American Society of Gene + Cell Therapy

July 29, 2024

Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

RE: Comments for Docket No. FDA-2024-D-1243 "Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products; Draft Guidance for Industry"

Dear Sir/Madam:

The American Society of Gene & Cell Therapy (ASGCT) appreciates the opportunity to comment on the document "Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products; Draft Guidance for Industry." ASGCT is a nonprofit professional membership organization comprised of more than 6,200 scientists, physicians, clinicians, and other professionals working in cell and gene therapy (CGT) in settings such as universities, hospitals, and biotechnology companies.

The mission of ASGCT is to advance knowledge, awareness, and education leading to the discovery and clinical application of genetic and cellular therapies to alleviate human disease. Many of our members have spent their careers in this field performing the underlying research that has led to today's robust pipeline of transformative therapies.

General Comments

ASGCT appreciates FDA for its proactive effort to address the evolving landscape of cell-based therapies. To date, FDA's Center for Biologics Evaluation and Research (CBER) has approved three human allogeneic cell and gene therapy products with many more products in the pipeline. By establishing clear safety testing protocols and integrating with existing guidelines, 21 CFR 610.18(c)(1) and 312.23(a)(7), the FDA is demonstrating a commitment to enhancing patient safety and streamlining the regulatory process for developers, ultimately creating greater patient access to therapies.

ASGCT believes that the coordinated release of this draft guidance with Considerations for the Use of Human- and Animal-Derived Materials in the Manufacture of Cellular and Gene Therapy and Tissue-Engineered Medical Products is an effective strategy to address the use of cells and materials across sources and ensure consistency and clarity across related documents.

We do note, however, that the Human Gene Therapy Products Incorporating Human Genome Editing guidance does not include the same recommendation for the use of whole genome sequencing (WGS) to test for off-target mutations. We request that this discrepancy be addressed, and that the Agency evaluate other guidance documents for similar discrepancies.

The Society commends the organization of the guidance which addresses the safety testing of three categories of primary cells - extensive expansion, limited expansion, and cells administered to a few individuals. This differentiation is valuable as it addresses the varying levels of risk and necessity for safety measures in a structured manner; that approach will help stakeholders better understand and comply with the appropriate testing protocols for their particular use. We do, however, request that FDA provide additional clarification in the final draft to better distinguish between these categories.

Finally, we appreciate that the guidance distinguishes between testing required at different cell banking stages. This ensures the safety testing is thorough and addresses potential risks at each critical point in the cell banking process. By outlining these requirements, the guidance helps streamline the development and regulatory approval process for allogeneic cell-based medical products.

In addition to these general comments, the Society respectfully submits the following line edits and additional comments for consideration:

Specific Comments

III. Background		
Lines/Section/Text Reference	Comment	Text Recommendation
59-63 “Viral and microbial contamination is a potential risk for all cell-based medical products, especially when the cells are cultured extensively during manufacturing. Contamination may be present in the source cells, or the cells may become contaminated with adventitious agents during	This section describes risks to allogeneic cells as: 1) viral/microbial contamination and 2) genomic changes that can occur during extensive culturing. Considering that the guidance also addresses gene edited allogeneic products, and specifically discusses test methods to be used on genome edited cells (Line 301), we suggest listing a third risk as the gene editing step, which may	“Viral and microbial contamination is a potential risk for all cell-based medical products, especially when the cells are cultured extensively during manufacturing. Contamination may be present in the source cells, or the cells may become contaminated with adventitious agents during manufacturing. In addition, genomic changes that result

manufacturing. In addition, genomic changes that result in tumorigenic cells can occur during extensive culture."	introduce additional unintended mutations.	in tumorigenic cells can occur during extensive culture. Furthermore, if gene editing is used in an allogenic product, it may also introduce additional unintended mutations."
IV. Considerations for Cell Safety Testing		
Lines/Section/Text Reference	Comment	Text Recommendation
148-151 "Primary cells with a limited expansion potential can be expanded to make a cellular therapy product, or to create small to midsize cell banks or a single lot of cells to manufacture cell-based products capable of being administered to a limited number of individuals."	ASGCT requests a definition or parameters that delineate what constitutes a "small to midsize cell bank."	N/A
162-163 "It is not recommended that primary allogeneic cells that are minimally expanded in culture to be administered to only a few individuals, or a single individual, undergo cytogenetic analysis or adventitious virus testing."	ASGCT also seeks clarification on the phrase "a few individuals." Specifically, we request a definition or numerical range that specifies what constitutes "a few individuals."	N/A
V. Testing Recommendations For Highly Expanded Cells		

Lines/Section/Text Reference	Comment	Text Recommendation
Section V. Testing Recommendations For Highly Expanded Cells	We appreciate the detailed recommendations provided for master cell bank and working cell bank testing for highly expanded cells. However, we suggest including an additional section on final product testing. The new section on final product testing could provide specific recommendations for the final cell-based product to ensure all critical stages, from cell banking to final product, are thoroughly addressed.	
222-233 “In vitro adventitious virus testing – Three cell lines should generally be used: human diploid (e.g., MRC5 cells), monkey kidney (e.g., Vero cells), and another cell line of the same species and tissue type as that used for production (e.g., HeLa cells if the product was made using human cells). However, different cell lines may be appropriate depending on the manufacturing process. For instance, when insect cells are used during manufacturing, BHK21 cells may be used to detect viruses such as rhabdoviruses. In this example, testing for adventitious viruses using	Although this section is comprehensive in recommending the use of cell lines for in vitro adventitious virus testing, it would benefit from better alignment with the ICH guideline Q5A(R2) by incorporating advanced methods such as NGS or HTS.	ASGCT suggests including the following text at the end of the referenced paragraph: “Additionally, in alignment with the revised ICH guideline Q5A(R2) (Section 3.2.2), next-generation sequencing (NGS) or high-throughput sequencing (HTS) can be considered as alternative or complementary methods to traditional in vitro adventitious virus testing.”

BHK21 cells would address the recommendation of testing for viruses in cells of the same species in which product production occurs. The BHK21 cells would be the third cell line recommended for adventitious virus testing when used in addition to the human diploid and monkey kidney cell lines."		
242-243 "Alternatively, a high throughput sequencing method may be used instead of in vivo adventitious virus testing to detect contaminating viruses."	ASGCT also suggests referencing the specific section of ICH guideline Q5A(R2).	"Alternatively, a high throughput sequencing method may be used instead of in vivo adventitious virus testing to detect contaminating viruses as detailed in ICH guideline Q5A(R2) 3.2.3."
301-302 "Whole genome sequencing and analysis should be performed on cell banks of continuous cell lines and genome edited cells."	ASGCT suggests the addition of an advisory note for whole genome sequencing analysis, similar to the previous advisory (lines 247-249) for communicating with FDA prior to implementation.	"Whole genome sequencing and analysis should be performed on cell banks of continuous cell lines and genome edited cells. We advise discussing the proposed whole genome sequencing method and validation plan with FDA prior to its implementation to ensure alignment with regulatory expectations."
304-305 "Cell lines that are cultured extensively often accumulate mutations during cell expansion."	We seek clarification on the guidance that cell lines that are "cultured extensively" should undergo WGS. Currently, using WGS to test cell lines derived from cell banks is not common practice, so interpreting this	N/A

	guidance depends heavily on how “cultured extensively” is defined. To provide clarity, we suggest FDA define "cultured extensively" by specifying a threshold based on the number of cell divisions or passages, potentially using human somatic mutation rates as a reference. This will help determine when WGS is necessary. Additionally, if this requirement represents a higher standard for cell bank testing, it should be tied to a risk-based approach based on the stage of drug product development, ensuring that the testing is appropriate and practical at different phases of development.	
308-311 “The whole genome sequencing method used should have a read depth of at least 50X, and at a minimum, the results should be compared to a database of cancer associated mutations.”	We seek clarification on the requirement for 50X coverage by WGS. Is the “50X coverage” an expected average across the genome or is the requirement to obtain 50X coverage at every nucleotide position? The latter is technically challenging as some regions of the genome are hard to sequence due to low complexity (e.g. repetitive sequence) or population variations (e.g. known duplications). We suggest FDA: • Clarify 50 X coverage as an average across the genome.	N/A

	• Allow for a risk assessment and/or orthogonal testing for any regions not sufficiently covered by WGS. • As discussed above, align with and reference the Gene Editing guidance and clarify if the testing of an expanded clonal MCB is sufficient to not require repeated testing at later manufacturing stages if no further gene editing is done.	
311-313 "Justification should be provided for the sequencing method, read depth, and for conclusions related to the safety of the product."	ASGCT appreciates the clarity on the preferred type of analysis performed on cell banks (lines 301-302) of continuous cell lines and genome edited cells. However, there are some challenges and uncertainties regarding the interpretation of the large amount of data generated by WGS. For example, stakeholders are likely to encounter numerous genetic changes. The draft guidance does not provide clear enough instruction on how to conduct this analysis or address these genetic findings. To address the challenges and uncertainties associated with the interpretation of WGS data, we recommend the inclusion of a risk-based approach tailored	N/A

	to the stage of drug product development.	
315-319 “For highly expanded clones of genetically modified cells, whole genome sequencing with at least 50X read depth should be performed to identify off-target genome editing, on-target editing outcomes, vector integration events, and to screen for any mutations of concern.”	Please reference comments for lines 308-311.	N/A
321-324 “Cytogenetic testing or whole genome sequencing should be performed on highly expanded primary cells that contribute cells to the final product. Whole genome sequencing as described above is the recommended method of testing genome integrity.”	WGS is primarily intended for assessing the MCB, however the current guidance does not specify the type of stability testing expected through the end of the process. We suggest clarifying whether WGS is preferred beyond the MCB as part of ongoing stability testing, or if it is intended as a one-time integrity check of the cell bank.	N/A
326-327 “Alternatively, if cytogenetic testing is performed, G-banding analysis or other sensitive methods should be used to confirm the cells have a normal karyotype. The karyotypes of at least 20 cells should be analyzed.”	While karyotyping is a traditional method for confirming normal cell karyotypes, it has certain limitations, such as the potential for abnormal data due to cell state and cell culture material, as well as not being a GMP method. We suggest FDA reconsider the value karyotyping adds to product safety testing and to	N/A

	evaluate whether more orthogonal methods could replace it.	
338-340 "Tumorigenicity testing, highly expanded cells – Under 21 CFR 338 610.18(c)(1)(ii), cell lines used for manufacturing biological products shall be described with respect to tumorigenicity."	We appreciate the guidance provided on tumorigenicity testing for highly expanded cells and continuous cell lines. However, the current section lacks specific recommendations for tumorigenicity testing once the cells are differentiated into final cell products. It would be beneficial to clarify what specific tumorigenicity tests are acceptable and relevant for these differentiated products.	N/A
342-344 "In cases where the cells present in the final product are phenotypically similar to those in the MCB, the tumorigenic potential of a product may be tested using cells from the MCB."	ASGCT appreciates the guidance provided for testing the tumorigenic potential of cells that are phenotypically similar to those in the MCB. However, we seek further clarification on the recommended testing approach for cases where the cells in the final product are phenotypically distinct from the cells in the MCB, such as differentiated progeny of iPSCs.	"In cases where the cells present in the final product are phenotypically similar to those in the MCB, the tumorigenic potential of a product may be tested using cells from the MCB. When the cells in the final product are phenotypically distinct from the MCB cells, such as differentiated progeny of iPSCs, we recommend that the tumorigenicity be evaluated in preclinical studies using representative material."
VI. Testing Recommendations for Cells with Limited Expansion Potential		

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Lines/Section/Text Reference	Comment	Text Recommendation
408-411 Table 1. Cell Safety Testing Recommendations for Allogeneic Cells Expanded for Use in Cell-Based Medical Products “Limited number of individuals” “A few individuals or a single individual”	We appreciate the comprehensive nature of Table 1. However, as discussed earlier, the terms “a limited number of individuals” and “a few individuals or single individuals” are too vague and ambiguous. Clear and precise definitions are crucial to application of the guidelines. ASGCT suggests defining requirements based on disease prevalence. For example, “limited number of individuals” could refer to products intended for diseases with a prevalence of less than 1 in 10,000 and “a few individuals or a single individual” could refer to ultra-rare diseases with a prevalence of less than 1 in 50,000.	N/A

Thank you for the consideration of these comments. If you have any questions, please do not hesitate to contact Margarita Valdez Martínez, Chief Advocacy Officer, at mvaldez@asgct.org.

Sincerely,

David Barrett, J.D.
Chief Executive Officer