ASSESSING FEASIBILITY OF A STANDARD

for Methods for the Evaluation of T-Cell Therapies

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STANDARDS COORDINATING BODY
REGENERATIVE MEDICINE

NEXIGHT GROUP

DISCLAIMER

This report was prepared for the U.S. Food and Drug Administration (FDA), Center for Biologics Evaluation and Research by Nexight Group and The Standards Coordinating Body for Gene, Cell, and Regenerative Medicines and Cell-Based Drug Discovery (SCB) under order number 75F40122F80406. The information and perspectives contained in this report are those of the authors and should not be attributed to the FDA. The mention of trade names, commercial products, or organizations does not imply endorsement of same by the U.S. Government.

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Introduction

Since the *21st Century Cures Act* was signed into law in December 2016, the U.S. Food and Drug Administration (FDA) has been engaged in ongoing efforts to fulfill its provisions to accelerate medical product development through the advancement of standards. The Standards Coordinating Body for Gene, Cell, and Regenerative Medicines and Cell-Based Drug Discovery (SCB) is supporting the FDA's efforts by coordinating the activities of the regenerative medicine community to accelerate regenerative medicine standards development.

A key element of SCB's support in accelerating standards development is engaging regenerative medicine stakeholders to help assess the feasibility of needed standards using the methods SCB outlined in *Realizing the Promise of Regenerative Medicine Therapies: Strengthening the Standards Development Process*. Assessing a needed standard's feasibility early in the standard advancement process is critical to ensuring efficient use of community resources.

SCB developed this report in partnership with Nexight Group to outline the results of its feasibility assessment of potential standards for the evaluation of T-cell therapies.

Need Overview: Methods for the Evaluation of T-Cell Therapies

T-cell therapies use modified T cells—white blood cells closely involved in regulating cell-mediated immunity—to recognize and eliminate cancer cells. T-cell therapies can lead to a variety of negative reactions, including neurotoxicity, incorrect targeting of non-cancerous cells, and anaphylaxis. The introduction of T-cell therapies can also trigger cytokine release syndrome (CRS), an influx of inflammatory cytokines that produces a potentially life-threatening immune response. Standards for product evaluation would be valuable to help ensure the quality, safety, and consistency of T-cell therapies.

Process

Phase 1: Initial Priority and Feasibility Assessments

After this area of standard need was identified, SCB assembled a working group to further assess the priority and feasibility of the needed standard. SCB conducted facilitated meetings in December 2022 and January 2023. See below for a breakdown of meeting participants by stakeholder group.

December 2022 and January 2023 Meeting Attendance by Stakeholder Group

Count	Stakeholder Type
4	Industry
1	Regulatory
1	Academia
3	SCB
2	Nexight Group

Feasibility Assessment Factors

The feasibility assessment considered four factors: technical feasibility, expert availability, implementation feasibility, and other related factors. Together, these factors offer a comprehensive overview of whether a standard is scientifically ready to advance and has sufficient buy-in from experts who are willing to support the standard advancement effort and community stakeholders who will ultimately adopt the standard.

- **Technical feasibility** assesses whether an adequate technical and scientific foundation exists for creating the standard and seeks to ensure that the standard will serve its intended purpose.
- **Expert availability** assesses whether there is sufficient interest from experts in the community to advance the potential standard, as well as buy-in from potential standards development organizations (SDOs) to publish a formal standard.
- **Implementation feasibility** considers factors that influence an individual firm's adoption of the standard such as costs; the standard's compatibility with existing equipment, materials, and technology; and required in-house expertise.
- **Other feasibility factors** include development costs, time to develop, accessibility, and legal feasibility.

Phase 2: Community Workshop for Detailed Need Assessment

FDA co-organized a workshop in November 2023 with SCB and the United States Pharmacopeia (USP) to build on the results of the feasibility assessment and identify specific subtopics that are feasible to standardize and would make a significant positive impact on the regenerative medicine field.

The workshop was a hybrid two-day event held at USP headquarters in Rockville, MD. It was attended by more than 50 in-person stakeholders and 180 virtual stakeholders from industry, academia, SDOs, and government agencies, among other regenerative medicine stakeholder groups.

Each day centered around a breakout session that offered participants a chance to engage in **detailed discussions of standards needs for gene and cell therapy product assessment.** After identifying needs, the groups voted on potential standards that would have the greatest positive impact in the field.

Findings

During the initial feasibility meetings, participants discussed current challenges surrounding the topic of T-cell therapies and a variety of sub-topics that should be considered for standardization. They identified three high-priority subcategories to potentially standardize:

- Language and terminology (e.g., definitions, T-cell subcategories)
- Assessment of modified T-cells in the body (e.g., reactivity, side effects, particulates, dosage)
- Testing/analytics requirements (e.g., assays, instrumentation, acceptable result ranges, measurement units, data logging)

Participants determined that a potential standard within one of the proposed sub-topics would have few significant technical, expert, or implementation feasibility barriers, but standardization efforts could experience slight pushback from manufacturers in terms of defining phenotypes.

After assessing each potential sub-topic, the group concluded that it would be most beneficial to focus specifically on standards advising on how to select, implement, and validate assays related to chemistry, manufacturing, and controls (CMC) such as phenotypic and functionality assays.

These initial discussions are described in depth in **Appendix: Detailed Phase 1 Findings.**

FDA identified a need to **further refine the scope of the standards needs identified during Phase 1 with input from the community** about current challenges and gaps, which was accomplished through a community workshop. The Phase 2 workshop focused on narrowing the testing/analytics topic from Phase 1 to focus on a specific assay or set of assays. Workshop participants identified the following two priority topics:

1. Best practices for statistical approaches to comparability analyses
2. Phenotype - flow cytometry markers/antibodies/controls

Challenges and Standards Needs

The workshop discussion on cell therapies touched on numerous challenges in the field; some of the major challenges included:

- The need for statistical methods to assist with analyzing complex data
- Limitations from the standpoint of sample validity and generating enough statistical power to allow meaningful comparisons
- Difficulty convening experts and encouraging information sharing
- Uncertainty around the right markers to evaluate for product quality and safety

Participants also discussed various action items that could support standardization, including identifying existing standards to leverage, characterizing existing kits, and improving communication to raise awareness across stakeholders about relevant standards development activities and existing standards resources.

Participants then identified standards topics that could address each of the challenges they had discussed. They ranked the identified topics by priority; the full list of identified topics in order of priority includes:

1. Best practices for statistical approaches to comparability analyses
2. Phenotype – flow cytometry markers/antibodies/controls
3. Killing assays for CAR-T therapies
4. Assays to detect the presence of replicating viruses
5. Standard approach to IL-2 independent proliferation
6. CAR expression assays for rapid CAR-T cell manufacturing
7. Assays for oncogene mutation assessment at the induced pluripotent stem cell (iPSC) stage
8. Standard cell line transduced with platform/universal targets for vector copy number assays
9. Artificial intelligence/machine learning-based cell counting
10. Specific functional assays and expected readouts for signaling domains commonly used in CARs

Priority Topic 1: Standards for Best Practices for Statistical Approaches to Comparability Analyses

During statistical analysis for comparability assessments, cell therapy product developers must make numerous decisions around topics such as confidence intervals, how to transform data, and whether data will be normalized, among others. The field would benefit from a statistical analysis standard to inform stakeholders of current best practices and offer a common decision-making framework.

Components of a Standard

A standard on this topic could address:

- Stimuli companion documents (e.g., via USP)
- Education (e.g., when equivalence evaluation is needed and when it is not)
- Prescriptive guidance on methods, including:
    - Testing pre- and post- change product in the same assay
    - Sample sizes on different risk score critical quality attributes (CQAs)
    - Clinical study requirements for non-comparable products (patient safety)

Stakeholders to Involve in Standards Advancement

Stakeholders to involve in the standard advancement effort could include statisticians, professional societies, industry, smaller manufacturers, and contract development and manufacturing organizations (CDMOs).

Potential Barriers to Standardization

Feasibility Assessment Factor	Description of Barrier
Technical Feasibility	There may be insufficient consensus on choice of assays
	Small sample sizes
Expert Availability	It may be difficult to identify contributors with interest and relevant expertise, particularly from academia
Other Feasibility Barriers	There may be difficulty harmonizing with global regulations and standards

Based on the workshop discussion, the group determined that expert availability and difficulty harmonizing with existing regulations and standards would present significant barriers and **a standard on this subtopic would not be feasible at this time.**

Priority Topic 2: Standards for Phenotype – Flow Cytometry Markers/Antibodies/Controls

Determining the phenotype of cells in a sample is important for assessing the purity and potency of a cell therapy product, among other attributes. The regenerative medicine field would benefit from standards in this area to help ensure that assay results are comparable and reliable.

Components of a Standard

A standard on this topic could address:

- Quantitative understanding of mitochondrial strength
- Analysis of T-cell penetration and penetration of serological materials passing the blood-brain barrier and targeting neoplastic cells
- Gating strategy/data analysis
- Standard controls for assay performance and tracking
- Markers and impurity profiles for specific T-cell lineages/populations
- Qualified controls for each step of the most critical phenotypic assay
- Limits of dynamic range of assays
- Protocol for choosing parameters for titrated reagents
- Expectations for release vs. characterization assays, including:
    - Parameters for method qualification and validation
    - Gating controls
    - Method bridging

Stakeholders to Involve in Standards Advancement

Stakeholders to involve in the standard advancement effort could include the International Society for Stem Cell Research (ISSCR), the National Institute of Standards and Technology (NIST) Flow Cytometry Consortium, American Society of Hematology (ASH), manufacturers, CDMOs, and end users.

Potential Barriers to Standardization

Feasibility Assessment Factor	Description of Barrier
Technical Feasibility	Variability in reagents, instruments, and analysis approaches
	Rapid change in availability of new markers
	Difficulty aligning on phenotypes of interest
	Difficulty identifying a minimal panel given what currently exists
Implementation Feasibility	Challenge with implementation if the standard is too complex or deviates too much from current industry practice
	Issues with availability of reference materials that work across instrument platforms
	High cost and time investment

Based on the workshop discussion and follow-up discussions with the NIST Flow Cytometry consortium, the group determined that technical feasibility barriers such as variability in equipment and a lack of physical reference materials for standardization of other measurements would make it **too difficult to develop a standard on this subtopic at this time.**

Several efforts are underway by the Clinical & Laboratory Standards Institute (CLSI) and the NIST Flow Cytometry consortium that may lay the groundwork to make a phenotype standard more feasible in the future, including interlaboratory studies to increase instrument standardization.

Next Steps

SCB plans to organize new working groups to further assess the feasibility of the prioritized topics for standardization and potentially advance them to SDOs for development.

Due to the major feasibility barriers identified with the top two priority topics, SCB will focus initially on advancing a standard for the **killing assays for CAR-T therapies** topic, which was the third-highest priority subtopic identified.

Next steps for the standard advancement effort are described below.

Goals for 2024–2025

- **Assemble a working group and seek relevant expertise,** focusing on the expertise areas identified in the feasibility report.
- **Conduct discussions with the working group** to confirm whether to move forward with the creation of a standard and further refine the standard's scope.
- **Identify interested SDOs** and formalize a plan to advance the standard within a particular SDO. Once the scope of a potential standard is finalized, SCB will reach out to contacts at relevant SDOs to evaluate their interest.
- **Make a final assessment** of whether the standard should be advanced, researched further through independent efforts, or held for future reconsideration. Based on the feasibility assessment, SCB expects the standard to move forward if community enthusiasm and participation remain high.
- **If the standard is expected to move forward,** SCB will begin to outline the potential standard and support its advancement through the relevant SDO development process.

Appendix: Detailed Phase 1 Findings

Technical Feasibility

Standards require strong scientific and technical bases to build community consensus. If too many unanswered technical questions remain at the time of standard development, the standard may be held up indefinitely until the field matures. Technical feasibility assesses whether an adequate technical and scientific foundation exists for creating the standard and seeks to ensure that the standard will serve its intended purpose.

The feasibility assessment group believed that the field is sufficiently mature to support the development of standards for T-cell therapies. While some disagreements remained about technical details of the potential standard (e.g., the specific T-cell subcategory to focus on), the group believed it would be possible to resolve these disagreements with further discussion.

SUB-TOPIC: Language and Terminology

Currently, the terminology used in the field of T-cell therapy is ambiguous and inconsistent, and T-cell subcategories that have different roles in immune response are not consistently defined. Clarifying terminology, language, and characterization will benefit both researchers and manufacturers.

OPPORTUNITIES	BARRIERS
• It would be helpful to **define various T-cell subcategories** and the phenotypic characterizations. • **Standardizing markers to correspond to specific T-cell subcategories** and applying multiple markers to subcategories would help to avoid discrepancies. • The field would benefit from further understanding and definition of what is considered **acceptable quality versus unacceptable quality for central memory cell capacity.** Assays can be used to assess quality or collect quantitative data.	• Scientific agreement regarding how to define and characterize different **T-cell types, subcategories, markers, and phenotypes** is lacking. • There is the potential for pushback from T-cell manufacturers on a standard establishing **phenotypic characterization**. This also includes the description of the subcategories and proper assays to clearly distinguish them.

SUB-TOPIC: Assessment of Modified T-Cells in the Body

As the development of T-cell therapies continues, it is critical to develop an understanding of how product components and technical choices affect the patient (e.g., immune responses, toxicity, product particulate safety). Testing products for safety, potency, and reactivity is crucial in the clinical production process. Defining the forms of assessment and the markers to target could also support overall therapy design.

OPPORTUNITIES	BARRIERS
• It would be valuable to accurately define and determine **a safe level of particulates (e.g., viral capsids, reagents) left in the body** after dose administration. • After dose administration, there is a need for better understanding and standardization of **leftover T-cells in the body** that were unused or are now considered "residue."	• There is a need for additional maturation of the science related to **biodistribution and assessment of the body.** • Currently, there is no standard that defines when healthy human tissue should be used in the production process. Using non-human tissue in the development of the product could cause issues and reactions down the line. There is a need to further discuss bridging the **gap between human and non-human tissue testing** in development stages. • There is a need to determine **clear and definitive levels of potency** that are suited for the patient's needs and immune response. However, there is not yet a clear way to approach this challenge.

SUB-TOPIC: Testing/Analytics Requirements

The quality of T-cell therapy products, or what is considered acceptable and unacceptable, is defined through analysis and testing. Currently, manufacturers and researchers use different markers to confirm product quality, making it difficult to compare and replicate results.

OPPORTUNITIES	BARRIERS

• Meeting participants determined that the following knowledge gaps would be valuable to address through standards:
  ○ Collectively deciding what assays to use to **assess certain T-cell subcategories**
  ○ Refining the **type of assays and markers used to analyze and collect data** in order to replicate and compare information
  ○ **Standardizing the production of assays** and defining the markers and antibodies to use in specific assays
  ○ Clarifying the **different outcomes in using different brands and instruments** when collecting T-cell data
  ○ Determining the **appropriate use of analytics tools** (e.g., multicolor flow cytometry, characterization of phenotypes, sequencing)
  ○ Determining analytics tools that are **effective at detecting cell damage** (e.g., cytokine release assays) compared to other testing methods
  ○ **Streamlining analytical methods for FDA review**, especially for therapies undergoing regulatory submission (currently at the Biologics License Application [BLA] stage)
	• **Varying results depending on the brand of analytics tools** could cause difficulties in replication and inability to compare data across research and products.
	• **Antibody use is challenging in flow cytometry**. Multicolor compensation beads can vary from instrument to instrument, and there is not a clear way to address this challenge with standardization.

Expert Availability

Standards development requires committed technical experts who can advance the potential standard and help communicate the standard's value to the regenerative medicine community. If there is insufficient interest from experts in the community, the working group may be unable to obtain the necessary technical information to include in the standard. Likewise, buy-in from an SDO is needed to publish a formal standard, although best-practices documents and other informal guides can be produced independently.

The feasibility meeting participants determined that it would be valuable to involve the following groups in standard development:

- Multicolor flow cytometry experts from NIST
- Gene editing specialists
- Quality assurance/quality control experts
- Analytics leads in the field
- Experts in novel T-cell testing products
- Biomanufacturers
- Contract Research Organizations (CROs)
- FDA

Implementation Feasibility

Implementation feasibility considers factors that influence an individual firm's adoption of the standard: incurred costs; the standard's compatibility with existing equipment, materials, and technology; and required in-house expertise. If a standard is developed that does not have the support of the community, adoption rates may ultimately be too low for the standard to have significant impact.

Overall, the participants believed that implementing this standard would not create a large financial strain on manufacturers or researchers. They also did not anticipate major disagreements in the field around the standard.

OPPORTUNITIES	BARRIERS
• **Low monetary expense** for the implementation of this standard would likely result in minimal pushback from manufacturers and researchers. • As long as the **T-cell therapies are already being produced at sufficient quality** prior to implementation of the standard, implementing standards should not strain the manufacturer.	• Standard implementation could be more burdensome for those who **harvest T-cells**, which could include smaller organizations.

Other Feasibility Factors

Several other factors—including development costs, time to develop, accessibility, and legal feasibility—can impact the feasibility of developing and adopting a potential standard.

Participants did not identify any additional major feasibility concerns.