange and Samantha Maragh

Biochemistry DOI: 10.1021/acs.biochem.2c00431

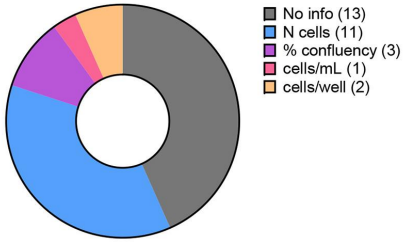
A Reporting of CRISPR reagents in 30 publications

		Cas9				
		No info	grams	mols	g/L	M
gRNA	No info	9				
	grams		14			
	mols		1	2		
	g/L				1	
	M					3

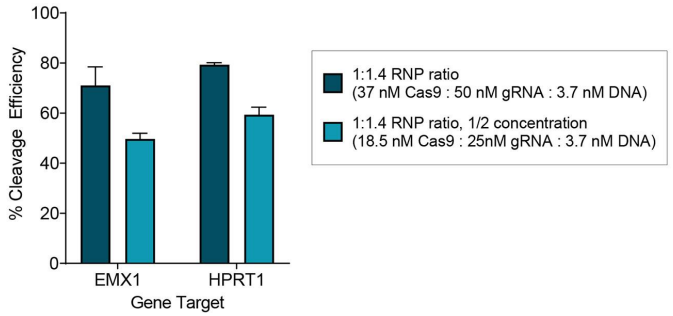
B Editing Molecule Type



C Reporting of cell quantity



D



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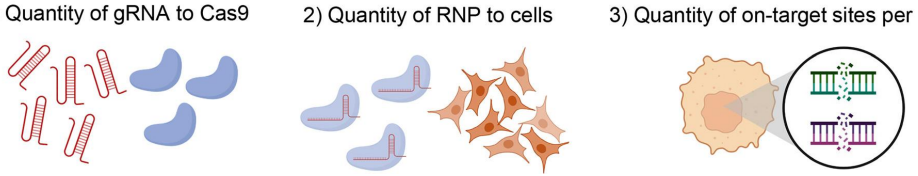
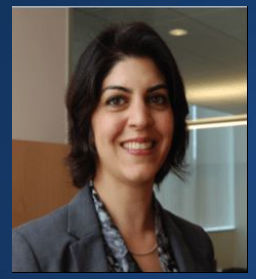


concept	recommendation
editing formulation	• report the formulation type (plasmid, RNA, RNP, etc.)
	• report any co-introduced reagents (HDR donor or fluorescent tracer)
reagent source	• report the source for each reagent used
	• from a donating lab: include citation to previous work where available
	• commercially purchased: vendor and item number
	• formulated in lab: details on how reagent was generated
numerical values and units	• reporting molar amounts is recommended; this could be reported as the starting molarity and the volume used, or the final molarity in the editing formulation
	• if reporting relative ratios (stoichiometry) of editing biomolecules
	• report the molecule identity to which the ratio corresponds, for example, 1:1.4 Cas9:gRNA
	• report numerical value and units of mass, concentration, and/or molarity for at least one component in the formulation so that the other component values can be calculated (see Figure 2D for an example)
	• if a plasmid is used, sufficient information should be provided to calculate the number of plasmid molecules in the formulation. This could be reported as follows
	• molar amount of plasmid, for example, 100 nM plasmid
	• mass amount of plasmid with nucleotide length, for example, 1 μg of plasmid, 8505 bp
	• mass amount of plasmid with molecular weight (MW), for example, 1 μg of plasmid, 5.26×10^6 g/mol
	• mass amount of plasmid with sequence or reference by which a reader can calculate the nucleotide length or MW of the plasmid construct, for example, 1 μg of plasmid, Addgene #71814
cell quantity	• reporting the number of cells that were treated with editing formulation is recommended
	• if reporting cell quantity in other units, sufficient information should be provided to obtain the cell number. This could be reported as follows
	• cell concentration and volume, for example, 200 μL of 1×10^6 cells/mL
	• cells per well and plate dimensions, for example, 1×10^5 cells/well in a six-well format (9.6 cm^2)
	• percent confluency and plate dimensions, for example, 70% confluency in a six-well format (9.6 cm^2)
delivery method	• report the delivery system used (lipid encapsulation, microinjection, electroporation, etc.)
	• report the source/vendor, instrument information, and the delivery parameters (include values and units)
assessments	• separate the concepts of delivery, localization, and editing when designing, executing, and interpreting experimental results
	• report the values and calculation used when describing a measure of performance (percent editing, delivery efficiency, etc.)
	• report on the time point(s) at which measurements were made

Thank You NIST Colleagues!


Natasha Kolmakova


Alex Tona


Tara Eskandari


Sierra Miller


Simona Patange


Patty Kiesler


Ayah Shevchenko

NIST Genome Editing Consortium Members & Other external collaborators


Justin Zook


Nate Olson


Zach Trautt


Arlin Stoltzfus


Hua-Jun He


Jamie Almeida

National Institute of Standards and Technology
U.S. Department of Commerce

Samantha Maragh
samantha@nist.gov
https://www.nist.gov/programs-projects/nist-genome-editing-consortium

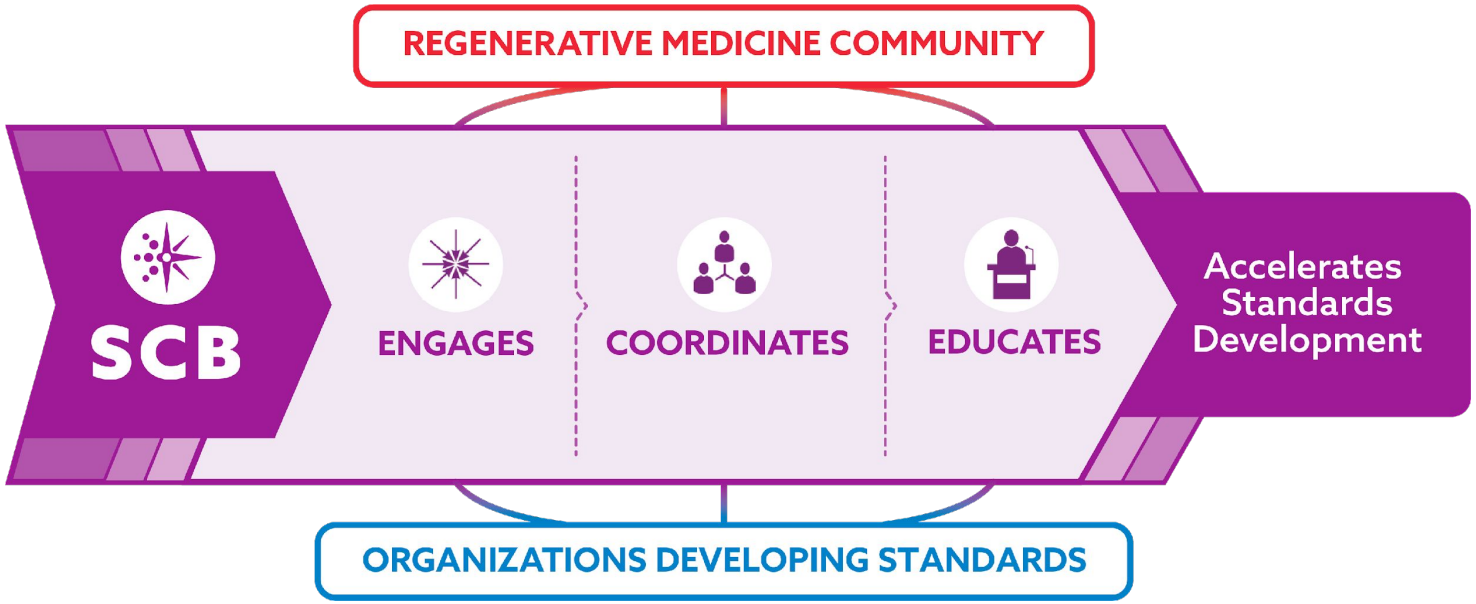
Standards Coordinating Body

November 16th, 2023
Standards workshop

Dawn Henke
Senior Scientific Program Manager

STANDARDS COORDINATING BODY
REGENERATIVE MEDICINE

Connecting the regenerative medicine community to standards development



Established in 2016 and launched in January 2017, SCB is an **independent 501(c)(3)** organization

Occupies unique niche within field with **no vested interests in specific scientific, commercial, clinical or policy approaches**

SCB is **not an SDO**, but rather **coordinates** the standards development process

Serves as **communication vehicle** among all stakeholders, including government agencies, critical to the development of standards

SCB Bringing value to patients and the community

MISSION: Coordinate the accelerated advancement and improved awareness of standards and best practices that address the rapidly evolving needs of the global regenerative medicine advanced therapy community

VISION: Improve patient lives through the widespread use of standards that enhance the consistency, availability, efficacy, quality, and safety of regenerative medicine therapies

STANDARDS COORDINATING BODY
REGENERATIVE MEDICINE

Benefits of Standards

Standards Recognition Program

Regulations, Guidances, and Standards

Regulations:
Have the force and effect of law and are usually mandatory, setting out specific requirements that regulated 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:
Formal documents issued by a government agency **to clarify** the agency's **thinking on existing laws or regulations** and offer guidelines for how industry **can comply with these regulations**.

Standards:
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.

Different standards types include:

- **Documentary Standards**
- Standard Reference Material
- Standard Reference Data

Regulatory perspective on standards

- Regulatory have clearly expressed the preference for the use of consensus based standards in the approval process when applicable

Voluntary Consensus Standards Recognition Program for Regenerative Medicine Therapies

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Guidance for Industry

Recognition program for Standards

- Finalized on Oct 20th
- Guidance outlines a program for the FDA to **vet and formally recognize standards** that are applicable to regulatory approval of regenerative medicine products
- Standards must apply to re**gulatory approval for regenerative medicines** and be consensus
- Public can submit standards they believe fit criteria for vetting by the FDA
- The list of recognized standards is not available yet but will be shortly
- SCB plans to work with the FDA to develop a **webinar in January** to go into detail about this program and the standards that are recognized

Standards Resources

Regenerative medicine standards portal

The SCB Regenerative Medicine Standards Portal offers an easily searchable and filterable database of hundreds of regenerative medicine standards across 25+ organizations.

Custom search by

- Keywords
- Sector
- Functional area
- Standard organization
- + more

Updated at least monthly to ensure accurate information on the entire regenerative medicine standards landscape.

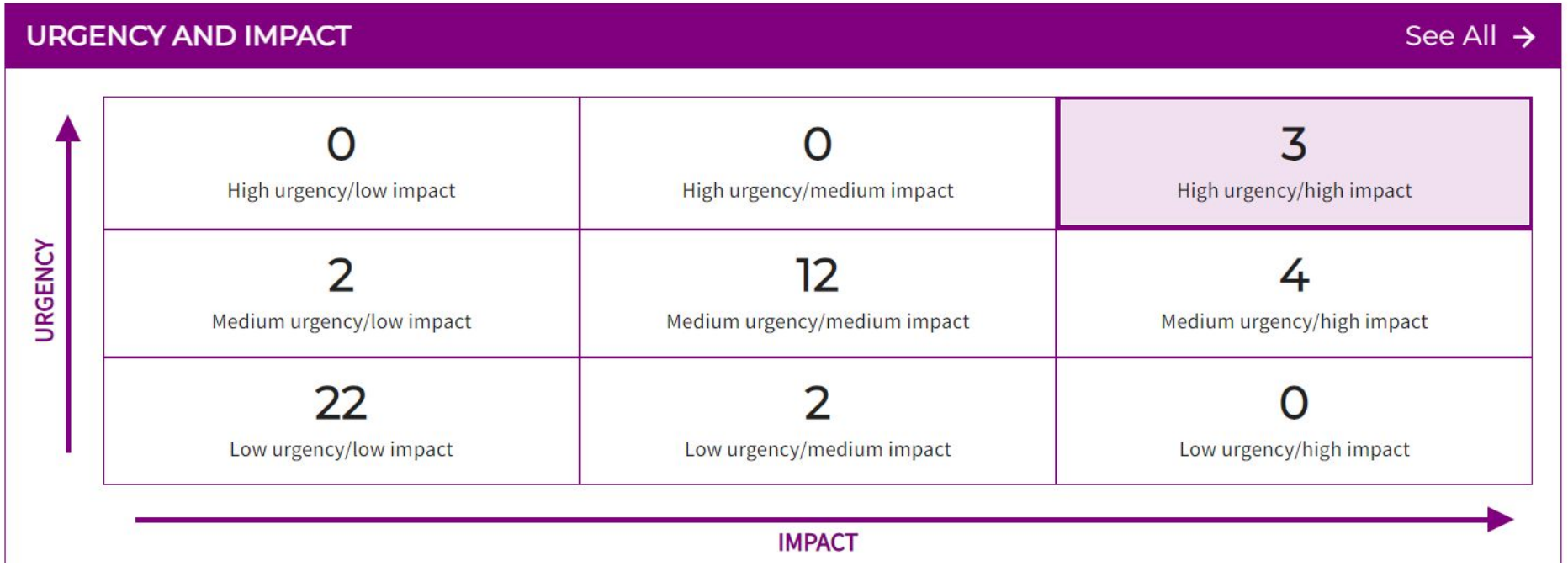
Needed regenerative medicine standards

To provide feedback for the next update, please fill out the needed standards survey.

Currently we are collecting responses for the update as part of the FDA contract. We will pull responses from the survey on 10/30

Based on the feedback, the chart below is updated semi-annually to reflect the community's prioritization perspectives.

TAKE THE SURVEY



The needed standards survey can be found at: https://www.standardscoordinatingbody.org/needsurvey

Open Ballots

1. ISO/NP 23494-2 Biotechnology — Provenance information model for biological material and data — Part 2: Common Provenance Model
   Closing: 30-Nov-23

1. ISO 20387:2018 Biotechnology —5 Biobanking — General requirements for biobanking
   Closing: 2-Dec-23

1. IG-050 Now Open for Public Comment - ICCBBA implementation guide for CoI identifier guidance
   Closing: Nov 20

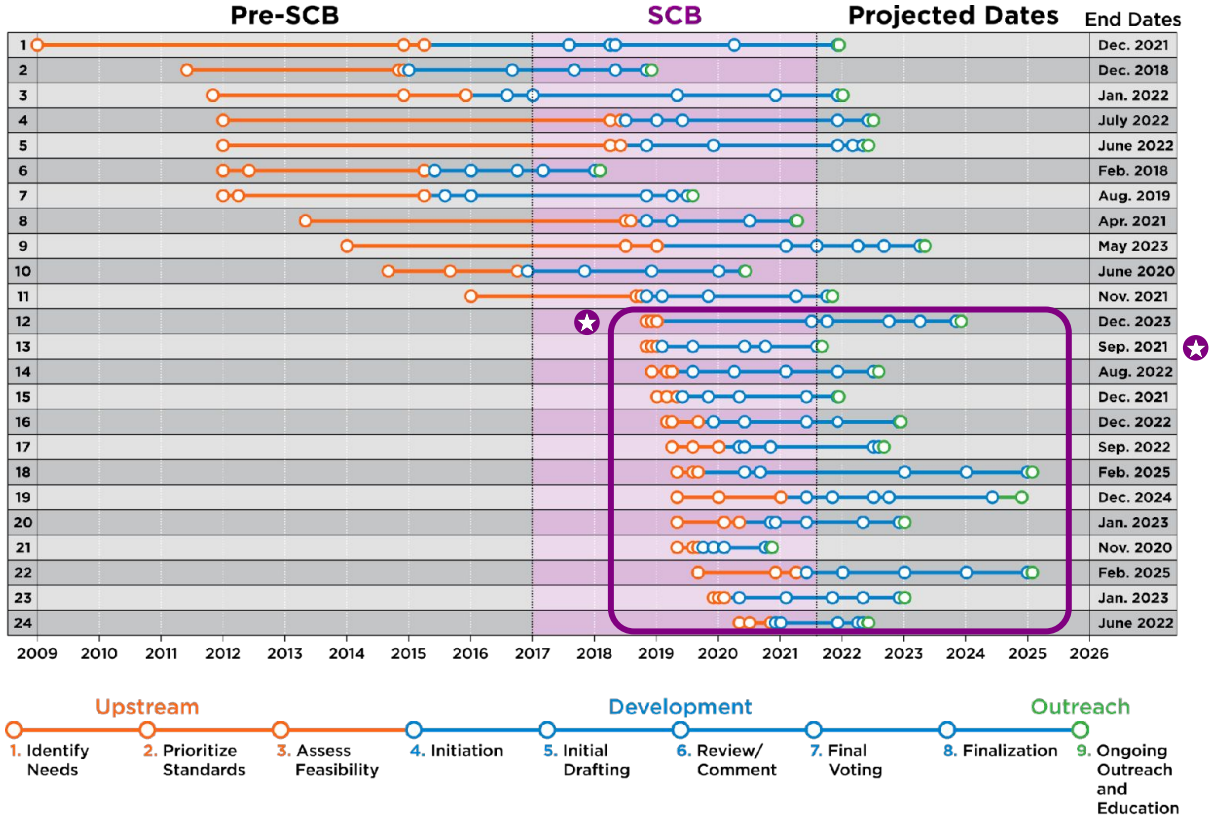
1. Guide for Bioinks Used in Bioprinting WK74668 PDF (1144K)

1. ISO/PWI 21085 Biotechnology — General requirements for the measurement of ultra-low concentration samples of target nucleic acid sequences
   Closing: 07-Dec-23

Standards Coordination

SCB: accelerating standards advancement

SCB involvement has significantly reduced the time spent on upstream steps, allowing needs to be addressed more quickly.



1. Characterization of Human Cells for Therapeutic Use
2. Ancillary Materials Used in Cellular Therapy Production (3-Part TS)
3. Requirements for Cell Therapy Manufacturing Equipment
4. Rapid Microbial Testing Method Design and Validation Framework
5. Sampling Methods of Tissue Engineered Medical Products for Sterility Assurance
6. General Guidance on Cell Counting Part 1
7. General Guidance on Cell Counting Part 2
8. Characterization of Fiber-Based Scaffolds
9. Cell Collection Standards for Cell Therapies
10. Transportation Requirements of Cells for Therapeutic Use
11. Bioink Printability Test Method
12. Evaluating Pre-existing Immunity to Adeno-Associated Viruses
13. Cryopreservation of Cells (PDA-led project)
14. Bioprinter Hardware
15. Ancillary Materials used in Cellular Therapy Production (IS)
16. Bioprinter Software/Data Governance
17. ASME Thermal Medicine Tissue Properties
18. Base Requirements for Digital Platforms for Providers
19. Viral Vectors (Lenti/AAV) for Gene Therapy
20. Microphysiological Systems
21. Base Labeling Requirements for Regenerative Medicine Products
22. Cell Viability
23. Tissue Engineering Lexicon
24. Chain of Custody (COC)/Chain of Identity (COI)

Key: Prioritized for Coordination Feasibility Reports Both

**Availability dates are estimates only. Development of a standard depends on SDO timelines, which can be time intensive and may vary significantly (particularly for reference materials).

FDA/Nexight Standards Development for Regenerative Medicine Therapies Contract

Timeframe: 9/22/2022-9/21/2024 (base year and 1 option year)

Status: Base Year approved and work began

Description: Accelerate and increase community engagement in the development of regenerative medicine standards

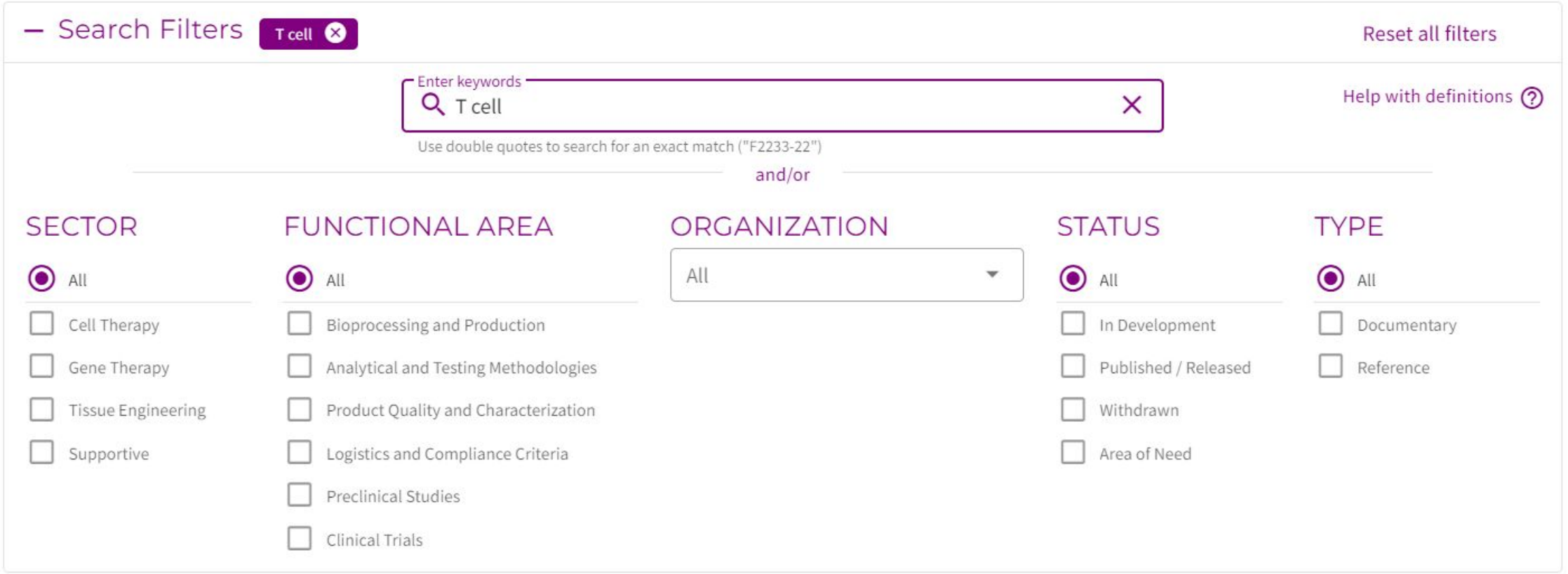
Highlights:

- Conduct Workshop on feasibility topics:
  - Methods for the Evaluation of Endogenous T-Cell Therapies
  - Methods for Assessing Gene Therapy Product Activity and Comparability
  - Feasibility assessments planned for spring 2024
- Update the Needed Standards report Work with experts to coordinate high-priority standards efforts
  - Needed standards will be updated by the end of this month
- Integrate and maintain the landscape of standards in the standards portal
- Provide ongoing reporting of engagement with experts and experts' engagement with standards

SCB Supported NIST Consortia

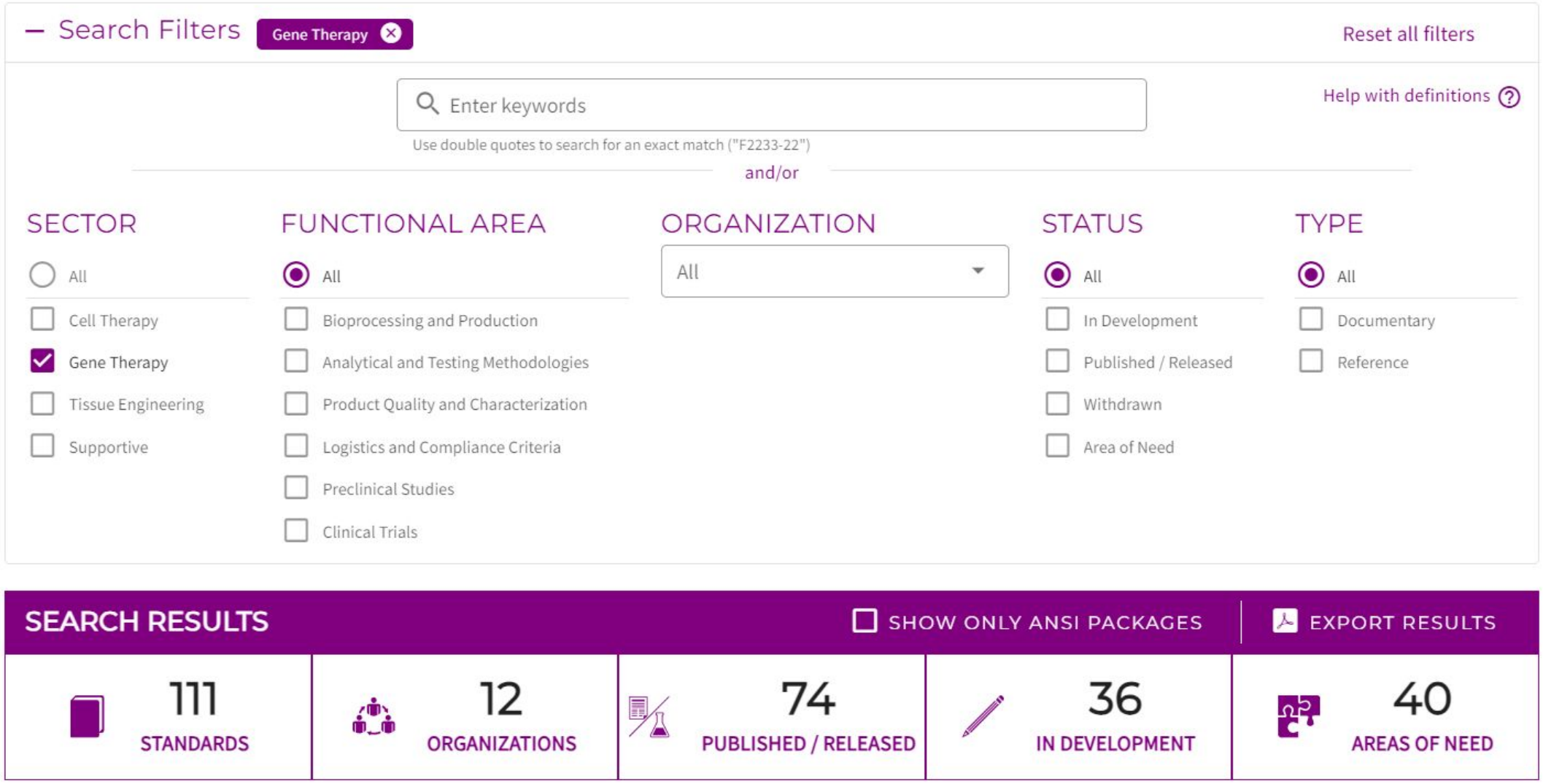
NIST RMTM Consortium			NIST Flow Cytometry Consortia		
WG01 - Reference Materials First task is to design and produce a candidate reference material	**WG02 - Methods and Validation** Provide guidance on the selection and considerations for use of appropriate reference standard materials for the qualification and/or validation of rapid sterility methods for ATMP's	**WG03 - Interlaboratory Studies** Designing planning activities for Q2 and Q3; starting with a survey to gauge availability of laboratory resources, instrumentation, and materials	**WG1 - ERF Bead Calibration and Instrument Standardization** Advance the utility of ERF assigned beads for flow cytometry calibration. Study will compare the intensity values for unknown samples among instruments based on ERF bead calibrations. Reagents, beads, and SOP will be supplied through the Consortium	**WG2 - Assay Standardization** Cell Count and Health (e.g., viability, exhaustion, and apoptosis) is the first test case Study design include various assay control materials to enable comparability of assay results across different cytometer platforms. Study outputs include SOP, standard panels, assay control materials, and reference data	**WG3** Database creation and data analysis

Relevant Standards:



Relevant Standards:

Note: This portal searches on standards titles and summary descriptions, not within the full text of the standard itself.



ISO Evaluation of quantification for nucleic acids

—	International Organization for Standardization — ISO	✓	ISO 20395:2019	Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR	Published 2019	Documentary

Applicable Sector(s) Gene Therapy

Functional Area(s) Analytical and Testing Methodologies; Product Quality and Characterization

Description This document describes the quality, reliability, and reproducibility of targeted nucleic acid quantification measurements employed in bioproduct development and characterization and in bioprocesses, supporting the regulatory-driven requirements of the biotechnology industry.

Additional Keywords quantification of DNA, deoxyribonucleic acid, RNA, ribonucleic acid, target sequences, digital, dPCR, quantitative real-time PCR, qPCR amplification technologies

Availability https://www.iso.org/standard/67893.html

Curated ANSI Package(s) Tissue Engineering Standards Addressing Analytical and Testing Methodologies Package

Feedback or updates on this item Export

ISO Cell Characterization

—	International Organization for Standardization — ISO	ISO 23033:2021	Biotechnology — Analytical methods — General requirements and considerations for the testing and characterization of cellular therapeutic products	Published 2021	Documentary

Applicable Sector(s) Cell Therapy

Functional Area(s) Analytical and Testing Methodologies

Description This standard defines terms related to characterization of human cells for therapeutic applications. It also provides a general description of cell characteristics and common cell measurement methods. This document defines terms and provides general requirements for the testing of cellular therapeutic products intended for human use.

This document also provides considerations for the characterization and testing of cellular therapeutic products, including approaches to select and design analytical methods that are fit-for purpose and considerations for setting specifications for the analytical methods. Such considerations can be used to establish critical quality attributes (CQAs) for a cellular therapeutic product.

Aspects of this document are applicable to starting materials (including those for tissue-engineered products) and intermediates of cellular therapeutic products. This document excludes tissues used in transplantation.

Additional Keywords Biotechnology, analytical methods, testing and characterization of cellular therapeutic products

Updates and Calls to Action This standard has been published (SCB-coordinated project).

Availability https://www.iso.org/standard/74367.html

Feedback or updates on this item Export

ISO Optical Measurements

ISO 24421:2023 Biotechnology Minimum requirements for optical signal measurements in photometric methods for biological samples

https://www.iso.org/standard/78742.html?browse=tc

1 Scope

This document specifies minimum requirements to support accurate measurement of optical signals in photometric methods used for qualitative or quantitative characterization of biological samples.

This document is applicable to optical signals that are generated, for example, by bioluminescence, chemiluminescence and fluorescence, and optical signals that are detected as changes of light due to absorption.

This document addresses the verification of optical signal measurement instruments used in photometric methods for measurement of biological samples including considerations for the use of optical references.

This document does not provide sector- or application-specific performance criteria for the workflow of measuring biological samples. When applicable, users can also consult existing sector- or application- specific standards, or both.

ASTM quantitative fluorescence measurements

—	ASTM International	✓	ASTM F3294-18	Standard Guide for Performing Quantitative Fluorescence Intensity Measurements in Cell-based Assays with Widefield Epifluorescence Microscopy	Published 2018	Documentary

Applicable Sector(s)

Cell Therapy | Tissue Engineering

Functional Area(s)

Bioprocessing and Production | Analytical and Testing Methodologies | Product Quality and Characterization

Description

Relative intensity measurements made by widefield epifluorescence microscopy are used as part of cell-based assays to quantify attributes such as the abundance of probe molecules (see ASTM F2997), fluorescently labeled antibodies, or fluorescence protein reporter molecules. The general procedure for quantifying relative intensities is to acquire digital images, then to perform image analysis to segment objects and compute intensities. The raw digital images acquired by epifluorescence microscopy are not typically amenable to relative intensity quantification. This guide offers a checklist of potential sources of bias that are often present in fluorescent microscopy images and suggests approaches for storing and normalizing raw image data to assure that computations are unbiased.

Widefield fluorescence microscopy is frequently used to measure the location and abundance of fluorescent probe molecules within or between cells. In instances where Random Illumination Microscopy (RIM) comparisons are made between a region of interest (ROI) and another ROI, accurate normalization procedures are essential to the measurement process to minimize biased results. Example use cases where this guidance document may be applicable include:

- Characterization of cell cycle distribution by quantifying the abundance of DNA in individual cells
- Measuring the area of positively stained mineralized deposits in cell cultures (ASTM F2997)
- Quantifying the spread area of fixed cells (ASTM F2998)
- Determining DNA damage in eukaryotic cells using the comet assay (ASTM E2186)
- The quantitation of a secondary fluorescent marker that provides information related to the genotype, phenotype, biological activity, or biochemical features of a colony or cell (ASTM F2944)

This guidance document was developed to facilitate the collection of microscopy images with an epifluorescence microscope allowing quantitative fluorescence measurements to be extracted from the images. The document is tailored to cell biologists who often use fluorescent staining techniques to visualize components of a cell-based experimental system. Quantitative comparison of the intensity data available in these images is only possible if the images are quantitative based on sound experimental design and appropriate operation of the digital array detector, such as a charge coupled device (CCD) or a scientific complementary metal oxide semiconductor (sCMOS) or similar camera. The document considers issues involving the array detector and controller software settings, including collection of dark count images to estimate the offset, flat-field correction, background correction, benchmarking of the excitation lamp, and the fluorescent collection optics.

This document was developed around epifluorescence microscopy but may also be applicable to quantitative imaging in other fluorescence microscopy systems such as fluorescence confocal microscopy. This guide was developed around single-color fluorescence microscopy imaging or multi-color imaging where the measured fluorescence is spectrally well separated. This document also discusses metrology issues related to relative measurements and experimental designs that may be required to ensure quantitative fluorescence measurements are comparable after changing microscope, sample, and lamp configurations.

Additional Keywords

Chemical Analysis, Imaging Technology, Microscopy, Optical Properties, Semiconductor Devices

Availability

https://www.astm.org/Standards/F3294.htm

Curated ANSI Package(s)

Tissue Engineering Standards Addressing Product Quality and Characterization Package
Cell Therapy Standards Addressing Product Quality And Characterization Package
Tissue Engineering Standards Addressing Analytical and Testing Methodologies Package

Feedback or updates on this item | Export

ASTM Osteoblast differentiation

—	ASTM International	✓	ASTM F3106-22	Standard Guide for in vitro Osteoblast Differentiation Assays	Published 2022	Documentary

Applicable Sector(s) Cell Therapy; Tissue Engineering

Functional Area(s) Product Quality and Characterization

Description This document describes the components and conditions used for in vitro osteoblast differentiation assays used to screen for the osteogenic capability of progenitor stem cells from various human or animal sources. These sources include mixed tissue-derived connective tissue progenitor populations and cell populations that may be selectively isolated or manipulated through culture expansion, processing, transfection, or genetic modification.

Additional Keywords Biological Test, Biomaterials, Bone Mineralization, Cell Culture, Cells, Cultivation, Forensic Anthropology, In Vitro Bioassay, Mesenchymal Stem Cells, Osteoblasts, Progenitor Cells

Availability https://www.astm.org/Standards/F3106.htm

Curated ANSI Package(s) Tissue Engineering Standards Addressing Product Quality and Characterization Package
Cell Therapy Standards Addressing Product Quality And Characterization Package

Feedback or updates on this item Export

Standards projects: Cell Therapy & crosscutting

- **ISO Ancillary Materials**: Published
- **ISO certificate of analysis:** currently drafting. Submitting initial comment period in early Nov
- **Framework for cryopreservation:** currently drafting. Submitting initial comment period in early Nov
- **ISO RMTM Framework**: Published
- **ISO Cell Viability:** Current draft is available for review
- **ISO Minimum Requirements for Cellular Morphological Analysis —** Image capture, image processing, and morphometry. At DIS stage.
- **ISO cell line authentication**: Published

Cell Therapy* Sector

Assess potential standards that could improve the safety, quality, and efficacy of cell therapy products and enable more efficient product development processes, such as by establishing common methods to measure cells' functional response to their environment.

* Cell therapy products use living cells as a means of replacing or repairing damaged cells to treat disease.

Standards projects: Gene Therapy

- **Pre-Existing Immunity to AAV**: Actively drafting. Preparing for NP ballot.
- **Validation of database used for nucleotide sequence evaluation:** At DIS stage
- **ISO Nucleic Acid Synthesis — Part 2: General definitions and requirements for the production and quality control of synthesized gene fragment, gene, and genomes**: Final stages before publication
- **ISO Massively parallel sequencing** — Part 1: Nucleic acid and library preparation. Published
- **ISO Gene delivery systems — Part 1, 2, and 3 –** Passed NP Ballot, Looking for SMEs for comment

Gene Therapy* Sector

Evaluate the potential for standards that can help improve the safety and efficacy of gene therapy treatments, such as by improving screening for pre-existing immunity to common viral vectors.

* Gene therapy involves the use of a vector, such as an inactivated virus, to insert a new copy of a gene or relevant nucleotide sequence into a patient's cells to treat a genetic health condition.

Opportunities to Impact Standards Development and Implementation

ISO/TC 276 Biotechnology Meetings

WG2 Biobanking: Nov 13th, Nov 14th, Nov 15th, Nov 16th

WG 3 Analytical Methods: Dec 6th, Dec 7th, Dec 8th

WG 4 Bioprocessing: Nov 28th, Dec 4th, Dec 5th

WG 5 Data processing and integration: Dec 4th, Dec 5th, Dec 6th, Dec 7th, Dec 8th

If interested in joining a working group, please contact Dawn at DHenke@regenmedscb.org.

Call to Action

- *ISO Cell Viability Project
- ASTM WK70143, New Guide for Sampling Methods of Tissue Engineered Medical Products (TEMPs) for Sterility Assurance
- Cryopreservation Framework
- Containers for Cryopreservation
- Certificate of Analysis for Ancillary Material

If interested in joining a working group, please contact Dawn at DHenke@regenmedscb.org.

Education Updates

Workforce Development Course Update

Pilot training program for standards:

Began to design and implement a pilot training program (with ARMI | BioFab USA) to help manufacturers to avoid/minimize many of the common front-end issues of the manufacturing process.

ISO Cell Counting Part 1&2: We've recently signed an MOU for training partnership with ISCT: The SCB certificate course will be offered through ISCT's LMS. The course should be available online through ISCT very soon.

ISO Ancillary Materials: we are still recruiting experts to help with the creation of course content.

If interested in serving as a subject matter expert or to contribute case studies, please contact Katie at CZander@regenmedscb.org.

Focus Areas

Since our inception in 2017, SCB has accelerated the advancement of 34+ standards. But we need additional community support to respond to the interrelated challenges facing the regenerative medicine field. To address this need we have developed SCB Focus Areas.

The first three Focus Areas are:

1. Standards Implementation Education and Workforce Development
2. Data Management
3. Cryopreservation

Donor Benefits Include:

- SCB's formal recognition of your contribution in meetings and media
- A seat on the Focus Area's Steering Committee
- Discounted course registration for your employees

Focus Areas

If interested in learning more about SCB Focus Areas, please contact Justin at JBarch@regenmedscb.org.

Standards Implementation Education and Workforce Development

KEY CHALLENGE:
Standards can only benefit the community if there is broad understanding of how to implement them to meet regulatory expectations.

FOCUS AREA SOLUTIONS:
Develop courses on how to use specific regenerative medicine standards to demonstrate regulatory compliance and improve the efficiency and repeatability of internal processes.

Data Management

KEY CHALLENGE:
Variability in data management practices and manufacturer requirements are common pain points for collection centers and can even overburden staff to the point of limiting their capacity to support clinical trials of potentially lifesaving therapies.

FOCUS AREA SOLUTIONS:
Identify common data needs and approaches to streamline data management practices.

Stay up to date on standards

Follow us on social media to stay up to date on news surrounding regenerative medicine standards, including webinars, FDA guidance documents, NIST consortium opportunities, open ballots, and new working groups.

Linkedin:https://www.linkedin.com/company/standards-coordinating-body

Twitter: https://twitter.com/SCBRegenMed

FOR MORE INFORMATION VISIT
www.standardscoordinatingbody.org

OR CONTACT dhenke@regenmedscb.org

Breakout Groups (will be opening these breakout groups to virtual participation)

Identification and Prioritization of Needed Standards

One of the main goals of SCB is to identify and prioritize needed standards to determine where resources should be allocated

SCB focuses on determining:

- What standards will be most beneficial for the community
- What standards will have the most impact
- What standards will help the therapy development process and get therapies to patients faster
- What standards will make therapies safer and more effective

Feasibility Assessments

The next stage is assessing feasibility of developing identified standards

SCB organizes feasibility assessment meetings for a selection of high-priority standards identified by the community in the Regenerative Medicine Standards Portal. These meetings bring together regenerative medicine stakeholders with diverse expertise and viewpoints to consider feasibility factors such as:

Technical feasibility: Whether an adequate technical and scientific foundation exists for constructing the standard

Implementation feasibility: Factors that influence an individual firm's adoption of the standard such as incurred costs; the standard's compatibility with existing equipment, materials, and technology; and required in-house expertise

Expert availability: Level of interest from technical experts in the field who can advance the standard

The results **inform SCB's standards priorities and often spur the creation of new working groups** for any standards or pre-standards outputs selected to move forward.

Breakout Groups

Today we want to use two separate breakout sessions to identify and prioritize needed standards for

A) Assessing Gene Therapy Product Activity

B) Assessing T-Cell and Other Cell Therapy Product Activity

We are looking to determine what standards need to be created to move the regenerative medicine field forward.

Breakout Group Goals

Day1:

Identify and prioritize specific standards needs and topics that are ripe for standardization for cell and gene therapy activity

Day 2:

Define more of the specifics of the standard topics identified on Day 1 and their feasibility

Focus Questions

What assays or related processes, if standardized, would help address current challenges? Standards can include specific protocols about how to conduct a process (e.g., a technical specification or validation protocol) as well as less prescriptive guides that aid in decision making.

Which two topics would have the greatest positive impact on the field if standardized in the near term? (voting exercise)

For the top 2 prioritized topics:

- What components of the assay or related process need standardization (e.g., test selection, measurement methods, interpreting results, validation)?
- What key questions should be answered by a standard on this topic?
- Do you anticipate any barriers to standardizing these assays (e.g., lack of scientific consensus, difficulty or expense of implementation, potential resistance from the community)?

Day 1
Breakout Instructions

Breakout Instructions

- You will break out into two groups to participate in a facilitated discussion of standards needs.
- Please bring your laptops, as this will make it easier to submit input via Xleap
- Virtual participants are welcome to join the breakout sessions and submit comments via Webex chat or XLeap; however, the focus will be on the in-person meeting

Gene Therapy Breakout Group

Facilitator: Dawn Henke

Room: Spaulding

Meeting Link: https://uspevents.webex.com/uspevents/j.php?MTID=md10004a2621f9cb0e66ea28fa68fe32b

XLeap Link: https://39049718.xleap.net/gene

T-Cell and Other Cell Therapies Breakout Group

Facilitator: Sarah Lichtner

Room: Bache & Wood

Meeting Link: https://uspevents.webex.com/uspevents/j.php?MTID=mcc9a0eabb2b516b90c15526242a2972f

XLeap Link: https://00689413.xleap.net/cell

Day 2
Breakout Instructions

Breakout Instructions

- You will break out into two groups to participate in a facilitated discussion of standards needs.
- Please bring your laptops, as this will make it easier to submit input via Xleap
- Virtual participants are welcome to join the breakout sessions and submit comments via Webex chat or XLeap; however, the focus will be on the in-person meeting

Gene Therapy Breakout Group

Facilitator: Dawn Henke

Room: Spaulding

Meeting Link:
https://uspevents.webex.com/uspevents/j.php?MTID=mf5650bce7f8d2321b56240803733a9ff

XLeap Link: https://39049718.xleap.net/gene

T-Cell and Other Cell Therapies Breakout Group

Facilitator: Sarah Lichtner

Room: Bache & Wood

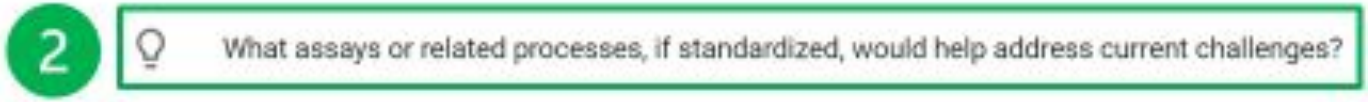
Meeting Link:
https://uspevents.webex.com/uspevents/j.php?MTID=m8844776ad851f3432b905f5a6dce21b8

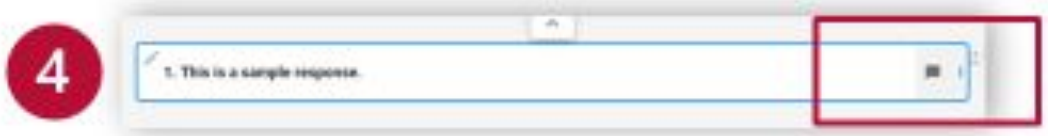
XLeap Link: https://00689413.xleap.net/cell

Gene Therapy Breakout

XLeap

1. Click on the navigator icon in the upper left to view the open discussion spaces.
2. Click on the discussion title to enter the space.
3. Type in and post your idea in the “Your idea box” at the bottom of the screen.
4. Click on the speech bubble icon on the right of an idea to open the comment panel.
5. Type in and post your comment in the “Your comment” box.





Please join XLeap using a laptop, tablet, or smart phone using the following link:

https://39049718.xleap.net/gene

T-Cell Therapy Breakout

XLeap

1. Click on the navigator icon in the upper left to view the open discussion spaces.
2. Click on the discussion title to enter the space.
3. Type in and post your idea in the “Your idea box” at the bottom of the screen.
4. Click on the speech bubble icon on the right of an idea to open the comment panel.
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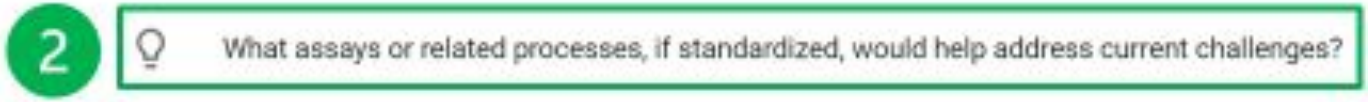
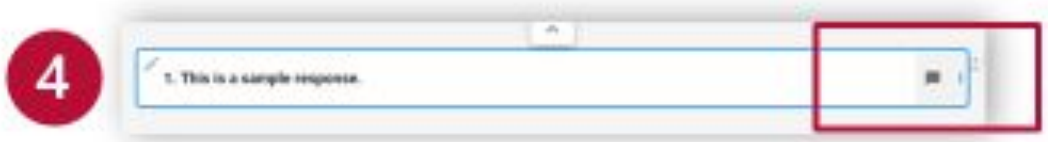


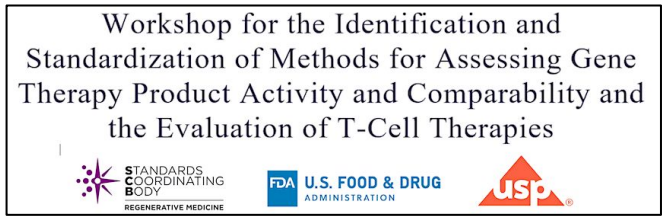


Please join XLeap using a laptop, tablet, or smart phone using the following link:

https://00689413.xleap.net/cell

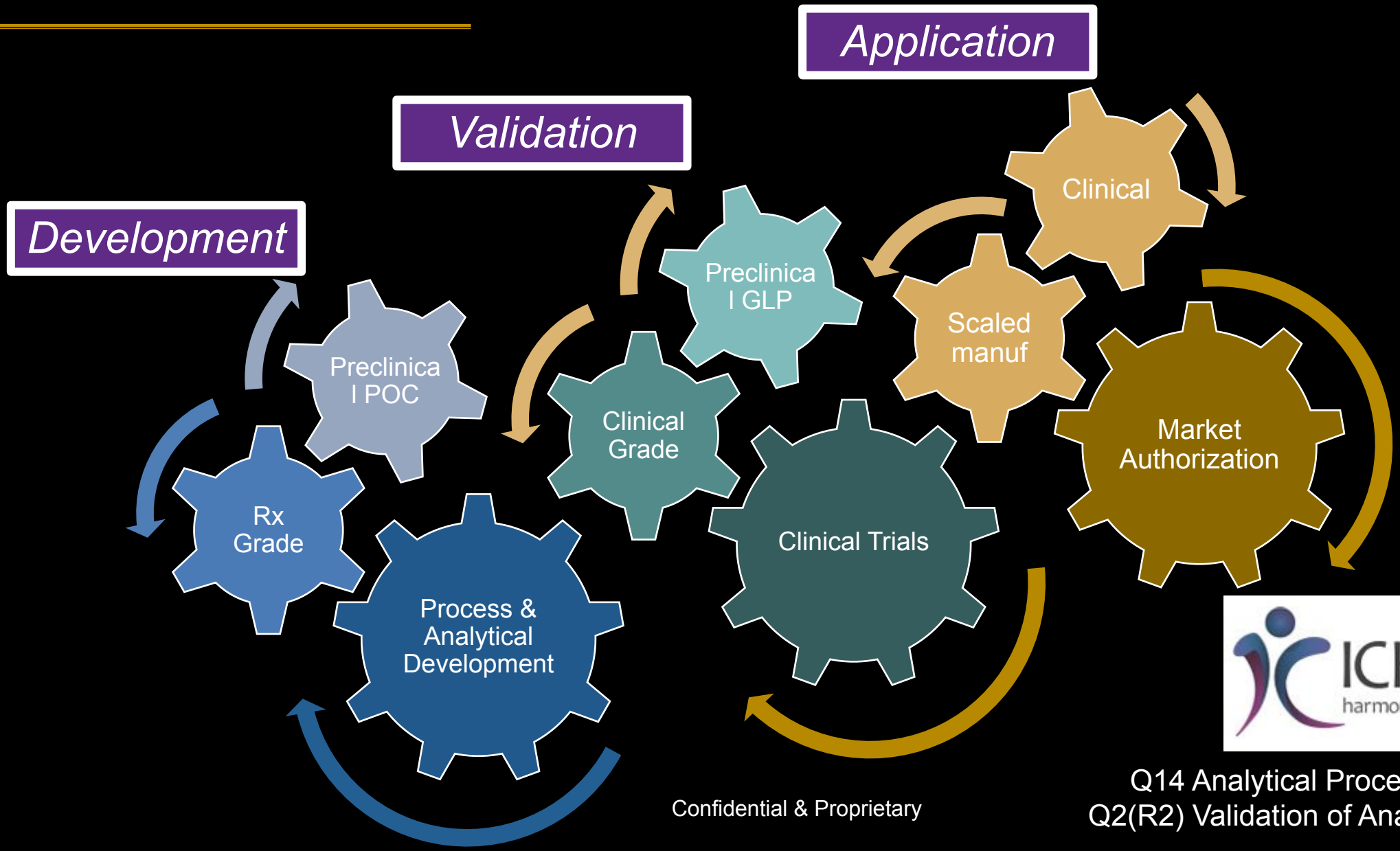
Navigating regulatory milestones throughout development

November 17, 2023



Patrick Bedford
weCANtranslate Network

Analytics Throughout Development

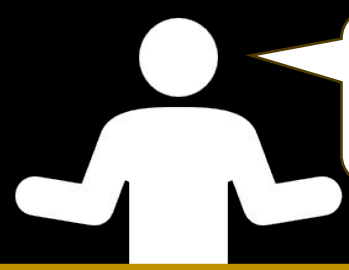


ICH
harmonisation for better health

Q14 Analytical Procedure Development
Q2(R2) Validation of Analytical Procedures

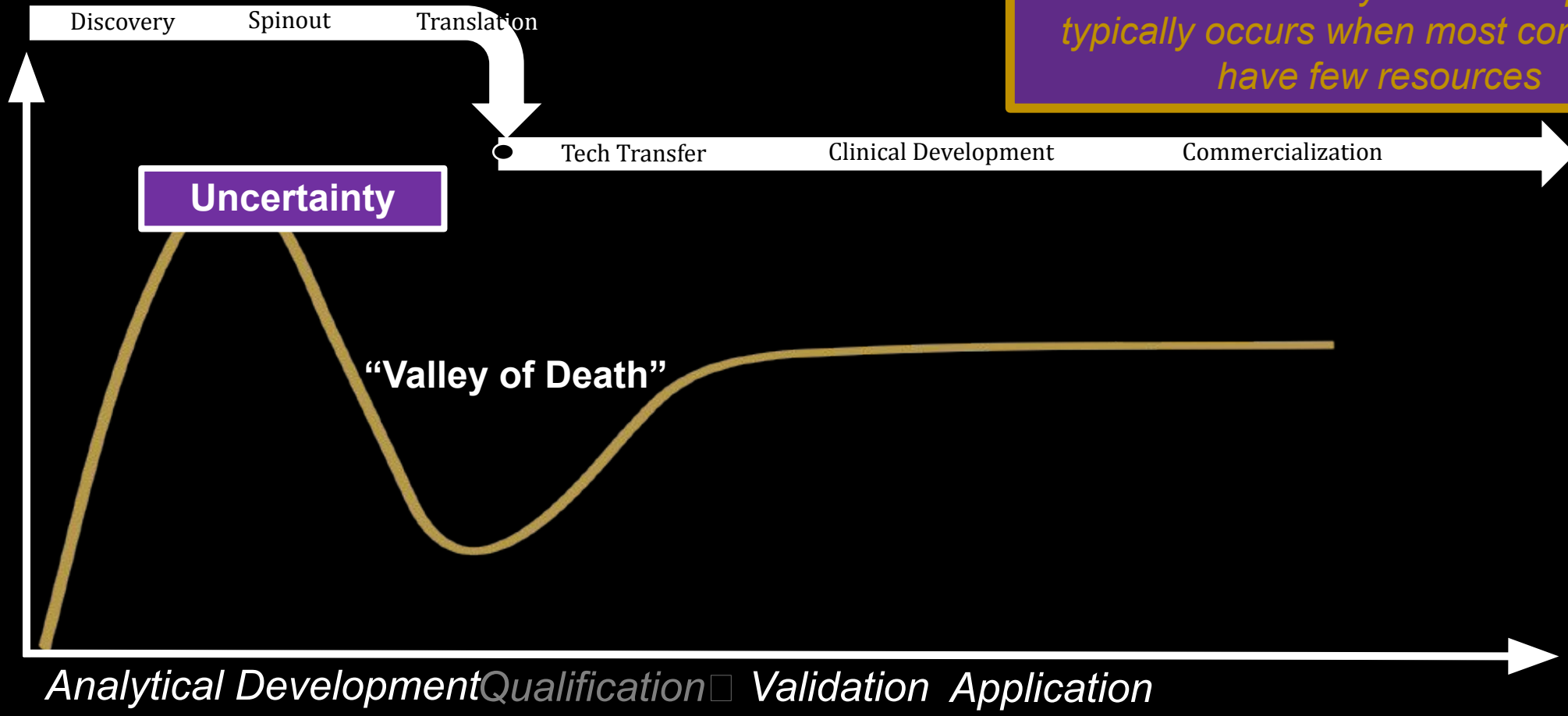
Confidential & Proprietary

Observed challenges over time



should I invest in THIS

Reminder: *Analytical development typically occurs when most companies have few resources*



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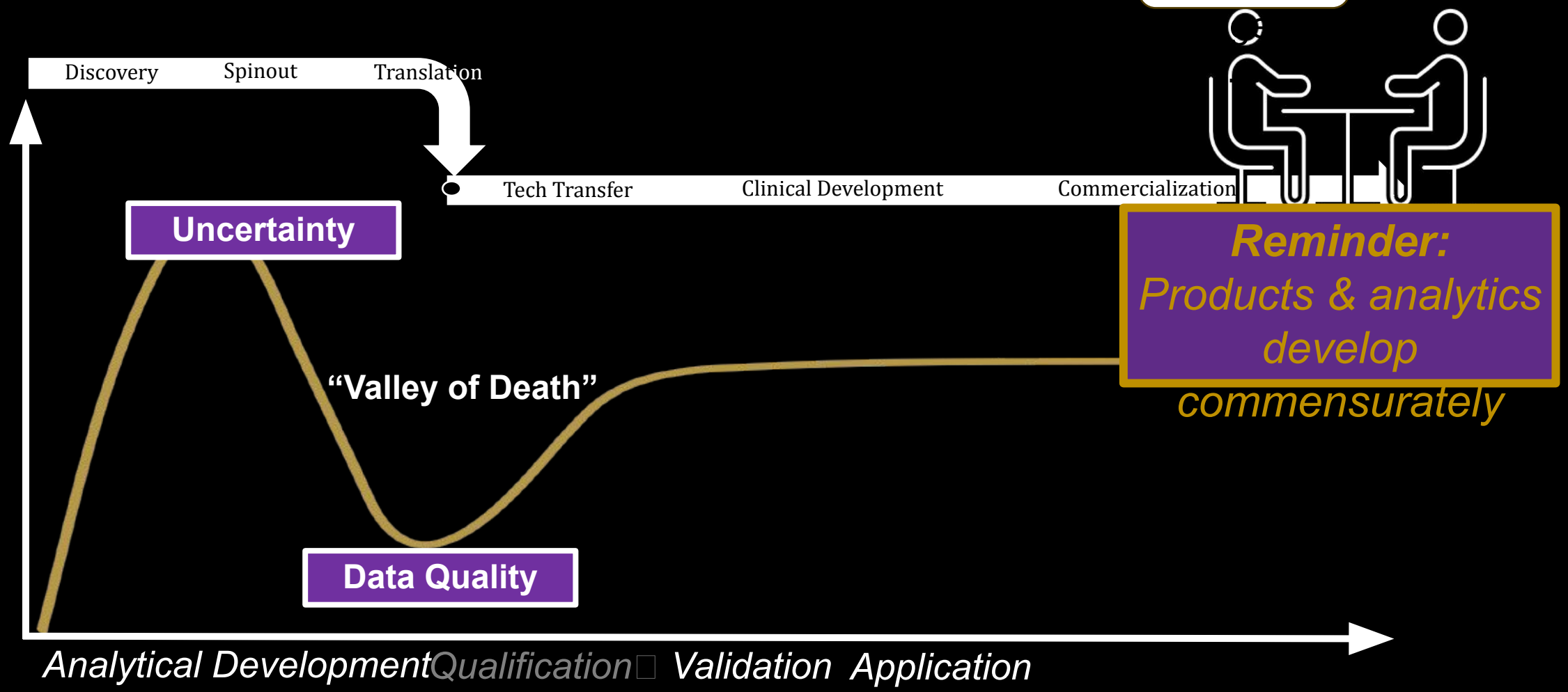
Observed challenges over time



Reminder:
Products & analytics develop commensurately

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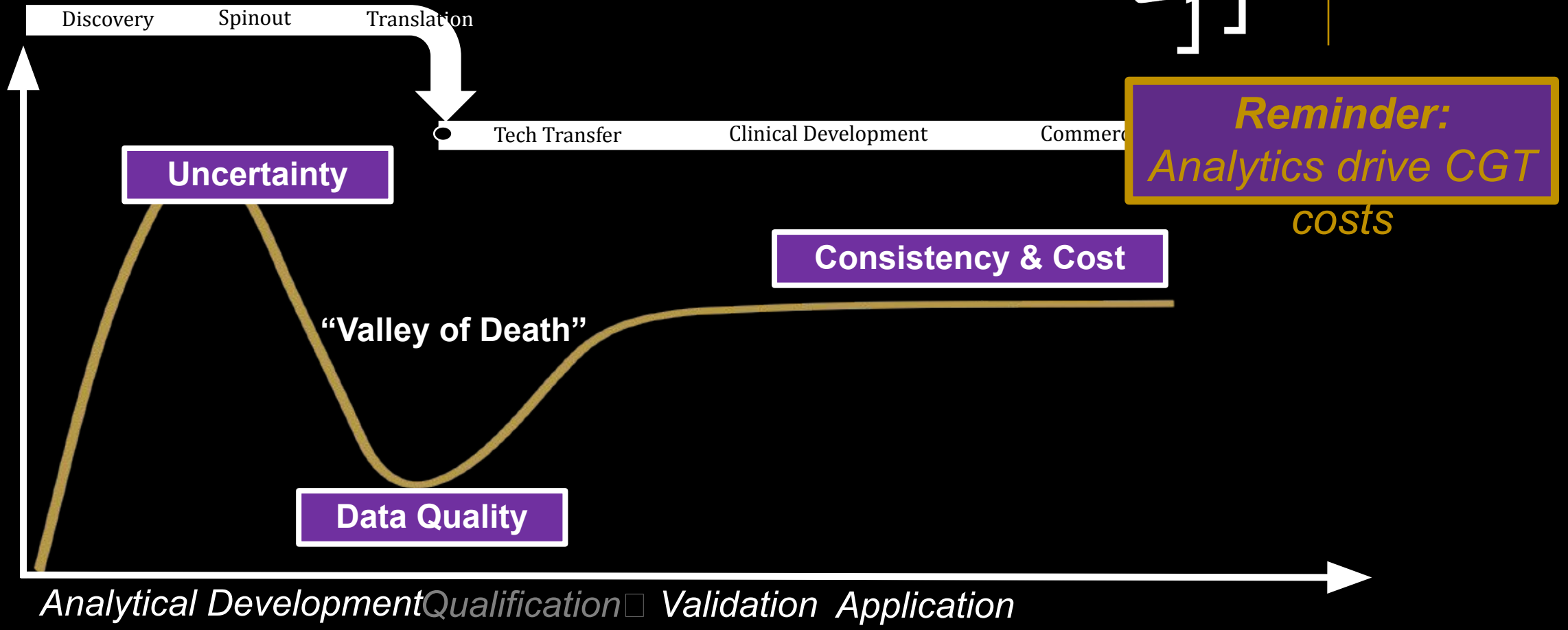
Observed challenges over time

that assay when we have more money & ne.

more cost-effecti



Reminder:
Analytics drive CGT costs

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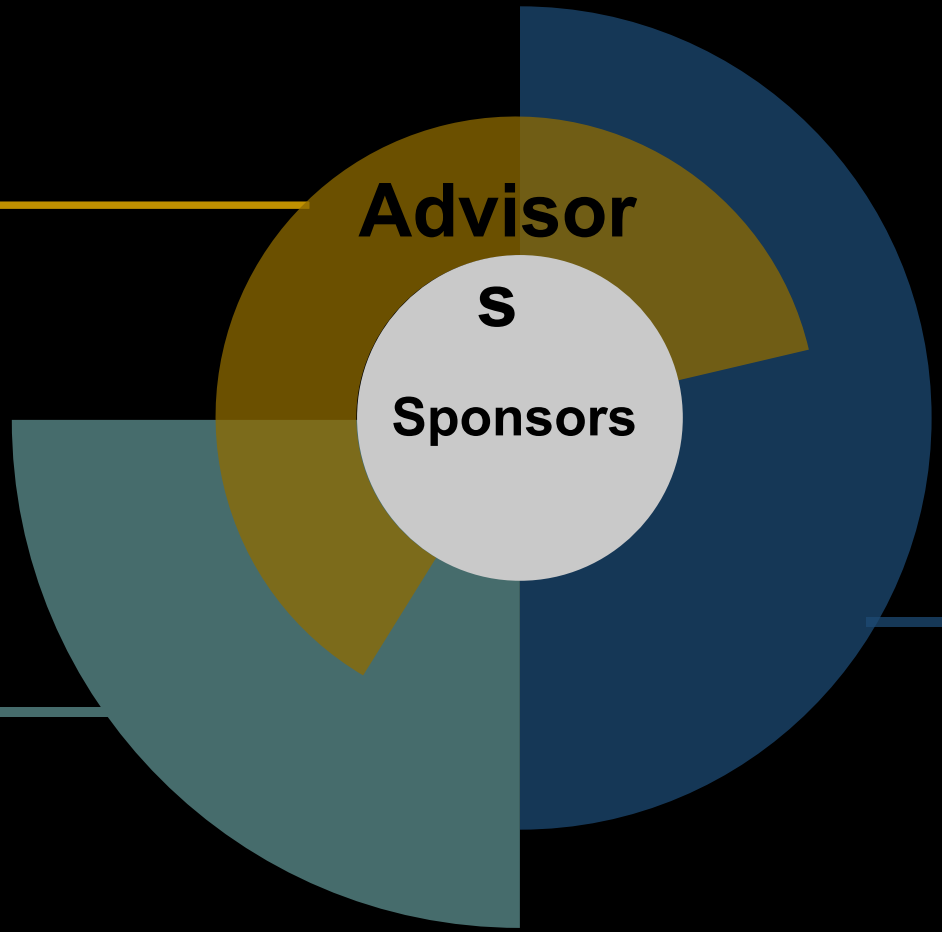
Multiple Interrelated Players

SDOs, Associations & Consultants

Provide expert knowledge & experience



CDMOs and/or GMP facilities

Off-the-shelf & custom develop analytics

Regulators

Provide guidance & assess sufficiency

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Enabling a Healthier World

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Navigating the milestones in developing a commercially-viable CGT Product

A CDMO perspective on the analytical methods for T-cell products

Krishna Panchalingam | Associate Director, CGT Technical Operations/Development Services

SCB Gene Therapy Product & Comparability & The Evaluation of T-Cells Workshop
November 17, 2023

Additional Information and Disclaimer

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Cell and Gene Therapies Come With Specific Challenges

Compared to traditional biologics

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Accelerated timelines

Expedited approval pathways lead to reduced development times

Scaling up & out

CGT manufacture is complex and cannot be ramped up linearly

Cost of development & manufacturing

COGs can range between $500K and $1 million, excluding R&D costs

Product quality & defining CQA

A process without fully developed CQAs and analytical methods does not provide any control. Thus, analytics are a critical part of process development.

Analytical methods development

Navigating the regulatory landscape

Evolving regulations and a lack of harmonization between health authorities

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Key challenges: Analytics

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- Timeline to optimize / develop new, potentially complex analytics
- Timeline for provision of data needed for in-process decision-making
- Timeline for characterization / release testing

- Specificity
- Precision: repeatable & reproducible
- Accuracy
- Data integrity compliance

- Low-throughput assays
- Customization often needed
- Cost of reagents

- Using phase-appropriate assays that meet regulatory requirements
- Balancing “gold standard” *vs.* rapid alternatives
- Selecting adequate controls & references

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Typical Allogeneic CAR-T Cell Processes with Scaled Up Expansion

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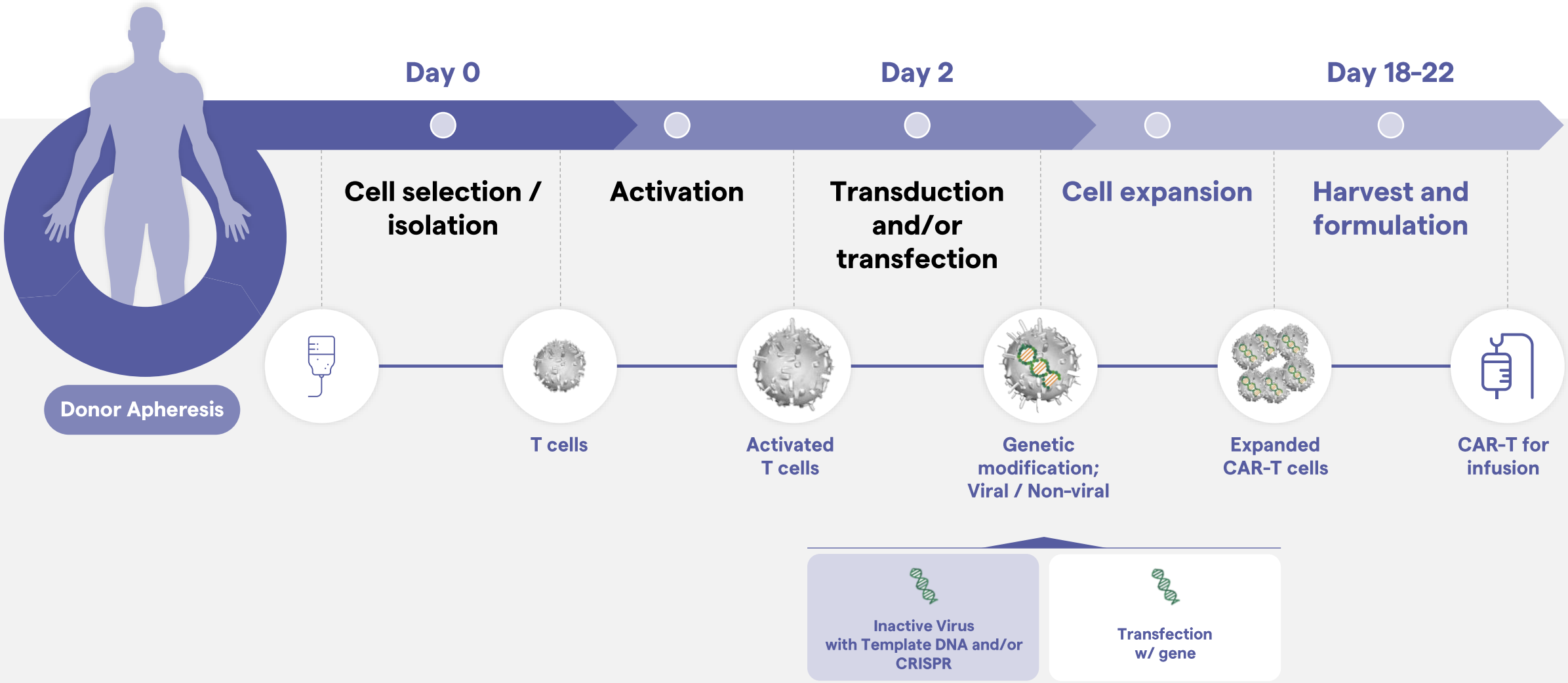

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The Path To Commercial Manufacturing Begins With a Well-defined Process

For Consistent Manufacturing of High-quality Product

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Starting Material → **Process** → **Product**

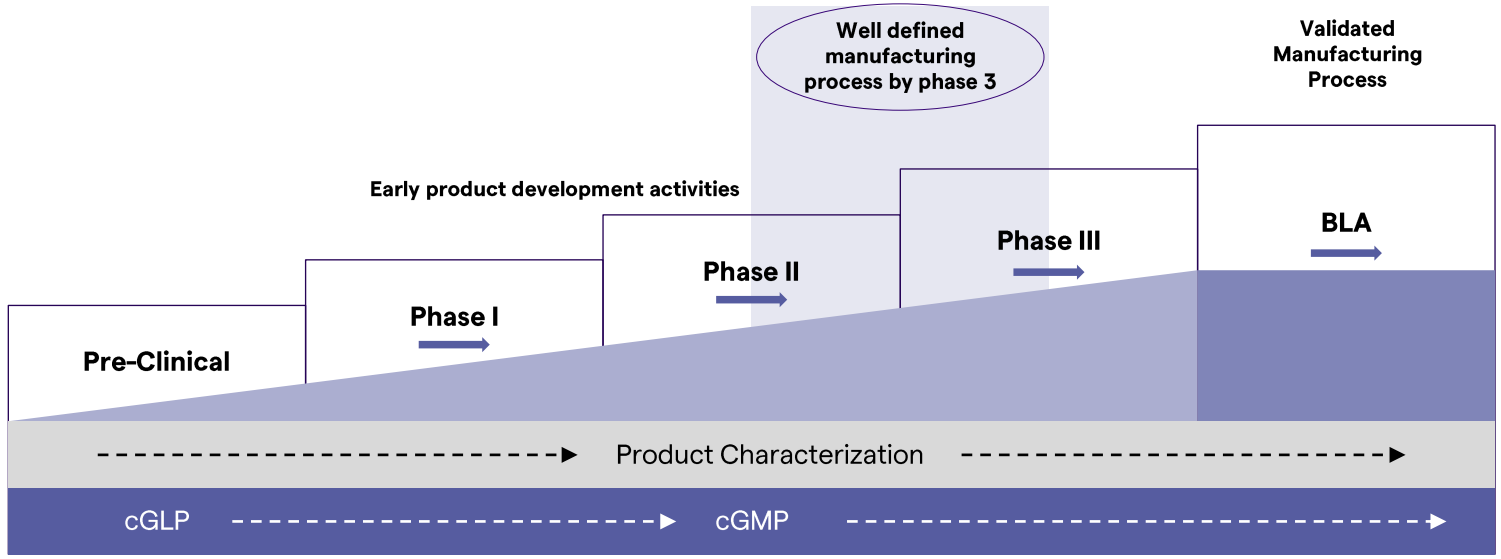
Critical process Parameters (CPP)

Process Characterization

- **CQAs**
- **Release Specs (COA)**

Regulatory requirements for manufacturers:

- Identify critical process parameters (CPPs) in the manufacturing process
- Critical product attributes (CQAs) to ensure the desired clinical effect of the final product



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In-Process Controls

For monitoring the process

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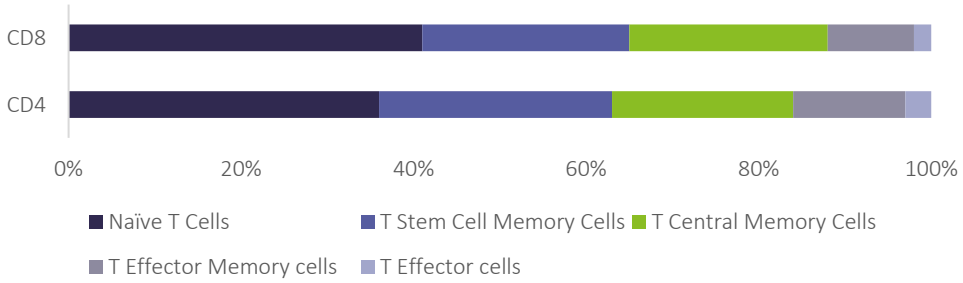
- Define control and action limits around each unit operation to ensure adequate control of the manufacturing process
- Some of the suggested process controls for CAR-T cell therapies
  - CD3, CD4 and CD8% of the starting material (Apheresis)
  - Post Selection T cell recovery
  - Post selection cell purity (CD3%) and phenotype (Memory subsets)
  - Post-selection Impurities (RBC, Platelets, tumor cells)
  - Transduction or Transfection or Transposition efficiency
  - Monitoring for Memory Phenotype, activation State and exhaustion markers during expansion
  - Cell count and cell viability throughout culture and downstream processing
  - Process residual clearance



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Assays for Allogeneic CAR-T cell Therapy

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Assay Release				
Assay	Objective	Method	Evaluation Criteria	Category
Cell Count & Viability	Dose	NC-200	% viability >70; minimum cell number /vial or Cell concentration / mL	Release Assay
Phenotype	Identity & Purity	Flow cytometry	CD3%, CD4%, CD8%, CAR%	Release assay
Phenotype	Cellular Impurities	Flow cytometry	CD19/CD20, CD56, CD14, CD15, Tumor cells	Release assay or FIO
Copy Number	Gene Modification	qPCR/ddPCR	No of copies of corrected gene	Release assay
Knock-out %	Gene Modification	Flow cytometry	% TCR-alpha beta negative cells	Release assay
Knock-in %	Gene Modification	ddPCR/ Targeted Sequencing	% of gene modified cells in product	Release assay
Mycoplasma Testing	Safety	MycoPCR or USP	Negative	Release assay
Sterility Testing	Safety	USP or BacT	Negative	Release assay
Endotoxin Testing	Safety	USP	Standard QC release (<0.5 EU/ml)	Release assay
Visual Inspection	Safety	USP	No visible particles	Release assay
Potency Assay	Potency	Various	Indication specific potency (cell activation, cytotoxicity and cytokine secretion)	Release assay
Characterization Assays				
Process Residuals	Residual clearance	Various	Demonstrate clearance of process related impurities such as Serum, Albumin, cytokines, RNPs, ssDNA	FIO*

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Additional Characterization Assays for Allogenic CAR -T Gene Editing

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Assay	Objective	Method	Evaluation criteria	**Category**
sgRNA design	Maximizing specificity and minimizing off-target cleavage	In-silico design	Evaluation of candidate sgRNAs	Development testing
On-Target Cleavage Detection	Confirm efficient and specific cleavage at desired site	ddPCR and endonuclease digestion	% of knock-out in pooled T cell post transfection	Development testing
On-Target Clone Validation	Confirm efficient and specific cleavage at desired site	Sanger Seq/ NGS	% of knock-out in T cell single clones post transfection	Development testing
Off-Target Validation	Safety	In-silico prediction/ Targeted Seq/ NGS	Zero off-target mutagenesis	Development testing

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Lonza support across the Analytical Lifecycle

Bioassay Services manages the maturation of assays throughout the product lifecycle

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- Lonza's CGT Analytical Team has the experience and expertise to fulfill all analytical needs across the analytical lifecycle.

Creation

Ideation (proof of concept, method creation/design and selection)

Development

Transfer

Feasibility

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Client or Source

Gap Analysis

Development & Optimization

- Accuracy
- Linearity
- Range
- LLOD/LLOQ
- Precision
- Matrix Inhibition Specificity

QC Training

Qualification

- Establishment of Reference Controls
- GMP Demonstration of Specificity
- GMP Demonstration of Linearity & Range
- GMP Demonstration of Accuracy
- GMP Demonstration of Precision

Qualification Execution

Qualification Experimental Design

Qualified

Validation

Assay Robustness

Validation Execution

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What do we provide in analytical development?

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Assay transfer from client and feasibility studies

- Gap assessment of client protocol according to each type of assays per ICHQ2R1 and USP1225

Assay optimization:

- Develop a robust, locked down method with suitable controls
- Performance verification to define the criteria including assay range e.g. assay precision, accuracy, limit of detection

Assay qualification

- Early phase activity to generate documented evidence that the method consistently delivers correct results through an evaluation of the performance characteristics of the test method relative to the intended purpose or expected performance

Assay validation

- Late clinical phase activity used to confirm the performance of tests using GMP material based on pre-set acceptance criteria

Development of in process testing and control assays, and stability testing
Product characterization

Product characterization

Seamless transition to QC for GMP testing

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What is the analytical pathway?

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STEP 1
Identify the analytics required to define the process/product

STEP 2
Evaluate the analytics and how they correspond to TPP in Pre-Clinical Studies

STEP 3
Early Phase Clinical Assessment and Develop Understanding of Process and Analytics and Qualification of Critical In-Process and Release Assays
Develop Potency assay to define MOA

STEP 4
Late-Phase/Commercial Process: Define and Validation of Critical In-Process Assays and Release Assays

STEP 5
Assays In-Place to Support Commercial Launch

Behind every successful commercial launch is detailed planning and preparation

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Off-the-shelf Bioassay Library

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Timeline reduction with off-the shelf assays

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~5-7 months ***after*** contract signature

Current Standard

Feasibility/ Development → Optimization → Qualification

Individual assays are Optimized and Qualified per client/product, **often recreating previous work on similar products**

Lonza develops ***prior to*** contract signature

Platform assay Development

Development (if needed) → Optimization → Qualification

A representative product can be **Optimized and Qualified, eliminating the need to repeat** these activities for each similar product in the future

~2-3 months *after* contract signature

Product-specific Qualification using Platform Assays

Feasibility – Apply to new product → Qualification – Sample Matrix Verification

Minimal experiments to verify the validity that the Platform Optimization/ Qualification applies to the new product

~0-2 months *after* contract signature

Implementation of Qualified Assays

Implementation & Verification study

Focusing on implementation / minimal verification studies to ensure there is no variability or product / process impact

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Assay families covered*

Safety	Identity	Strength	Purity	Quality
CAR-T/TCR/NK cells for both allogeneic and autologous therapies	AAV	Lenti	Adenovirus	hiPSCs

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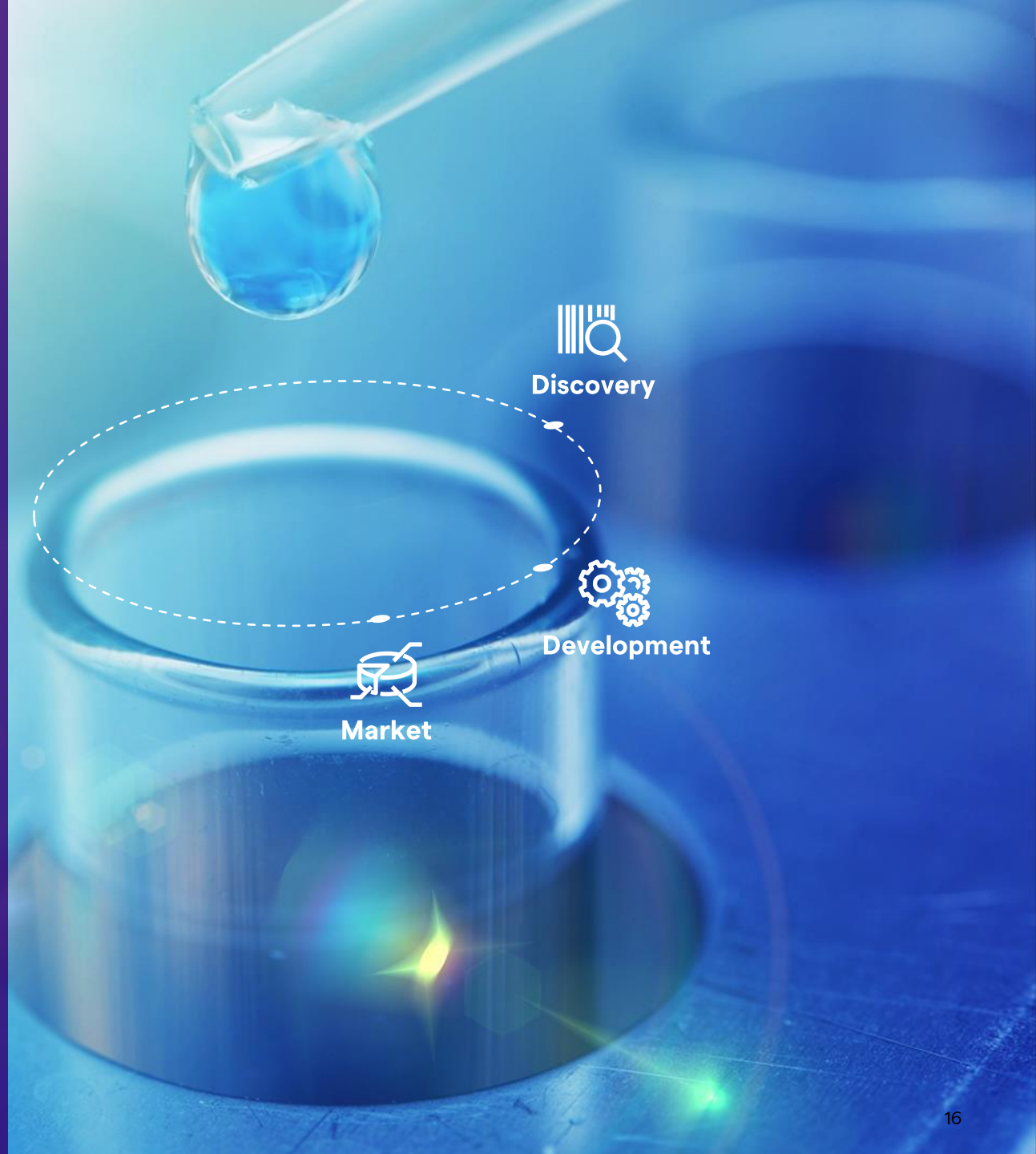
*This is not an exhaustive list

How will it work?

As part of feasibility gap assessment, we **recommend pre-developed assays based on your therapy**

and

We will **test and verify** the assays for your therapy and **ensure that they meet your critical quality attributes**



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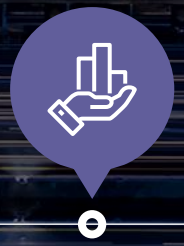
Meet Your Timelines

...

...

and Map Out the Future

Reduce timelines by up to 2-6 months*

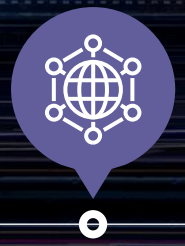


Utilize pre-developed catalog of assays

That still fits to your specific product

Ensure a smooth transfer of analytical methods into GMP manufacturing

De-risk your clinical development

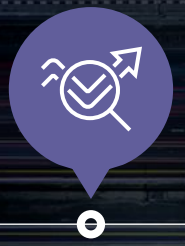


Meet the critical quality attributes (CQAs) of your product

Leverage Lonza's 20+ years of technical expertise in CGT

Navigate regulatory pathway with ease

Have a clear path forward



Clear contractual terms

Tech transfer package and documentation defined

Flexibility

*If applicable, to be verified based on specific customer process

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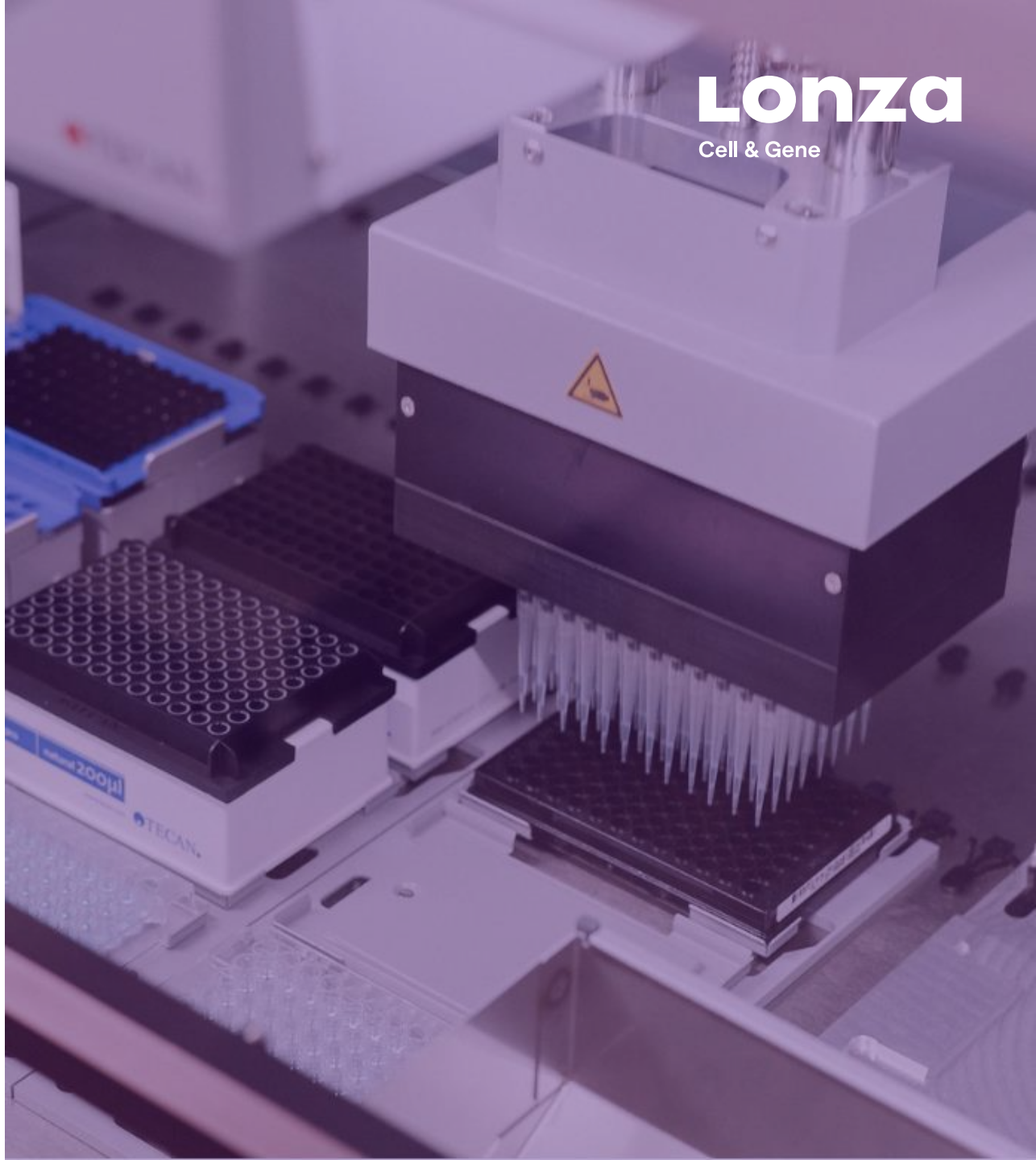
Assay Automation

Solving the Assay Pain Points in Commercial Cell & Gene Therapeutic Product Manufacturing



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Lean to Automated Sample Preparation

Liquid Handling Systems

Implementing consistency for complex and/or time-consuming manual-based procedures

- Reduce analyst variation and potential deviations/errors
- Increase consistency of assay performance

BD FACS Lyric

BD FACS Duet

Tecan

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ELISA Automation Overview

Tecan (Freedom EVO) at Lonza Houston

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Automation advantages

- Relieve operators from repetitive tasks: in-process and routine testing
- Robotic arms for pipetting and labware movement
- Optimize assay precision, accuracy and consistency
- Meet throughput, cost saving and productivity targets
- Automation extension for other assays
  - ELISA dilution preparation
  - qPCR and ddPCR sample preparation
  - TCID50 assay sample dilution
  - NGS sample preparation - Automated library preparation, quality control and pooling
  - Colony picking & plating

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Shortened Timelines, Faster Turnarounds

Leveraging our standard *New Product Introduction* & Lifecycle Process (1/2)

New Product Introduction

A New Paradigm

De-risked product introduction

1. Robust fit assessment to ensure alignment with Lonza requirements
2. Standard requirements to identify issues early on
3. Reduced compliance risk
4. Avoidance of delays and rework

Standardization across 6 key workstreams

1. Raw materials
2. Sterility assurance
3. Analytics
4. Manufacturing Processes
5. Facilities & equipment
6. Tissue acquisition

Well-defined processes turned into manufacturing successes

1. Clearly defined deliverables at each stage gate with checklists
2. Standard tech transfers across the globe
3. Support from capability assessment to commercial production

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Shortened Timelines, Faster Turnarounds

Leveraging our standard *New Product Introduction* & Lifecycle Process (2/2)

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Lifecycle Module

0	A	B	C	D	E	F	G
Pre-contract	Process Development	Technology Transfer and Pilot Runs	Engineering Runs	Clinical Production	PPQ Lots	Pre-approval Inspection Readiness	Commercial Production

Stage gate Checkpoints

- Capability & Manufacturability Assessment
- Ready for Tech Transfer – Pilot Runs
- Ready for Engineering Runs
- Ready for GMP/Clinical
- Ready for PPQ
- Ready for PAI

FOCUS AREAS

Process
- FMEA for the Assessment of Process Parameters
- Block Flow Diagram for the process incl. relevant parameters

Sterility Assurance
- Pre-Technology Transfer Sterility Assurance Assessment Report
- Microbiological Contamination Risk Assessment

Facility & Equipment
- Engineering & Commissioning
- Equipment and Facility Validation, Calibration and Maintenance

Analytics & Equipment
- Analytical Gap Assessment for all methods incl. Raw materials, In-process and Final Product Testing
- Sampling Plans
- Master Validation Plan

Raw Materials
- Raw Material List
- Material Testing Requirements & Specifications
- Supplier Qualification

Tissue Acquisition
- Donor Program creation and maintenance; Starting Material (cells/tissue) Assessment; Chain of Custody; Chain of Identity

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Established Leading Partner for Innovators in the Cell & Gene Space

Cell & Gene Technologies value proposition

Lonza
Cell & Gene

Offering across all cell and gene modalities	 **Autologous & Allogeneic cell and gene therapies**	 **Viral Vectors (inc. Lentiviral vector and AAV)**	 **Exosomes and EV-based therapeutics**	
Integrated Solutions	 **Process & Assay Development**	 **Clinical & Commercial Manufacturing**	 **In-house Tissue Sourcing**	 **Regulatory Support**
Expertise and track record	**>20 years** of GMP Experience	**>160** Customers Served Globally	**>200** Process Development Projects Delivered	**>15** Phase III/ Commercial Projects
Global network and capabilities	**4 sites** across **3 continents** for CGT manufacturing	**2** sites dedicated to **Exosomes** (US & EU)	**3 Commercial CGT Products** at a single site (Houston)	**>1,200 Employees** dedicated to CGT

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Enabling a Healthier World

Lonza
Cell & Gene

Thank You!

Enabling a Healthier World

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Extra Slides

Established Commercial Manufacturing

Across a global network

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Cell & Gene

Lonza Houston

PAI approvals in Feb 2021, Aug 2022, Sep 2022

Certified CGT commercial site, manufacturing both viral vectors and cell therapy for 3 commercial CGT products

Capacity expansion:

- 32,500 square feet of manufacturing space expansion
- >200 clinical and commercial batches annually
- Additional shell space for rapid and flexible modular expansion

20+ years of experience in viral vector manufacturing

Over 8 years of extensive experience in LVV production (over 60 GMP batches)

R&D Innovation & Process Development expertise to support process industrialization and de-risked manufacturing

Off-the-shelf, pre-developed assay library

Global regulatory support through entire program life cycle

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Established Commercial Manufacturing

Across a global network

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Cell & Gene

Lonza Houston

CGT PAI approvals in Feb 2021, Aug 2022, Sep 2022

Manufacturing >200 clinical and commercial batches annually of **both viral vectors and cell therapy for 3 commercial CGT products**

Expansion space available

Lonza Portsmouth

World's most commercially licensed biological site

over 50 licensed commercial products, including the **Moderna COVID vaccine** in 2020 (mRNA).

Expansion space available

Lonza Netherlands

Highly flexible, established commercial site in Europe combining CGT and mRNA

Experience manufacturing **EU approved commercial products + ongoing PAIs**

Expansion space available

Lonza Singapore

One of the most commercially licensed Biological sites in Asia with 9 commercial products

20+ successful inspections from various agencies with **no critical observations**

Expansion space available

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Gene therapy comparability challenges and practices – an industry perspective

David Litwack, PhD

Prevail THERAPEUTICS
A Wholly Owned Subsidiary of Eli Lilly and Company

Disclaimer

- I am an employee of Prevail Therapeutics, a wholly owned subsidiary of Eli Lilly
- Views and opinions expressed are my own

Special challenges posed by new technologies

- Technology constantly improving
- Many gaps in scientific knowledge
- No uniform practices (assays, manufacturing, etc.)
- Effects of product quality on safety and efficacy not well understood
  - Too few patients in any given gene therapy trial

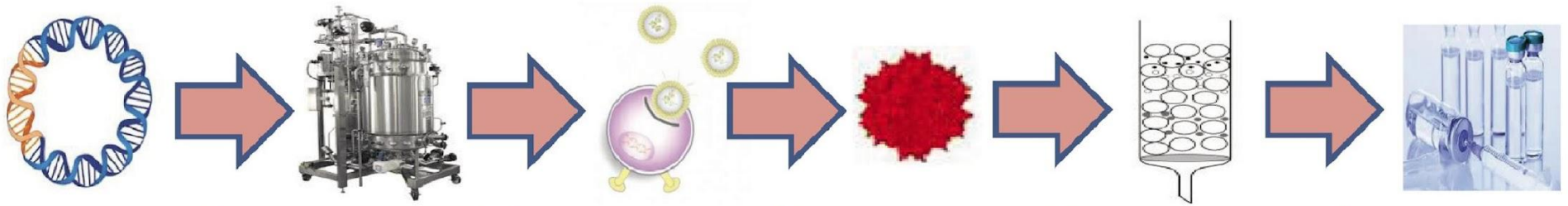
Gene therapy manufacturing challenges

- Complex biology
- Inconsistent manufacturing
- Mixed results with both HEK and baculovirus platforms
- Manufacturing changes during product development
- Limited material for characterization, comparability
- No standard cutoff for CQAs
- Lack of standard methods for measuring CQAs
- Assays are not standardized
  - Differences in sensitivity, precision, etc.
  - No ground truth
- High-priority examples
  - Partial capsids
  - Aggregates

Quality Attribute Category	Quality Attribute
Safety	Bioburden
	Endotoxin
	Sterility
Content/ strength	Appearance/particulates
	pH
	Osmolality
	Vector genome titer
	Potency (protein expression)
	Potency/infectious genome titer
Identity	Capsid identity
	Genome identity
Process impurities	Residual cell culture media components
	Residual host cell protein
	Residual plasmid DNA
	Residual host cell DNA
	Residual transfection reagent
	Residual chromatography ligand
	Replication-competent AAV
Purity	Capsid protein purity
	Capsid protein ratio
	% full capsids
	Total capsids
	Aggregates/subvisible particles

Project A-Gene

AAV viral vector manufacturing workflow

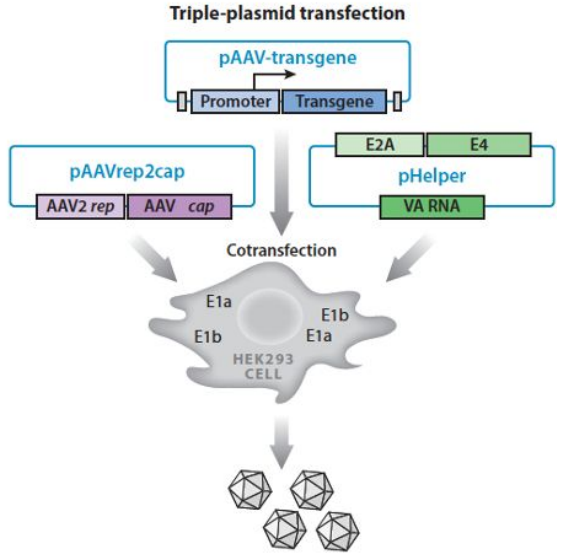


Srivastava (2021)

HEK293 vs Sf9/baculovirus

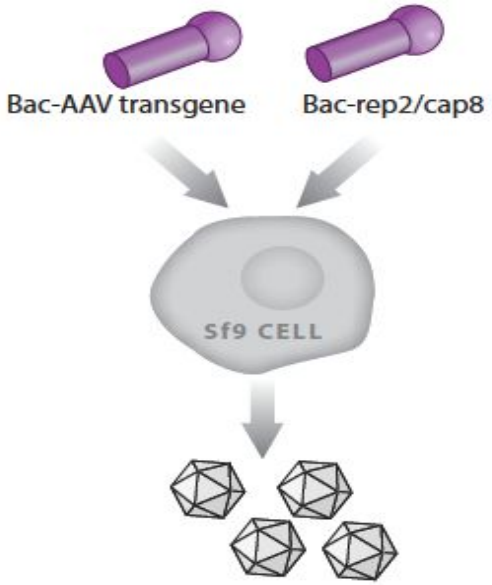
HEK process

- Yield up to ~1E14 vg/L harvest titer
- Upstream production <20% full capsids
- Adherent and transfection-based systems, and traditional ultracentrifugation difficult to scale
- Long history, more experience
- Faster time to AAV production



Sf9/Bac process

- Yield >1E15 vg/L harvest titer (10-100X adherent process)
- Upstream production >50% full capsids
- Can be manufactured in bioreactors from 50 L to >2000 L scale
- Fewer CMOs with experience
- Overall higher upfront costs and lower long-term costs
- Longer time to AAV production



How to Show Comparability ?

Products need be "*highly similar*" with "*no adverse impact*" in:

Quality | Safety | Efficacy

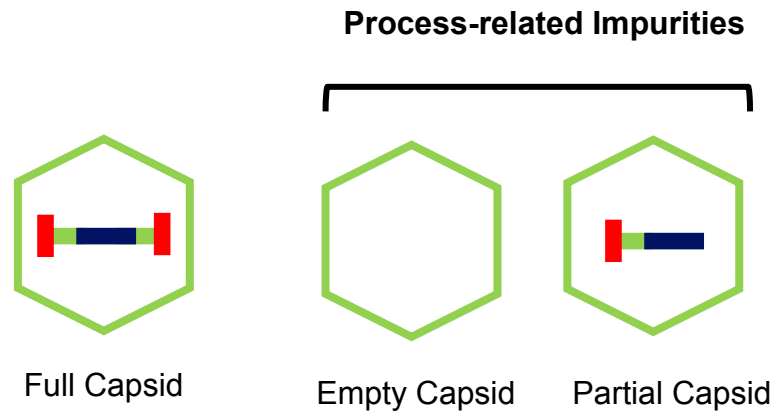
Analytical comparability | In-vivo NHP toxicology | Efficacy in animal models

Challenges	Approach
Material (few lots, material availability)	Include non-GM lots Prioritize testing
Methods (methods not qualified)	Test samples side-by-side Confirm w/ orthogonal method
Large set of data	Great organization and tracking

Quality: AAV Particle distribution

Analytical Ultracentrifugation

Process	Batch	Empty (%)	Partial (%)	Full (%)
HEK	Lot 1	3.9	47.7	39.9
	Lot 2	12.5	38.6	34.4
SF9	Lot 1	6.6	3.0	76.5
	Lot 2	8.6	3.5	80.9
	Lot 3	5.5	5.2	82.8
	Lot 4	3.1	9.2	84.3

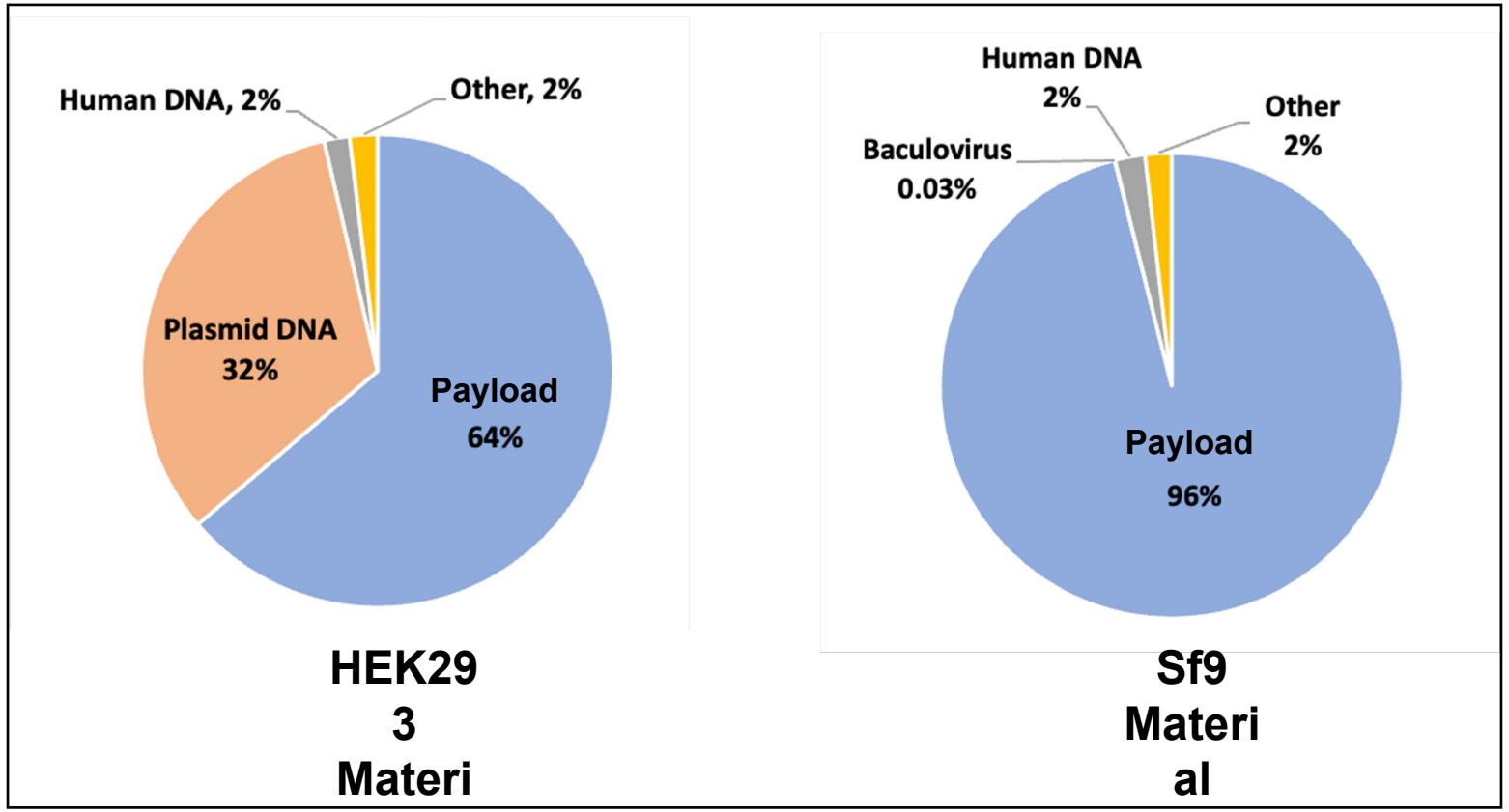


New platform: higher % Full, fewer Partials and Empty Capsids

***"No adverse impact"* on quality**

Quality: DNA Residuals

Next Generation Sequencing



Sf9 platform: Fewer DNA residuals

***"No adverse impact"* on quality**

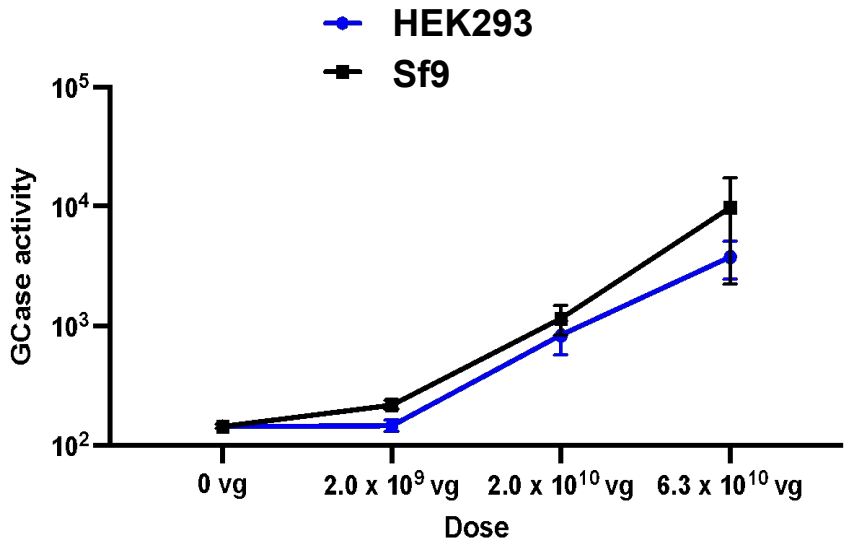
Efficacy: Comparable efficacy

***In vitro* Analytical Potency Assay**

Platform	Batch	Relative Potency
HEK293	Lot 1	153%
	Lot 2	143%
Sf9	Lot 3	142%
	Lot 4	93%
	Lot 5	113%

Assay variability 30% CV

***In vivo* Cerebral Cortex GCase activity in the CBE Mouse Model**



No statistically difference between lots, highly similar in-vivo efficacy

Comparable efficacy

Safety: Similar Safety

CMC Analytics

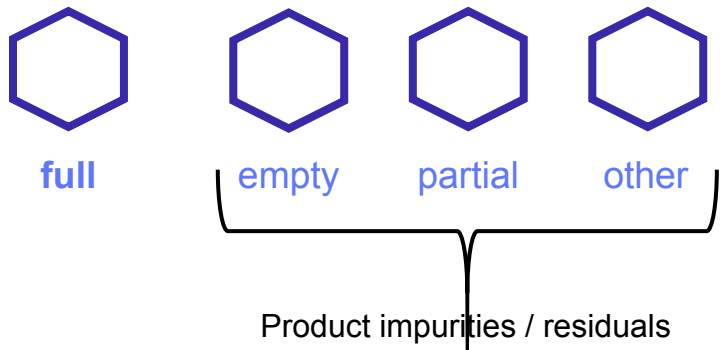
Test	PR001A (v1.0)	PR001A (v2.0)
Sterility	No Growth	No Growth
Endotoxin	≤ 0.5 EU/mL	≤ 0.5 EU/mL
Mycoplasma	Not detected	Not Detected
In- vitro Adventitious virus	Not Detected	Not Detected
In-vivo Viral contaminants	NT	Not Detected
rcAAV (Replicative competent AAV)	Not Detected	Not Detected

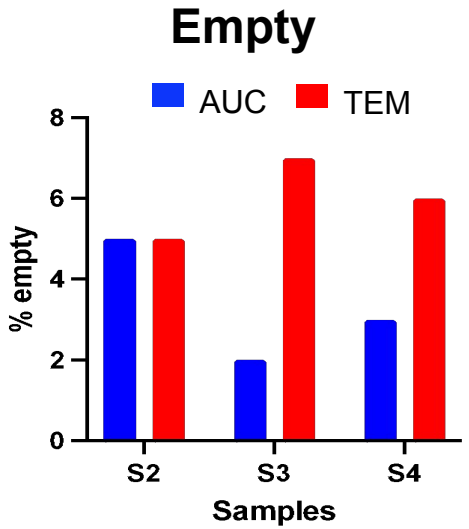
Toxicology Study in NHPs

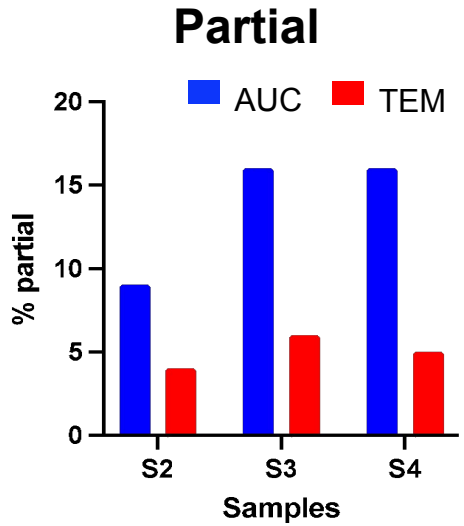
"No in-life or clinical or anatomic pathology findings related to the gene product were observed. Therefore, the dose levels were well-tolerated by male and female monkeys dosed via intracisternal injection to the cisterna magna."

Capsid proteins (V1, V2, V3): no change in

Partial capsids

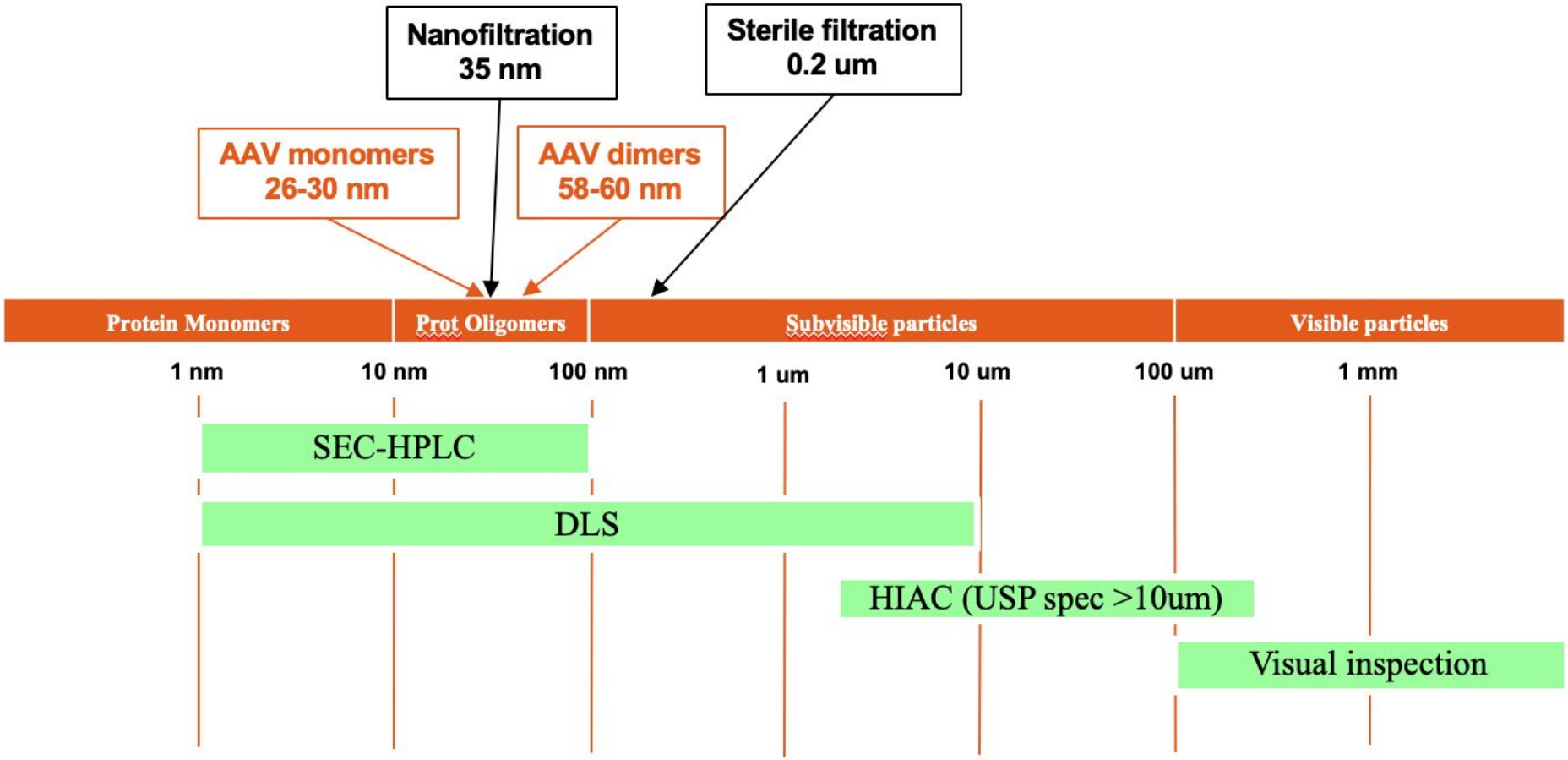






- Many techniques do not distinguish partial capsids
- TEM seemingly overestimates the number of empty species and underestimates the proportion of partial species compared to AUC

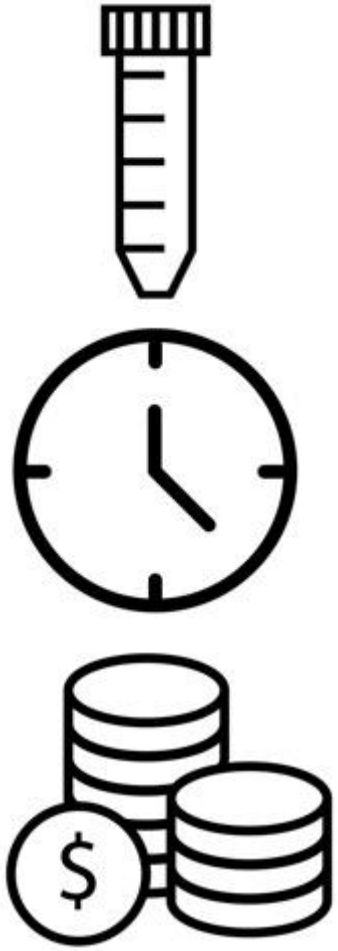
Aggregation Testing for AAVs



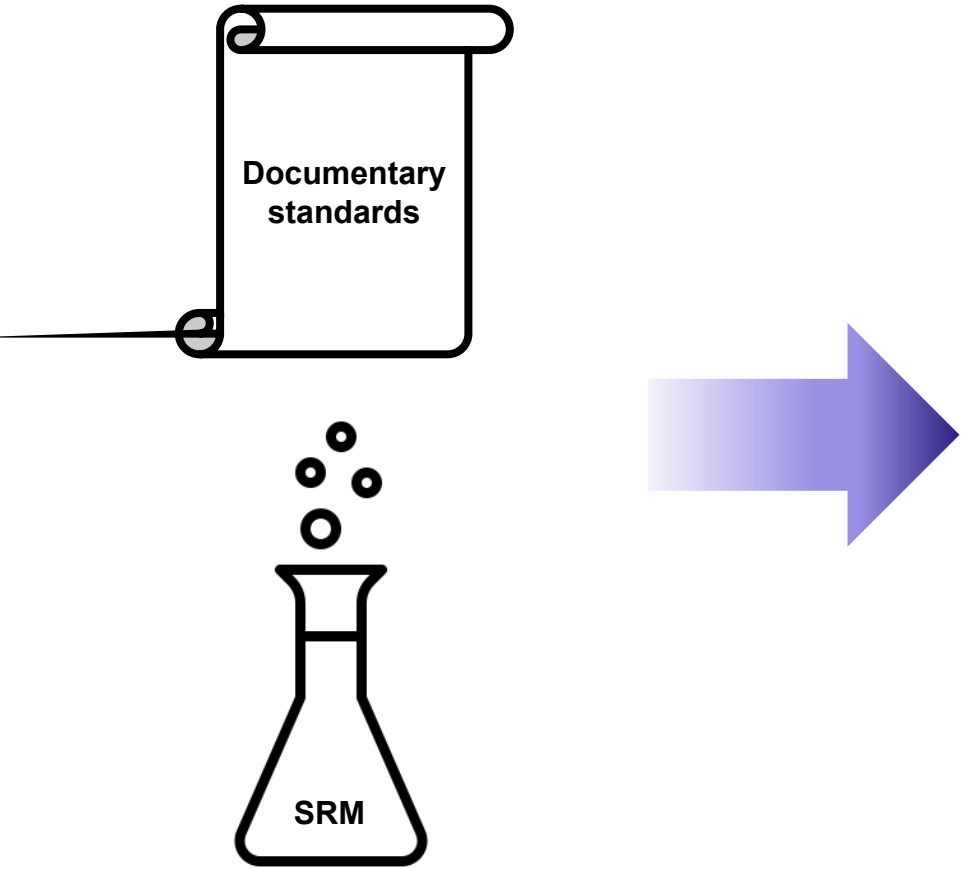
A combination of analytical techniques is required for assessing particle composition

S. No.	Technique	Detection of partial/intermediate species
1	Genomic Titer to Capsid Titer	✗
2	Analytical Ultracentrifugation	✓
3	CryoTEM	✓
4	SEC-MALS	✗
5	Anion Exchange Chromatography	✗
6	Mass Photometry	✓
7	Charge Detection Mass Spectrometry	✓

Additional requirements:



How can standards help?



- More uniformity in manufacturing
- Comparability of results
- Speed product development and regulatory review
- Enable data sharing
- Advance knowledge

Acknowledgements

- Jorge Haller
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