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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
Cell therapy upstream processing and materials

Guest Editor
Isabelle Rivière, Takeda

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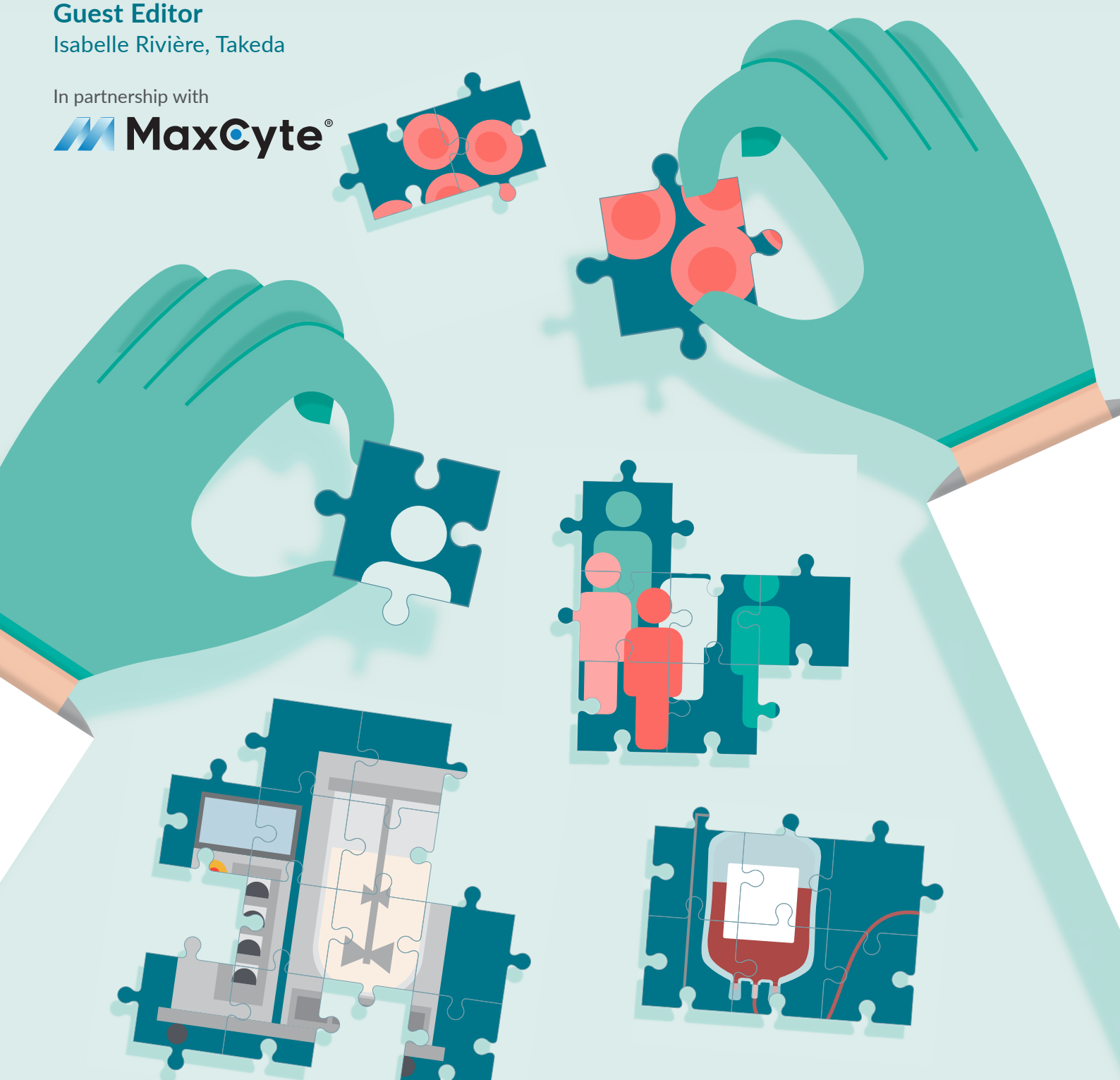

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Raw materials and supplies for cell therapies: end to end expectations and best practices

Lili Belcastro

Ancillary materials are the raw materials and single-use systems that are used during the manufacturing of cell and gene therapies. Selecting, evaluating, and qualifying these materials require careful consideration. In this article, the aim is to provide readers with an overview of available guidance documents, best practices, and points to consider for raw material and supplier qualification from the point of view of a CAR-T drug product manufacturer.

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INTRODUCTION

Ancillary materials (AMs), as defined by the *United States Pharmacopeia* (*USP*) chapter <1043> and the International Standards Organization (ISO) Standard 20399, are raw materials (RMs) and consumables used in the manufacturing of cell and gene therapy (CGT) products or advanced therapy medicinal products (ATMPs) [1,2]. These materials come into contact with the starting materials, product intermediates, and final drug products but are not intended to be present in the final drug product. The focus of this article is on the requirements and considerations of the ancillary materials used in the manufacture of chimeric antigen receptor-T cell (CAR-T) therapies from the perspective of end-users of AMs. The term 'ancillary materials' will be used for describing all RMs and single use systems (SUS) or polymeric consumables. RMs will encompass reagents, buffers, cell culture

media, proteins, supplements, etc. used in CAR-T manufacturing. SUS will refer to all single-use bioprocessing containers, single use assemblies, filters, polymeric components (e.g., tubing, connectors) used in CAR-T cell therapy manufacturing. The term 'supplier' will identify AM manufacturers, suppliers, and distributors, while 'user' will refer to AM users and ATMP manufacturers.

OVERVIEW OF THE MANUFACTURING PROCESS HIGH LEVEL UNIT OPERATIONS

Numerous AMs are utilized in the CAR-T manufacturing process. The overall CAR-T manufacturing process can be summarized by the following high-level unit operations:

- Leukapheresis collection
- T cell isolation
- Cell activation
- Cell transduction or gene editing
- Cell expansion
- Cell harvest
- Drug product (DP) formulation and cryopreservation

Subsequent sections will cover specific materials used and considerations for each of these unit operations.

OVERVIEW OF AVAILABLE STANDARDS, GUIDELINES, AND BEST PRACTICES FOR AMS

The ATMP industry is constantly evolving, leading to frequent updates in health authority regulations, guidance documents, standards, and best practices. Various resources are available to assist suppliers and users of AMs in navigating the regulatory landscape and conducting proper qualification activities. Table 1 [1–9] provides a non-exhaustive list of references along with a brief description of their scope.

Based on the documents mentioned in Table 1, suppliers and users can use these as a framework to establish control strategies for AMs. Table 2 [6,10,11] below provides a compilation of best practices for release, qualification, and stability testing, along with documentation considerations for animal-derived materials. It also includes relevant quality terminology to consider during the selection and evaluation of AMs.

SUPPLIER CONSIDERATIONS

The responsibility for selecting and qualifying a supplier lies with the AM user. AMs are not regulated by health authorities, i.e., they do not conduct audits of AM suppliers. Therefore, it is crucial for the user to ensure that the supplier has appropriate manufacturing controls, documentation, training, and other necessary measures in place. As suppliers adhere to GMP, it is essential for the user to understand which specific GMP principles are being followed and identify any gaps based on their requirements for AMs. In addition to best practices described above, the following tables provide a list of recommended best practices and documents that should be established when collaborating with an AM supplier (Tables 3 and 4) [12,13].

PROCESS/UNIT OPERATIONS ELEMENTS

The subsequent sections explore the various unit operation steps involved in CAR-T manufacturing. Each unit operation has its own unique bill of materials (BOM), although several raw materials may be shared across multiple steps. For instance, the same cell culture medium may be utilized in both the activation and expansion stages. However, it is important to note that the underlying principles to consider generally remain consistent throughout the process regardless of timing

TABLE 1

Overview of available documents addressing ancillary materials.

Organization	Document title	Scope
BioPhorum Operations Group (BPOG)	Raw Material Risk Assessments [3]	An article and tool available for free through BPOG that helps to standardize the raw material risk assessment process to allow users to identify requirements and compare associated risks of products available from multiple suppliers
BPOG	Perspectives on Raw and Starting Materials Risk Assessment for CGT Processes [4]	This article describes the unique challenges for AMs used in CGT manufacturing and provides three case studies using the raw material risk assessment tool described above
BPOG	Determining Testing Validation and Release Requirements for Single-Use Systems Through Risk Assessment [5]	Another article and tool available for free through BPOG. SUS validation testing standards are available from several standards organizations. However, it is often unclear for users what the requirements for batch release testing should be and this tool helps identify the risks associated with SUS and appropriate release testing
ISO	ISO 20399: 2022 Biotechnology—Ancillary Materials present during the production of cellular therapeutic products and gene therapy products [2]	Originally a three-part standard that was re-released in 2022 as a single comprehensive standard. This document provides clear qualification requirements for both the users and suppliers of ancillary materials
Bio-Process Systems Alliance (BPSA)	BPSA 2023 Single-Use Manufacturing Component Quality Test Matrices [6]	This third edition, updated in 2023, accounts for changes to common practices followed by single-use component and assembly providers as well as sterilization process compatibility, pre-use treatment, as well as sterilization (irradiation and thermal) exposure to test samples
American Society for Testing and Materials (ASTM)	ASTM E3244-23 Standard Practice for Integrity Assurance and Testing of Single-Use Systems [7]	A holistic approach to evaluate risks associated with an integrity breach in a SUS, considering its life cycle from development to disposal
USP	*USP* <1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products [1]	A general chapter published in 2020, providing a four-tiered risk evaluation matrix for best practices for the qualification of AMs based on the quality and risks of a given material
European Pharmacopeia (*EP*)	*EP* 5.2.12 Raw Materials of Biological Origin for the Production of Cell-Based and Gene Therapy Medicinal Products [8]	This chapter provides quality requirements for raw materials of biological origin used for the production of cell-based and gene therapy medicinal products for human use
US Food and Drug Administration (FDA)	Guidance for Industry: Contract Manufacturing Arrangements for Drugs: Quality Agreements [9]	This guidance describes FDA's current thinking on defining, establishing, and documenting manufacturing activities of the parties involved in contract drug manufacturing subject to current good manufacturing practice (CGMP) requirements
Future initiatives in draft		
BPOG	Materials and Components in CGT: A Consensus Approach	The BPOG Supplier Resilience Phorum is drafting a white paper to address gaps in the aforementioned documents. Suppliers and users are collaborating to provide guidance for AM best practices and resolve inconsistencies in material grade naming conventions
ISO	Guidelines for Certificate of Analysis (COA) of Ancillary Materials Used in Cell and Gene Therapy Product Manufacturing	The Standards Coordinating Body (SCB) is currently drafting an ISO standard that outlines the essential information and testing requirements to be included on an AM COA

TABLE 2

AM best practices and considerations.

Consideration	Best practices
Animal-derived materials	Minimize the use of animal-derived materials. Suppliers must provide documentation and assurances regarding source tracing of animal derived materials
Adventitious agents	Perform viral inactivation and/or clearance studies for animal-derived raw materials to ensure safety
Safety testing and controls	Controls should be implemented during AM manufacturing to minimize contamination risk. Suppliers should conduct sterility and endotoxins release testing. Some CAR-T cell therapy manufacturers may request mycoplasma testing, even if considered a very low risk. Given the nature of autologous CAR-T products, having only one chance to manufacture a safe and effective material for a very sick patient, DP manufacturers may prefer to see mycoplasma testing included on the COA regardless of the low risk. Users should also include safety testing in their internal material specifications and repeat it before release
Product-related impurities	Host cell DNA, host cell RNA, and host cell proteins (HCP) are inherently present for proteins derived from *E. coli* or mammalian cells. The supplier should perform in-process and release testing for these residuals. Acceptance criteria depend on the product, process, and end-use
Process-related impurities	Depending on the type of AM and manufacturing process, various solvents may be used, such as antibiotics, slip agents, and enzymes. It is crucial for the supplier to demonstrate removal of residual solvents through qualification activities, as well as in-process and/or release testing. This helps ensure the safety and quality of the AMs used in the user's manufacturing process
Conflict minerals, melamine, and nitrosamine risk assessments	AMs should also be assessed for conflict minerals, melamine, and nitrosamine risk. Suppliers should provide appropriate documentation for these components
Quality or 'grade'	Suppliers may offer different quality grades of their materials, each with its own terminology such as research use only (RUO), research grade, good manufacturing practices (GMP)-grade, clinical grade, commercial grade, pharmaceutical grade, for further manufacturing use, and compendial grade. The *USP* <1043> chapter, described in Table 1, provides a comprehensive understanding of these different grades and the appropriate qualification activities for each. *USP* recommends use of AMs manufactured under the principles of GMP whenever possible. It is important to note that most AMs are not registered drug products and therefore are not manufactured at GMP-certified facilities audited by health authorities. Consequently, regardless of the terminology used by a supplier (e.g., GMP grade, clinical grade, commercial grade), most suppliers manufacture AMs under GMP principles rather than GMP certification from a health authority. Understanding and confirming the quality level that aligns with a user's standards is the user's responsibility. The specific GMP principles a supplier should adhere to are not explicitly defined
Stability, storage, shipping	The supplier performs stability studies based on the ICH guideline Q1A(R2) 'Stability Testing of New Drug Substances (DS) and DP' [10]. This guideline, though written for DS and DP, can be useful for both suppliers and users. In some cases, users may need to conduct their own stability studies if they plan to store outside of the supplier's recommended storage conditions. For example, a reconstituted cytokine stored in cell culture medium for later use would need a stability study to confirm its suitability when stored this way. Additionally, the SUS supplier must perform shipping validation studies to ensure the integrity of the SUS upon arrival at the user's manufacturing site. Similarly, the RM supplier should also perform shipping studies. In some cases, the validated configuration upon arrival should be maintained, for example, bioreactor or final storage bags can be subject to damage and/or loss of sterility if removed from validated packaging configurations. An assessment can be performed with input from the supplier
Fit for use or suitability testing	*USP* <1043> and ISO 20399 AM guidelines recommend testing AMs to ensure their suitability for use, especially when used outside of their intended purpose. For instance, recombinant insulin, while approved for human use, can also be used in ATMP manufacturing. In-process testing should be conducted to confirm its suitability as an AM. Additionally, this assessment should include bag or other container measurements as a release criterion to ensure the materials are compatible with the user's manufacturing equipment

TABLE 2 (CONT.)

AM best practices and considerations.

Consideration	Best practices
Release testing/COA	Suppliers have specifications for the AMs they manufacture, which should capture the critical material attributes of the material. Validated assays are used to assess these attributes to established acceptance criteria. After each batch is manufactured, the supplier performs release testing according to the specifications and provides a COA with test results that meet the acceptance criteria. Users may rely on COA results in a phase-appropriate manner. In early phases of clinical manufacturing, the user's internal specification for a given AM may include limited testing, for example a COA check or the use of a method not yet validated. However, as a DP progresses into pivotal and commercial manufacturing, the user should reassess their internal AM specifications to ensure adequate acceptance criteria, inclusion of an identity verification test, and validated methods are in place
Extractables and leachables	The SUS supplier is responsible for conducting extractables testing. This data is typically generated during the qualification or validation of the SUS and should be readily available to the user. Based on the results provided by the SUS supplier, the user may need to conduct additional extractable and/or leachable studies. Similarly, RM suppliers should conduct extractable testing on the containers used for storing their final products. Alternatively, they may review and confirm the safety of the data provided by their container sub-supplier
Container closure integrity (CCI) or package integrity	SUS suppliers are expected to conduct package integrity testing during qualification/validation activities, and for each lot as part of lot release. Users are advised to consider implementing a point of use (POU) integrity test, as recommended in BPSA [6]. Similarly, RM suppliers should perform container closure integrity testing or package integrity testing. Leak testing and visual inspection on consumables as User release criteria can help to alleviate risk—and depending on the process (autologous processes in particular), visual inspection for particulates and damage can also be done at point of use by manufacturing staff
Materials of construction (MOC), composition, or formulation	SUS suppliers are expected to provide comprehensive design drawings of their SUS including all MOC. While RM suppliers may not always be able to provide full formulation information, such as for proprietary cell culture media, they are obligated to disclose the presence of any animal-derived materials (e.g., human serum albumin [HAS]). Additionally, RM suppliers should prioritize transparency in sharing formulation information, enabling users to verify the use of high-quality materials in the final raw material
Lot-to-lot variability	Suppliers are expected to establish a robust trending program that incorporates release testing data, for information only (FIO) data, and characterization testing. This program serves to evaluate and mitigate any potential lot-to-lot variability of their AMs, ensuring the implementation of effective controls to limit such variability. Similarly, users should also implement a trending program that considers the COA, internal release testing results, and any available FIO or characterization data. This program enables users to monitor and assess the consistency and quality of the materials they receive, facilitating proactive measures to address any observed variability
Identity testing	To ensure the integrity of the DP manufacturing process, users must conduct identity verification of the RMs used in manufacturing. Consequently, it is crucial for RM suppliers to supply a COA that includes identity testing. The SCB initiative mentioned earlier, which aims to standardize COAs for AMs, will enforce this requirement. While some suppliers of complex RMs, such as chemically defined cell culture media, may be hesitant to disclose identity test results on their COAs due to proprietary concerns, there are viable approaches for suppliers to address this requirement. One option is to provide users with a physical reference standard along with a representative FTIR or Raman spectrum, enabling users to perform internal identity verification tests. Another approach involves employing multi-attribute identity testing. For instance, pH, conductivity, density, and/or osmolality can serve as multiple attributes for identity verification of a cell culture medium. If the specifications for these attributes are unique to a specific RM, users can have confidence that the correct material is released into their manufacturing process

TABLE 2 (CONT.)

AM best practices and considerations.

Consideration	Best practices
Functionality testing	Functionality assays, such as potency, proliferation, specific activity, binding capacity, reporter, and cell health assays, are used to assess the performance of RMs. However, not all RMs require functionality assays on their COA. Simple materials like phosphate-buffered sodium solutions typically do not have functionality assays included. The need for functionality assays depends on whether the attribute being assessed is critical to the final quality of the drug product. Since RMs can be used in various ATMP modalities, it can be challenging for suppliers to provide a one-size-fits-all functionality test. Suppliers may include a functionality assay on their COA, but it may not align with the user's specific needs for assessing function. For example, if a supplier performs a proliferation assay on Jurkat cells, but the user requires a material that can activate primary T cells, the provided functionality assay may not be suitable. This is where 'fit for use/ suitability testing' comes into play as described previously
Cytotoxicity, biocompatibility	SUS should be tested for cytotoxicity and biocompatibility. In instances where a raw material is suspected to contain potentially toxic substances, such as a small molecule inhibitor or transfection reagent, it is crucial to conduct cytotoxicity testing
Residual testing in the final DP	Both *USP* <1043> and ISO 20399 recommend performing RM residuals testing and clearance studies of the final DP. This ensures the safety and quality of the product, contributing to improved patient outcomes
Regulatory	Certain SUS, such as blood transfer packs, are classified as registered medical devices. In Europe, if an SUS is a registered medical device, the European Medicines Agency (EMA) mandates the inclusion of a CE mark on the device. However, the global health authority requirements for SUS in ATMP manufacturing are currently unclear. To address this issue, BPOG has undertaken initiatives aimed at establishing guidelines and standards for SUS in ATMP manufacturing
Visible particulates	AMs should be free from visible particulates. However, SUS suppliers may have permissible ranges for visible particulates (embedded/free floating). It is crucial for users to understand these supplier-specified ranges to ensure they align with their own requirements
Subvisible particulates	While it is impossible to completely eliminate subvisible particulates in SUS, it is recommended that their levels adhere to the *USP* <788> acceptance criteria [11]. For RM, there's no explicit guidance on subvisible particulates. However, cell therapy manufacturers may aim to minimize subvisible particulates in their raw materials, as the final DP cannot be sterile filtered to remove these particulates. Some suppliers do offer RMs tested for subvisible particulates, but there is no industry-wide consensus or health authority guidance on controlling subvisible particulates in raw materials

in the process since the final DP cannot be sterile filtered.

Leukapheresis collection

Leukapheresis is a procedure that involves the separation and collection of white blood cells (including T cells) from a patient's blood. During leukapheresis, blood is drawn from the patient and passed through a machine that separates the different components of blood. The desired white blood cells, such as T cells, are collected, while the remaining components are returned to the patient's bloodstream. Leukapheresis is used in CAR-T manufacturing to obtain a concentrated of white blood cells for manufacturing use. Table 5 shows a list of materials commonly used during the leukapheresis step and some points to consider.

T cell isolation

T cell isolation is a process used to separate T cells from a mixed population of cells. There are various methods for T cell isolation, including positive and negative selection. This allows for targeting of T cells for further manufacturing. If in-process products are shipped between different manufacturing sites, the need for an identity test upon receipt should be considered. Table 6 shows some of the materials that may be used during the T cell isolation process. The user must ensure appropriate qualification of these

TABLE 3

Supplier documentation best practices checklist.

Document type	Description
Quality agreements	Several documents outlined in Table 1 offer recommendations on setting of quality agreements with suppliers. Quality agreements should encompass various aspects such as change notifications with sufficient lead time, data sharing, ensuring continuity of supply, defining roles and responsibilities, establishing effective communication channels, implementing audit procedures, dispute resolutions, and managing deviations
Supply agreements	A supply agreement is a crucial contractual arrangement between a supplier and user that outlines the terms and conditions for the procurement and delivery of materials. This agreement serves as a foundation for a mutually beneficial relationship, ensuring clear expectations and responsibilities for both parties involved
Drug master files (DMF) or regulatory support files (RSF)	In the United States, suppliers have the option to file DMFs with the FDA, which allows them to maintain proprietary knowledge of their processes and products. However, it is important for users to ensure that the RMs they use meet high quality standards, have adequate safety profiles, and are properly controlled, as required by health authorities. To facilitate effective information sharing, users and suppliers typically establish a combination of non-disclosure agreements (NDAs) and quality agreements. These agreements enable both parties to find a suitable path forward while ensuring the necessary information is shared. In some cases, suppliers may offer RSFs to customers under NDA and quality agreements. While RSFs provide additional information, they do not offer the same level of detail as those captured in a DMF. Users can request a letter of authorization to cross reference the DMF in regulatory filings. DMFs cannot be provided directly to users. It's worth noting that there are four different types of DMFs (types II through V) that can be submitted to the FDA. AMs can be submitted under different types of DMFs
Transmissible spongiform encephalopathies (TSE)/bovine spongiform encephalopathies (BSE) statements	Suppliers are obligated to inform users about the presence of any human or animal-derived materials in their products. If animal-derived components are used ensure that BSE/TSE statement is provided by the supplier and complies with the 'Note for guidance of minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev. 3—July 2011)' [12,13]
Certificates of origin (COO)	Suppliers are required to furnish documentation specifying the country of origin for animal-derived AMs. This documentation serves to address concerns related TSE and other diseases of concern
Safety data sheets (SDS)	Suppliers are required to provide SDS for all substances and mixtures that meet specific criteria for physical, health, or environmental hazards. Additionally, SDS must be provided for mixtures containing components that exceed documented cut-off limits for carcinogenicity, reproductive toxicity, or target organ toxicity
Validation guides	SUS suppliers should have qualified assemblies and components. The qualification activities should be summarized in a validation guide which can be shared with user
User requirement specifications (URS)	Use of a URS where users provide suppliers with the needed specifications for a specific process or product. BPOG offers a single-use user requirements toolkit which includes a URS template for SUS

materials as related to downstream patient safety. Adventitious agent risk assessments and/or testing must be performed as a requirement from health authorities due to the presence of recombinant proteins of animal origin or raw materials manufactured in the presence of animal-derived materials [1,2,14].

Cell activation

T cell activation refers to a process in which mature T cells can express antigen-specific T cell receptors on their surface to recognize antigens and respond by entering the cell cycle, secreting cytokines, and initiating the cell-based functions of the immune system. In CAR-T manufacturing, this prepares the cells for the introduction of genetic material by transduction or non-viral methods. The user must ensure appropriate qualification of these materials as related to downstream patient safety. Adventitious agent risk assessments and/or testing must be performed as a requirement from health authorities due to the presence of recombinant proteins of animal origin or raw materials manufactured

TABLE 4

Supplier assessment, qualification, and relationships.

Activity	Description
Suppler relationship management	Supplier relationship management (SRM) refers to the systematic approach and practices used by an organization to effectively manage its relationships with its suppliers. It involves developing and maintaining positive and mutually beneficial relationships with suppliers to optimize the value and performance of the supply chain SRM encompasses various activities, including: ▶ Supplier selection and evaluation ▶ Contract management ▶ Collaboration and communication ▶ Performance monitoring ▶ Risk management ▶ Continuous improvement Effective SRM can lead to benefits such as improved quality, reduced costs, increased supply chain efficiency, enhanced innovation, and better overall business performance
Forecasting	RM forecasting involves predicting the future demand for specific materials used in manufacturing. It uses historical data, demand patterns, and other factors to estimate the quantity and timing of material requirements. The goal is to ensure a timely supply while minimizing costs and avoiding shortages or excesses. Accurate forecasting optimizes the supply chain, reduces costs, minimizes stockouts, and improves planning and decision-making for procurement, inventory, and production scheduling
Regulatory support	Suppliers can provide regulatory support to their customers by offering assistance and guidance in navigating regulatory requirements and ensuring compliance through documentation, regulatory expertise, compliance assistance, audits, regulatory updates, training and education, and collaboration with regulatory bodies. It is important to note that the extent of regulatory support provided by suppliers may vary. Users should communicate their specific regulatory needs and expectations to suppliers to ensure a mutually beneficial partnership
Technical capability	Assessing a supplier's technical capability is crucial to ensure they can meet the user's requirements and deliver high-quality materials. Some steps to take to assess a supplier's technical capability: ▶ Defined user requirements ▶ Requests for information (RFIs) ▶ Evaluate past performance ▶ Site visits ▶ Technical documentation review ▶ Technical expertise and support ▶ Continuous monitoring and feedback Remember that assessing a supplier's technical capability is an ongoing process. It is important to establish clear communication channels, maintain regular dialogue, and address any concerns or issues promptly to ensure a successful supplier relationship
Quality audit	A supplier quality audit is a structured evaluation of a supplier's quality management system, processes, and performance. The purpose of a quality audit is to assess the supplier's ability to consistently meet quality requirements, comply with applicable standards and regulations, and deliver materials that meet the user's expectations
Continuity of supply	Continuity of supply for AMs refers to the uninterrupted availability and delivery of AMs needed for manufacturing processes. It ensures that the user has a reliable and consistent flow of AMs to meet production demands and avoid disruptions in the supply chain. To ensure continuity of supply for raw materials, companies can take several measures: ▶ Diversify suppliers and materials ▶ SRM ▶ Supply chain visibility ▶ Inventory management ▶ Contingency planning

TABLE 5

AMs used in the leukapheresis process.

Ancillary material	Points to consider
Cryopreservation or apheresis medium	Consider whether the apheresis material will be shipped fresh or frozen before manufacturing. If frozen, a cryopreservation reagent, such as DMSO, will be needed. Be sure to source SUS that are compatible with DMSO
Anticoagulants	To prevent blood clotting during the apheresis procedure, anticoagulants may be used. Commonly used anticoagulants include citrate-based solutions, such as ACD (acid-citrate-dextrose) or heparin
Collection bags and tubing sets	Leukapheresis is commonly performed with blood collection bags that may be registered medical devices. These traditional blood collection bags are often made of PVC. PVC film and tubing does have some additional negatives to consider: ▶ Chemical leaching: such as plasticizers like phthalates ▶ Environmental impact: PVC is not biodegradable ▶ Temperature limitations: may not be suitable for applications that require extreme temperatures such as those required for CAR-T ▶ Mechanical strength: lower mechanical strength, prone to damage or failure ▶ Compatibility issues: as mentioned earlier, PVC may not be compatible with certain materials limiting its seamless integration into manufacturing ▶ Regulatory concerns: there are regulations and restrictions on the use of PVC due to its potential environmental and health impacts. Compliance with these regulations may require additional measures or alternative materials
Filters	In some apheresis procedures, filters may be used to remove unwanted substances or particles from the blood before or during the separation process

TABLE 6

AMs used in T cell isolation.

Ancillary material	Points to consider
Antibodies	Can be *E. coli*- or CHO-derived. Consider appropriate product impurity testing based on the expression system used
Magnetic beads	Ensure consistent removal from the final DP
Buffers and cell culture media	Buffers and media may contain animal-derived materials such as HSA. Consider using recombinant forms

TABLE 7

AMs used in T cell activation.

Ancillary material	Points to consider
Cytokines	Can be *E. Coli* or mammalian derived. Ensure that the supplier has performed viral inactivation or clearance studies. Host-cell impurities' acceptance criteria will be end-use dependent
Antibodies	Can be *E. coli*- or mammalian-derived. Consider appropriate product impurity testing based on the expression system used
Cell culture media	Media may contain animal-derived materials such as HSA. Consider using recombinant forms

in the presence of animal-derived materials (Table 7) [1,2,14].

Cell transduction or gene editing

Once the T cells have been activated, it is time to genetically modify the T cell to allow for the expression of the CAR. Expression of the CAR has been traditionally performed with the use of lentiviral vectors, and more recently with adeno-associated viral (AAV) vectors. As the industry progresses, non-viral techniques are being explored further. Table 8 shows a list of materials that may be used during the gene editing step of the CAR-T manufacturing process, and some points to consider.

Expansion

T cell expansion in CAR-T therapy refers to the process where a patient's harvested T cells, which have been genetically modified *ex vivo* through transduction with a CAR lentiviral vector, are grown in number. This process is crucial to ensure there are enough CAR-positive T cells to reintroduce into the patient for effective therapy. The materials used in the T cell expansion process overlap with materials described already. The same points to consider apply.

Cell harvest

Once the CAR-T cells have undergone the expansion phase and reached the desired cell count, they are harvested. Harvesting involves several steps:

- Washing: this is done to remove any remaining culture media, growth factors, or other components used during the expansion phase;
- Concentration: after washing, cells are concentrated to increase their cell density. Concentration is typically achieved through centrifugation or filtration. These methods help remove excess liquid and concentrate the cells into a smaller volume;
- Removal of impurities: during concentration, any remaining impurities, such as dead cells, debris, or residual media components, may be removed;
- In-process quality control: before the harvested cells are considered suitable for patient administration, quality control tests are performed. These include viability, purity, and potency. Viability determines the percentage of live cells, while purity ensures that the harvested cells are predominantly CAR-T cells and not contaminated with other cell types. Potency evaluates the functional activity of the CAR-T cells, such as their ability to recognize and kill target cells.

It's important to note that the exact harvesting process may vary depending on the specific CAR-T therapy and manufacturing protocols employed by different companies. The materials used in the cell harvest process overlap with materials described already. The same points to consider apply.

DP formulation and cryopreservation

Once the harvested CAR-T cells have passed the quality control tests, they are formulated into a final product for patient infusion. This may involve additional steps, such as cryopreservation, formulation in a specific infusion solution, or further manipulation depending on the specific CAR-T therapy and manufacturing process. Table 9 shows some additional materials that may be used in the DP formulation and cryopreservation steps.

TRANSLATION INSIGHT

This article has highlighted several key areas of consideration for AMs. However, it is crucial to note that specific requirements for

TABLE 8

AMs used in T cell transduction or gene editing.

Ancillary material	Points to consider
Viral vectors	While often considered DS in the CAR-T manufacturing process, the best practices and considerations discussed in this article may be applicable
Electroporation cartridges	Electroporation cartridges can be considered and qualified as a SUS
Small molecules	The supplier must perform cytotoxicity testing, and the user must ensure adequate removal from the final CAR-T DP
Transduction vessels or culture bags	While nuclease-free statements for SUS are not a common practice, users should ensure that adequate controls are in place by the supplier to avoid any potential nuclease contamination that could affect gene editing materials
Enzymes	Enzymes may be used in the gene editing process during CAR-T manufacturing. Users should ensure that suppliers are manufacturing enzymes under the appropriate GMP principles

TABLE 9

AMs used in final formulation.

Ancillary material	Points to consider
Cryopreservation media and formulation buffers	These materials are considered excipients and should be qualified and manufactured as such
Final DP container	The final DP container should be capable of being stored in liquid nitrogen and have adequate qualification activities to ensure robustness of the materials during shipment. These should also be DMSO-compatible if DMSO is the cryopreservant used in the final formulation

AMs may vary depending on the local health authorities. Therefore, it is recommended to consult with these authorities when preparing filings. As the ATMP industry continues to evolve, it is essential to adapt existing guidelines and best practices to develop a robust control strategy for AMs. By staying informed and implementing these strategies, suppliers and users can ensure the safety and quality of their products in this dynamic field.

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AFFILIATION

Lili Belcastro
Bristol Myers Squibb

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

EXPERT INSIGHT

The sweet cell of success: key considerations for the sourcing and production of pluripotent cell lines for therapeutic development

Samuel JI Blackford and Nathan C Manley

Cell sourcing and cell line production are two imperative challenges for the production of pluripotent stem cell (PSC)-based therapeutics. Regulatory guidelines published by the FDA, EMA, and other regulatory authorities around the world are still evolving to appropriately accommodate cellular therapies and have yet to achieve full harmonization between jurisdictions. In parallel, the industry continues to develop improved methods for production and characterization of PSC-based therapeutics that seek to enhance manufacturing performance and improve overall product safety. This article explores the current regulatory requirements for PSC sourcing, including donor eligibility, consent, and testing within leading and rapidly growing cell therapy jurisdictions. In the second section of this article, key considerations for PSC line derivation and banking are described, including methods for reprogramming, incorporation of genetic modifications, and cell bank production. Finally, strategies for PSC line testing are discussed, including considerations for genome editing and potency assurance, strategies to reduce downstream drug product testing requirements, and a potential approach to overcome theoretical BSE/vCJD risk in existing banked lines. Finally, thoughts on how best to align cell line production with critical product development activities, such as regulatory interactions and nonclinical studies are presented.

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The development of cell-based therapies is accelerating at an exciting rate. 2017 marked the dawn of a new therapeutic era, with the FDA approving both YESCARTA® and KYMRIAH®—autologous CAR-T products [1]. This was followed by the approvals of TECARTUS® and Libmeldy® in 2020 [2], StrataGraft®, RETHYMIC®, ABECMA®, and Breyanzi® in 2021 [3], and ZYNTEGLO®, SKYSONA®, and CARVYKTI® in 2022 [4]. Then last year, in 2023, we saw the approval of four notable cell-based therapies, the autologous CASGEVY™ (Vertex Pharmaceuticals, Inc.) and LYFGENIA™ (bluebird bio, Inc.), which are gene-modified hematopoietic stem cell products, as well as the allogeneic cellular products, Lantidra™ (CellTrans Inc.) and OMISIRGE™ (Gamida Cell Ltd.) for the treatments of type-I diabetes and hematologic malignancies, respectively [5].

In response to the rapidly expanding cell-based therapeutic industry, regulatory health authorities continue to update their guidance documents and regulations to ensure that new therapeutic products are developed in a manner that maximizes patient safety. Examples from this year include the now finalized FDA guidance documents for the development of chimeric antigen receptor (CAR) T cell products [6] and human gene therapy products incorporating human genome editing [7].

The increasing number of approved cell-based therapies in conjunction with new and updated regulatory guidance documents will help pave the way for future innovations with the potential to transform how a variety of diseases are treated or even cured. Accordingly, many look towards the promise of pluripotent stem cells (PSCs). Both induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) possess theoretically infinite self-renewal capabilities and can differentiate into cell types of each of the three germ layers [8]. The ability to exploit these characteristics makes PSCs highly desirable starting material for development of a wide range of cell-based therapies, including non-terminally differentiated progenitor cells that cannot be easily isolated from adult donors and for therapies requiring large scale production [9,10].

Indeed, a number of PSC-based programs are generating excitement in the US marketplace. These include two programs in Phase 1 clinical testing for Parkinson's disease: BlueRock/Bayer (allogeneic ESC-derived cell therapy; [11]) and Aspen Neuroscience (autologous iPSC-derived cell therapy; [12]). Additionally, the National Eye Institute (NEI)'s autologous iPSC-derived RPE/PLGA transplantation intervention for age-related macular degeneration is currently being evaluated in a Phase 1/2a trial [13]. Vertex Therapeutic's VX-264 (encapsulated) [14] and VX-880 (non-encapsulated) [15], allogeneic ESC-derived pancreatic islet cell therapies are in clinical testing for type-1 diabetes. Moreover, Fate Therapeutics Inc. is developing an off-the shelf allogeneic iPSC-derived product portfolio [16], which includes FT-819, a CAR-T cell therapy in a Phase 1 trial for treatment of relapsed/refractory B cell malignancies [17] and other programs focusing on cancer and autoimmune disease.

Globally, additional clinical trials of PSC-derived cell-based therapies are under evaluation for various indications. Examples include, Cynata Therapeutics Ltd's CYP-004 [18] and CYP-001 [19] products are undergoing Phase 3 and Phase 2 clinical evaluations in Australia (and the USA and Turkey for CYP-001) for the treatment of osteoarthritis and graft versus host disease. Heartseed Inc's HS-001 CS is undergoing Phase 1/2 clinical development in Japan for the treatment of ischemic heart disease [20]. In Europe, Repairon's BioVAT-HF Phase 1/2 trial is evaluating the safety and efficacy of iPSC-derived human myocardium in treating terminal heart failure [21]. Further information on clinical trials utilizing pluripotent stem cell-derived products can be accessed in recent review articles [22–24].

CELL SOURCING

Obtaining cellular starting material that meets regulatory expectations and enables proficient

and consistent manufacturing is essential to the development of cell-based therapeutics. Interestingly, sourcing of some PSC lines and in particular ESC lines, may occur in an academic setting [25–27] with initial focus more on identification of a technically appropriate and cost-effective production strategy as opposed to forward compatibility for therapeutic application. When such academically sourced PSC lines are then considered for therapeutic development, two equally important considerations are:

1. How the candidate line will perform in the intended manufacturing process; and

2. What potential gaps and associated mitigations must be considered to ensure regulatory compliance of the cellular starting material.

To address the first consideration, therapeutic developers may utilize research banks of established PSC lines for early preclinical applications prior to transitioning to a matched PSC bank produced in a manner that is suitable for clinical/commercial use [28]. For PSC lines with one or more gaps in regulatory compliance, it is essential for developers to understand the relative risk to patient safety posed by the existing gap(s) and whether suitable risk mitigating strategies are available. The concept of PSC line risk mitigation will be revisited below in the context of cell line production and testing, but first it is important to consider the current global landscape of cell-based therapeutics and how sourcing of cellular starting material is regulated.

Underscoring the importance of sourcing cellular starting materials with the potential for multi-jurisdictional application, the global cell therapy market is continuing to grow and increase in revenue [29]. The market can be divided geographically into North America, Europe, Asia-Pacific, Latin America, and the Middle East and Africa. As of 2022, North America and Europe were the two largest markets. However, the Asia-Pacific region is forecast to increase its market share due to the rising number of clinical studies, expansion of pipeline programs for CAR-T/immunotherapies, and favorable funding support for cell therapy research technologies. With the cell therapy market becoming more global, developers should become prepared to meet the regulatory requirements in multiple jurisdictions.

DONOR ELIGIBILITY

An initial critical consideration for cell sourcing for both autologous and allogeneic therapies is donor eligibility, which seeks to minimize the potential for transmission of pathogens/infectious diseases. Whilst pre-existing adventitious agents, such as viruses, do not pose a risk to an autologous donor/recipient and may be stored in hospitals [30], these pathogens still have the potential to be amplified within biomanufacturing processes or cross-contaminate other materials present in the same facility. However, transmission of pathogens poses a considerably larger concern for allogeneic products that have the potential to be administered to a large patient population. For allogeneic products being developed in the USA or Europe, there is an expectancy that the sourcing of cells will comply with guidelines recommended by the American Association of Blood Banks (AABB) [31] and the Center for Disease Control and Prevention (CDC) [32] which include a list of specific disease pathogens for which donors should be tested (Table 1). Donor testing in the EU is very much aligned with FDA expectations, however depending on the member state concerned, additional testing may be required, such as for hepatitis A, hepatitis E, and parvovirus B19 [33]. Whilst the FDA requires donors to be screened for West Nile virus (WNV), under EU guidelines only certain at-risk donor subpopulations (i.e., potentially exposed travelers) are selectively screened [34]. Additionally, the list of infectious diseases/disease agents shown in Table 1 largely align

TABLE 1

Testing requirements for infectious disease markers for the FDA and EMA.

Infectious disease/disease agent	Testing requirement	
	FDA	EMA
HIV, type 1	R	R
HIV, type 2	R	R
HBV	R	R
HCV	R	R
Treponema pallidum	R	R
West Nile virus	R1	N
Human T-lymphotropic virus, types I and II	R2	R4
Cytomegalovirus	R	R5
Chlamydia trachomatis	R3	R3
Neisseria gonorrhea	R3	N
EBV	R2	R5
Zika virus	R	N
Malaria	N	R5
Toxoplasma	N	R5
Trypanosoma cruzi	N	R5
Risks or evidence of prion disease (vCJD/BSE/TSE)	R	R

R: Required; N: Not required; 1: Living donor HCT/Ps; 2: Viable, leukocyte-rich HCT/Ps; 3: reproductive HCT/Ps (unless excepted in 1271.90); 4: HTLV-I antibody testing must be performed for donors living in, or originating from, high-incidence areas or with sexual partners originating from those areas or where the donor's parents originate from those areas; 5: Depending on donor history and/or characteristics of the donated tissue/cells. BSE: Bovine spongiform encephalopathy; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; EBV: Epstein-Barr virus; vCJD: variant Creutzfeldt-Jakob disease; TSE: Transmissible spongiform encephalopathy.

with testing requirements in other major jurisdictions such as Canada and Asia Pacific with some additional considerations for relevant communicable diseases specific to a given geographic locale.

In the USA, products containing cells with more-than-minimal manipulation, including PSC-derived products, are deemed human cells, tissues, and cellular and tissue-based products (351 HCT/Ps) under Section 351 of the Public Health Service Act (42 USC § 262) [35] and classified as biological products or medical devices. While the FDA's Advisory Committee has provided points to consider for preclinical safety testing and patient monitoring for ESC-derived therapeutic products (CTGTAC Meeting #45; 2008 [36]), no official guidance documents pertaining to PSCs have been published. Instead, PSC-derived therapeutic products are reviewed within the guidelines of 351 HCT/Ps more generally. Additional information on US donor eligibility requirements, donor testing, and relevant communicable disease agents are provided in 'Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)', published by FDA/CBER in 2007 [37], 21 CFR Part 1271.3(r) [7] and are further summarized below.

As per 21 CFR 1271.75(a) [38] all donors, must have relevant medical records reviewed and be asked questions about their medical history and relevant social behavior, including risk factors for relevant communicable disease agents and diseases, and communicable disease risks associated with xenotransplantation. The FDA does not mandate the use of any particular screening tool to fulfill the requirements set forth in 21 CFR

Part 1271 Subpart C for screening donors for risks from communicable disease. However, to standardize donor screening, donor history questionnaires have been developed by an AABB interorganizational task force [39]. FDA liaisons collaborated with the task force in the development of the questionnaires which were based on current FDA regulations and guidance documents, and incorporated requirements of accrediting organizations (AABB and the Foundation for the Accreditation of Cellular Therapy) and the National Marrow Donor Program.

To meet US donor eligibility requirements, the testing for infectious disease markers (IDMs) must be done using FDA-licensed kits at a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory using kits approved or cleared by the FDA specifically for that purpose [37]. Whilst there exist some CLIA-certified laboratories outside of the USA, developers should be aware that if donor material is transported outside of the USA for testing by international laboratories, then these laboratories are also subject to the CLIA regulation. The screening and testing requirements outlined in 21 CFR Part 1271 became effective on May 25, 2005, which are more expansive than the older 21 CFR 1270 rules. However, the FDA never revoked the 21 CFR 1270 rules, which also remain effective today. It should be noted that no guidance is provided in the 21 CFR 1271 rules for ESC-derived products made from starting material derived or recovered before May 25, 2005. As ESCs understandably were not considered in the older 21 CFR 1270 rules, it is not clear which regulations, particularly those regarding donor eligibility and screening requirements, should apply to products derived from ESCs derived or recovered prior to 21 CFR 1271 becoming effective [40]. For HCT/Ps created before 21 CFR Part 1271 came into effect, it is likely that few, if any, could meet the current criteria that determine suitability of these products for licensure.

In the USA, donor eligibility and screening requirements indicated that donor testing should be performed within 7–30 days of tissue recovery [37]. However, for ESC lines created from spare embryos originating from *in vitro* fertilization (IVF) clinics, the decision to donate frozen embryos may occur months or even years after initial collection of donor gametes. As testing of gamete donors for IDMs is not required under current IVF regulations, it is highly unlikely for IVF-sourced ESC lines to have undergone donor testing within the required 7–30-day window. Although FDA has granted exemptions for ESC products that do not meet 21 CFR 1271 Section 361 donor eligibility requirements and allowed them to enter clinical testing, no assurances exist that exemptions would be granted for licensure [40]. This uncertainty has created a burdensome regulatory paradigm for ESC-based products that is as of yet unresolved.

As noted at the bottom of Table 1, donated cellular material must be assessed for risk or evidence of prion disease to be eligible for use in USA and European jurisdictions. Indeed, the FDA imposed a ban commencing in 1999 on blood donations from anyone who has spent more than 6 months in the UK from 1980 to 1997 [41] because of the possible risk of transmitting the human form of bovine spongiform encephalopathy, known as variant Creutzfeldt-Jakob disease (vCJD). However, in May 2022 the FDA issued a new guidance for industry relating to measures for reducing CJD and vCJD possible transmission risks [42]. Consequently, the FDA removed the recommendation for deferral of individuals who spent time in the UK (from 1980 to 1996) and Ireland and France (from 1980 to 2001) and removed the recommendation for indefinite deferral of individuals who had received a blood transfusion in the UK, France, or Ireland from 1980–present. Therefore, donors previously deferred for geographic risk for time spent in the UK, France, and Ireland—or for receipt of a blood transfusion in the UK, France, or Ireland—can now be assessed for requalification under 21 CFR 630.35(b) [43] and may be eligible

for reentry, provided they meet all other eligibility requirements. However, at the time of writing, these new FDA recommendations to remove the UK-centric geographic deferral of blood donors has yet to impact the donor eligibility criteria that informs the suitability of cellular starting material for use in cell-based therapeutics—albeit it is possible that these criteria may soften in the future to be permissive to cell lines derived in the UK from UK donors.

DONOR CONSENT

A second key consideration for procuring donor-sourced cellular material is donor consent. As stated in the American Medical Association Code of Medical Ethics: II,V [44], human biological materials and their products may not be used for commercial purposes without the consent of the tissue donor. Moreover, profits from the commercial use of human biological materials should be shared with the tissue donor in accordance with lawful contractual agreements. Developers of cell therapies must ensure that the selected starting material has voluntary informed consent in place for research, clinical, and commercial applications. Donor consent questionnaires currently seek broad consent i.e., does consent expressly prevent development of commercial products? Or does consent expressly prevent financial gain from any use of the donated embryo/tissue, including any product made from it? A 2017 revision of the US Common Rule, the country's national research ethics guideline, explicitly endorses the use of broad consent in specific situations [45], albeit this remains controversial [46].

Pluripotent stem cells are unique in that theoretically only one donor collection may be required for a drug product's lifespan. In contrast, CAR-T therapies utilizing allogeneic apheresis material will continuously collect from donors through the various stages of preclinical and clinical development. Therefore, ensuring commercial readiness of the material is essential at a considerably earlier development stage for iPSCs and ESCs. Gaps in donor consent have limited many PSC lines to being suitable only for research. This is even the case if the material (lacking informed consent) has been de-identified to ensure confidentiality [47,48].

National authorities are responsible for defining the process of obtaining and recording consent for cell, tissue and organ donation in accordance with international ethical standards, the manner in which organ procurement is organized in their country, and the practical role of consent as a safeguard against abuses and safety breaches. The World Health Organization (WHO) has published Guiding Principles intended to provide an orderly, ethical, and acceptable framework for the acquisition and transplantation of human cells, tissues and organs for therapeutic purposes. Each jurisdiction determines the means of implementation of the Guiding Principles [49]. Additionally, differences in remuneration occurs between the jurisdictions. For example, in the US, remuneration for peripheral blood stem cell collection is legal in states within the Ninth Circuit [50], whereas in the European Union (EU), the Tissues and Cells Directive (EUTCD) (2004/23/EC) [51] encourages Members States to ensure cellular donations are voluntary and unpaid, but allows compensation for expenses and inconveniences incurred as a result of donation for human transplantation; donation for research purposes falls outside of this scope. Likewise, the UK prohibits the commercial trading of tissues and cells for human transplantation [52].

CELL SOURCING REGULATION IN EUROPE AND JAPAN

In both the EU and in the UK, cell-based therapeutic products utilizing iPSCs or ESCs as a starting material are categorized as Advanced Therapy Medicinal Products (ATMPs)—as the cells have undergone substantial manipulation and are to be used for a different essential function. Refer to Article 2(1)(a) of Regulation (EC) No.1394/2007 [53] and

Directive 2001/83/EC Annex I Part IV [54] for detailed definitions of the different groups of ATMPs.

The European Medicines Agency (EMA) is responsible for assessing marketing authorization applications for ATMPs in the EU. Moreover, member states can enforce state-specific regulation on PSC products. For example, heavy restrictions on the use of human ESCs are enforced in Germany under the Embryo Protection Act (Embryoenschutzgesetz) [55], which makes the derivation of human ESCs a criminal offence. The 2002 Stem Cell Act (Stammzellgesetz), which was amended in August 2008 (StZG- Stem Cell Act) [56], allows the import of human ESCs under strict conditions, with the ESC line needed to have been derived before May 1, 2007, and can only be used in research if they are developing new medical/scientific knowledge.

If tissues and cells are being used as starting materials in a medicinal product, the donation, procurement and testing of the cells are covered by the Tissues and Cells Directive (2004/23/EC) [51]. Member states have a designated competent authority or authorities responsible for implementing the requirements of this directive. However, this directive does not prevent a member state from maintaining or introducing more stringent protective measures, provided that they comply with the provisions of the treaty.

In the UK, the Medicines and Healthcare products Regulatory Agency (MHRA) is the standalone medicines and medical devices regulator. Since the UK left the EU, ATMPs are regulated in Great Britain by the MHRA according to the same principles that previously applied. In Northern Ireland ATMPs continue to be regulated according the EMA's Centrally Authorised Procedure [57]. Unchanged since leaving the EU, the two designated competent authorities in the UK are the Human Fertilsation and Embryology Authority (HFEA)—for the use of gametes and embryos which may be used in the derivation of cells for ATMP manufacturing, and the Human Tissues Authority (HTA)—for the licensing and inspection of all other cells. Accordingly, if an human ESC line is derived in the UK, a sample of the resulting cell line is required to be deposited at the UK Stem Cell Bank as a condition of their HFEA license [58].

Once the starting material has been made available under the Tissues and Cells Directive, medicines legislation (2001/83/EC) [54] applies and is regulated by the MHRA in the UK [59] (or EMA in the EU). If blood and/or blood components are being used as starting materials in a medicinal product, i.e., to reprogram into iPSCs, the donation and testing are covered by the Blood Directive 2002/98/EC [60]. The competent authority for blood in the UK is MHRA; moreover, the MHRA and HTA agree that the collection of blood as a starting material for an ATMP can be carried out under either a tissues and cells license or a blood establishment license.

The Pharmaceuticals and Medical Devices Agency (PMDA), which operates under the Ministry of Health, Labour and Welfare (MHLW), is in charge of pharmaceutical regulatory affairs in Japan. As the nation where the original iPSC breakthroughs were made by Shinya Yamanaka in 2006 [61] and 2007 [62], Japan has been a pioneering nation for iPSC technologies. Therefore, it should not be a surprise that Japan is the nation offering the highest number of iPSC-based physician-led clinical trials [63]. The MHLW/PMDA has issued several notifications relating to cell-based therapeutics (categorized as Regenerative Medical Products), and overall, the guidelines are akin to those of the FDA and EMA.

The PMDA has issued five guideline documents (PFSB Notifications No. 0907-2–0907-6) [64–68] describing the basic technical requirements to ensure the quality and safety of cell-based therapies for each type of starting cell source. Moreover, unlike FDA-issued guidance for industry, three of these documents focus on PSC-derived Regenerative Medical Products—Notification No. 0907-4, PFSB (2012) for

autologous iPSCs [66], Notification No. 0907-5, PFSB (2012) for allogeneic iPSCs [67], and Notification No. 0907-6, PFSB (2012) for allogeneic ESCs [68].

For developers thinking of entering both the US and European markets, careful consideration should be given as to which test kits are used for IDM testing. In Europe mandatory donor testing must be carried out using tests that have been validated for the purpose in accordance with current scientific knowledge, and CE marked, where appropriate [69]. A limited number of the FDA licensed, cleared, or approved donor tests are also CE marked in the EU [70]. Regarding testing facilities, in the EU evidence should be available to show that any laboratory used for testing of donor samples has been accredited, designated, licensed and/or authorized by the appropriate authority to carry out such testing. This is more flexible compared to CLIA in the USA, however it does have the potential to prevent an allogenic cell line derived in Europe from qualifying for use in the USA unless an exemption is granted by FDA.

Regarding testing time window requirements, in Europe, for living donors, (except allogeneic bone marrow stem-cell and peripheral blood stem-cell donors), blood samples must be obtained at the time of donation or, if not possible, within seven days post donation. Where tissues and cells of allogeneic living donors can be stored for long periods, repeat sampling and testing is required after an interval of 180 days. In these circumstances of repeat testing, the donation sample can be taken up to 30 days prior to and 7 days post-donation. However, if this initial sample is additionally tested by the nucleic acid amplification technique (NAT) for HIV, HBV, and HCV, testing of a repeat blood sample is not required. Retesting is also not required if the processing includes an inactivation step that has been validated for the viruses concerned [69]. Moreover, in Europe for partner donation of gametes and embryos, serological testing of a sample taken within 2 years of the procedure is required [71].

Developers should seek confirmation that donor material has been appropriately tested for the market(s) they wish to enter. Alignment on testing time windows does not exist between all regulatory jurisdictions [72]. Therefore, confirmation should be sought that donor material has been appropriately tested for the market(s) they wish to enter.

INTERNATIONAL HARMONIZATION

It should be no surprise that there exists highly similar guidance for cell-based therapeutics between the forementioned regulators, as the USA, Japan, the EU, and the UK (added post-Brexit in June 2022 [73]) were the founding members of The International Council for Harmonization (ICH)—formally the International Conference on Harmonization. Since its establishment in 1990, the ICH has recognized a need for harmonized international guidelines and has a successful record in delivering harmonized guidelines for global pharmaceutical development and regulation [74]. For example, ICH Q5D published in 1998 provides guidance on standards for the derivation of human cells to be used in biotechnological/biological products, including recommendations for the preparation and characterization of cell banks [75].

Accordingly, many regulators in established cell therapy markets have aligned their guidance with that of the ICH founding nations. For example, in North America, Health Canada which is the regulator for pharmaceuticals in Canada, has full alignment with the FDA in terms of physical examination and the health history screening questionnaire (donor screening) [76] and the required IDM tests, testing timelines and IDM and HLA laboratory accreditation requirements (donor testing) [77]. However, differences exist in the testing equipment and collection equipment that is required. Health Canada (and also the MHRA and US FDA [78]) do

not currently provide specific requirements for Good Manufacturing Practice (GMP) application during the collection of cellular starting material. GMP is applicable when product manufacturing commences. Within the EU, member states can take different approaches to GMP. Notably, Germany requires a certificate of compliance for collection facilities (issued under EU directive 2003/94/EC) [79] whereas for other member states, a facility collecting starting material does not need to be GMP adherent if it is regularly audited and inspected by the regulatory authority [80].

Likewise, in Australia, the Therapeutic Goods Administration (TGA) has adopted many international scientific guidance documents from both the FDA and the EMA. PSC-derived products are categorized as Advanced Therapies by the TGA and regulated under the Therapeutic Goods Act 1989. For Advanced Therapies the TGA refers sponsors/developers to refer to ICH Q5D [75] as well as EMA guidance document EMEA/CHMP/410869/2006 [81]—the latter document informing that the specific requirements for donation, procurement and testing laid down in Directive 2006/17/EC [69] shall be met. The Therapeutic Goods Order (TGO) TGO 108 (which came into effect September 2021 and replaced TGO 88) and ARGB Appendix 12 version 2.0 (February 2023) [82] outline the regulatory requirements for donor screening and testing relating to human or tissue (HCT) product starting cellular material. The IDM testing requirements set out align with that of the EMA. Similarly, Singapore's regulator, the Health Sciences Authority (HSA), issued guidance on cell, tissue, and gene therapy products registration in Singapore in March 2021 (revised February 2022) [83] emphasizing adventitious agent testing and risk assessments for TSE agents. This guidance document included references for ICH Q5D and numerous EMA and FDA guidance for industry documents.

It is apparent that in the most established cell therapy markets, regulatory harmonization is becoming increasingly effective, which supports developers' efforts to expand into new regions and advance clinical trial activity. As stated earlier, the Asia-Pacific market is experiencing growth [84] and has the potential to overtake the EU market in revenue size within the next decade. However, not all countries in this region have the same degree of harmonization with the FDA and EMA/MHRA. Developers should be considerate that whilst some cellular starting material may be deemed appropriate for entry into certain Asia-Pacific countries, there may exist challenges if they wish to enter the same product in the future into the USA or a European country. For example, India and Korea do not require IDM screening for TSE/CJD and EBV (nor Taiwan for EBV), moreover, India currently has different requirements relating to the traceability of cell collection and preparation to the FDA [72].

While it may be expected for harmonization to further improve in the future, current developers may aim to source cellular starting material that complies across all major jurisdictions. In reality, this strategy is not always feasible, and whilst it is recommended that every effort be made to adhere to the cell sourcing regulations and guidelines pertaining to a developer's target jurisdiction(s), in some cases PSC lines with gaps in donor IDM testing may be tested into compliance during downstream production and development activities, as discussed in the next sections.

CELL LINE PRODUCTION

This section provides a general overview of PSC line production, including big picture considerations such as selection of a suitable iPSC reprogramming methodology, potential ways to reduce overall cell line production costs/timeline, and implementation of key process control measures. The cell line

production process generally can be broken up into the following two stages:

1. Initial derivation and seed stock generation; and

2. Production of a master cell bank (MCB) and downstream working cell banks (WCBs).

Each of these stages are discussed in turn below.

For developers looking to generate an iPSC line as their starting material, a critical initial decision is choice of delivery methodology for reprogramming factors. The predominant options for reprogramming methodology include episomal DNA, Sendai virus, and mRNA, each of which comes with its own benefits and drawbacks. First, each type of reprogramming methodology can result in varying degrees of reprogramming efficiency, which can be further impacted by starting cell type (commonly fibroblasts, peripheral blood mononuclear cells, or hematopoietic stem cells) and reprogramming conditions. When determining what level of reprogramming efficiency is needed, it is important to consider the number of successfully reprogrammed iPSC clones required to feed into downstream applications. With the advent of GMP-ready, single cell printing instrumentation (see below section on single cell cloning), it also is becoming increasingly feasible to select for rare events within a relatively large cell population, thereby decreasing the importance of high efficiency reprogramming. Another consideration for choice of reprogramming method is clinical precedent. Episomal DNA-based reprogramming now has a rather extensive track record of clinical programs, including more than 10 clinical trials in Japan targeting indications such as macular degeneration, cardiac failure, graft-versus-host disease, Parkinson's disease, and cancer [85]. In contrast, Sendai virus and mRNA-based reprogramming are mostly limited to preclinical-stage programs and therefore require further vetting by health authorities. Finally, the potential for reprogramming technology to impact product safety is a critical factor that should influence choice of methodology. Of note, use of episomal DNA-based reprogramming does carry the theoretical risk of genomic integration and insertional mutagenesis [86]; a safety concern that can be mitigated through sensitive screening measures applied post-reprogramming and upstream of drug product production, or avoided with non-integrating methods, such as Sendai virus and mRNA. Another reprogramming safety consideration is whether downstream manufacturing processes will be of sufficient duration to enable clearance of the reprogramming factors. This is most relevant to episomal DNA and Sendai virus, which are much more stable/long-lived than mRNA. For all three methods, demonstration of reprogramming factor clearance typically is required as part of downstream testing, as discussed further in the next section.

Sourcing of reprogramming reagents is another important factor in the decision process; currently, Sendai virus is commercially available as an off-the-shelf, GMP compliant product [87], whereas GMP episomal DNA and mRNA reprogramming reagents generally require bespoke engagements with research-focused manufacturers. In considering reprogramming and gene editing reagent suppliers, an additional key question is whether reprogramming reagents need to be GMP quality for PSC line production. Clarity on this has recently been provided by the FDA, as discussed in the next paragraph.

Once decisions about donor sourcing and iPSC reprogramming methodology have been made, the next critical consideration for cell line production is what level of manufacturing quality environment and control measures are appropriate for initial cell line derivation. Donor material collection most commonly occurs in a clinic or apheresis center, which should have measures in place to minimize risk of contamination, document

the collection process, and maintain chain of custody/chain of identity. In almost all cases, the collection process is not performed in accordance with GMP regulations, whereas GMP compliance typically is required for downstream drug product manufacturing activities. Historically, initial cell line derivation has occurred as a transition point between donor collection and drug product manufacturing, often occurring in a research lab setting rather than a GMP manufacturing environment. Despite an increasing number of GMP compliant facilities, the trend to perform non-GMP cell line derivation continues, largely driven by two factors:

1. Cell lines may be derived without knowing whether they are destined for clinical/commercial use; and
2. A desire to reduce overall product development costs and timelines.

From a regulatory perspective, derivation of PSC lines in a non-GMP environment can be acceptable in the context of allogeneic PSC-derived products for which cell line derivation occurs upstream of drug product manufacturing. The potential suitability of non-GMP PSC line derivation requires that sufficient control strategies are implemented, including laboratory environmental controls (e.g., use of a biological safety cabinet for open manipulations and/or use of closed instrumentation, appropriate cleaning procedures), material controls (e.g., tracking and segregation of critical raw materials and process intermediates), personnel controls (e.g., appropriate training, laboratory access restrictions), quality oversight (e.g., review and sign-off on procedures and development reports), and full documentation of each of these control measures. Importantly, the recently finalized FDA guidance on human gene therapy products incorporating genome editing acknowledges the potential suitability of this approach, noting that one-time gene editing, as might be used for allogeneic cell line production may require less documentation and testing of editing reagents as compared to gene editing performed as part of drug product manufacturing [7]. As this guidance suggests, genome editing of PSC lines may also be performed early in the process of line derivation to expedite platform development and reduce overall production costs. Regulatory success of performing reprogramming and/or genetic modification in a non-GMP environment is dependent on several factors, including:

1. Implementation of appropriate control measures as described above;
2. Regulatory buy-in on the PSC line derivation/modification strategy, and perhaps most importantly;
3. Downstream production of an MCB that is made in compliance with GMP regulations and fully tested in accordance with the relevant health authority guidelines.

Prior to MCB production, derived PSC lines typically will be used to generate one or more seed banks which are then evaluated for suitability as MCB starting material. Upstream of seed bank production, an additional control strategy to consider is clonal selection (i.e., isolation of single cells that are then propagated to yield clonal seed banks). Production of clonal seed banks is achievable by manual limiting dilution, or, for additional process control/assurance of clonality, via implementation of an automated single cell printing system such as the VIPS® Pro by Solentim or the SIGHT™ instrument line from Cytena (e.g., C.SIGHT™, F.SIGHT™). The main benefit of generating clonally-derived seed banks is that each resulting bank should be fully homogeneous with respect to cellular make-up and genome profile, thereby maximizing consistency for downstream applications. A homogenous genome profile is particularly advantageous in the context of genetically modified PSC lines; if the genetic modification step is performed upstream of

clonal selection, the resulting clones can then be screened for the desired genetic modification as well as for unwanted genetic changes (e.g., off-target editing, translocation events, and/or insertional mutagenesis). Similar to initial PSC line derivation and genetic modification, clonal selection and production of PSC seed banks may be performed in a non-GMP/development lab setting, providing that appropriate control strategies are implemented as outlined in the preceding paragraph.

Where there can be leniency with respect to performing initial PSC line derivation through seed bank production in a controlled, non-GMP environment, downstream MCB production should be performed in full compliance with GMP regulations. This is because the MCB serves as a key opportunity to de-risk any non-GMP activities occurring upstream (e.g., donor material sourcing or initial cell line derivation), address potential gaps in donor or pre-MCB material testing, and ensures that the resulting cell bank is suitable as starting material for downstream drug product manufacturing. As such, MCB production typically represents an early major milestone for a therapeutic program development with respect to cost and planning. To inform MCB size, it is important to consider how much starting material will be needed to support the entire product lifecycle, including material needs for MCB characterization, release testing, stability testing, drug product manufacturing (clinical and commercial), and retains. In addition, developers should consider whether a two-tiered banking strategy will be needed to provide sufficient starting material for full product lifecycle, in which case the MCB is used to generate as series of downstream WCBs. Like the MCB, WCBs should be made in full compliance with GMP regulations, as they then serve as the cellular starting material for drug product manufacturing. Finally, MCB/WCB production planning should include modeling of the PSC passage number required to generate drug product at the intended commercial scale and confirmation that the manufacturing process is sufficiently robust at or beyond the maximum anticipated PSC passage. To fully de-risk the path to, and ultimate production of, the MCB and WCBs, a stepwise and comprehensive analytical testing approach is key, as described in the next section.

CELL LINE TESTING

During initial PSC line derivation, implementation of a stepwise or tiered testing approach can provide an effective method to balance between progressive de-risking of the cell line and resulting impact on development timeline and cost. In the tiered testing scenario, assays are grouped based on their relative importance to moving the PSC line forward, such that tests with the potential to yield 'no-go' results are conducted as early as possible during line derivation/seed bank generation and followed by assays of progressively lower priority. For example, it can be prudent to include key safety assays in the first tier of testing, such as sterility, mycoplasma, a method to detect large-scale chromosomal abnormalities and/or mutations with oncogenic potential, and clearance of detectable reprogramming factors. First tier testing typically also should include a method to confirm donor identity/absence of non-donor cells, such as short tandem repeat profiling, particularly in the case of autologous products or facilities where multiple products are processed in parallel. For gene modified PSC lines, first tier testing also should include a confirmation of the desired genetic modification and transgene expression (or lack of gene expression in the case of knock-out strategies) to warrant moving forward. If utilizing a clonal production strategy, first tier testing should ideally be performed on clonal progeny as soon as sufficient material has been generated. As early as is feasible in tiered testing, it also is important to confirm that a given PSC line or clone successfully performs in the intended differentiation process (i.e., can generate the target cell type),

however the timing of this must be aligned with the amount of required cellular material and the duration of the manufacturing process. Additional testing should then be assigned to a testing tier using a risk-based approach, considering factors such as impact on PSC line suitability, timeline to complete, and cost. Ideally the more expensive tests that also are less likely to yield a surprising result (i.e., *in vitro* and *in vivo* adventitious agent testing) can be saved for the MCB.

Testing of the MCB and WCB is the final and arguably most important step in overall risk management of PSC starting material and therefore should adhere to the expectations of health authorities. Key guidance documents that should be used to inform MCB and WCB testing include:

- 'ICH Topic Q5D: Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/ Biological Products' (1997) [75];
- 'Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals' (FDA, 1993) [88];
- 'Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications' (FDA, 2010) [89];
- 'Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products; Draft Guidance for Industry' (FDA, 2024) [90];
- 'Considerations for the Use of Human- and Animal-Derived Materials in the Manufacture of Cell and Gene Therapy and Tissue-Engineered Medical Products; Draft Guidance for Industry' (FDA, 2024) [91];
- 'Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells' (EMA, 2020) [92];
- 'Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)' (FDA, 2020) [93];
- 'Human Gene Therapy Products Incorporating Human Genome Editing: Guidance for Industry' (FDA, 2024) [7].

The MCB/WCB testing described in these guidances can be generally broken up into the categories of identity, purity, safety, and stability (which should include one or more measurements of potency). Identity tests should include accepted PSC markers that utilize a quantitative method such as flow cytometry, an assay to confirm donor origin (e.g., short tandem repeat), and in the case of gene edited cell lines, assessments of the intended gene edit and/or incorporated transgene inclusive of copy number. The array of purity tests will vary more based on the specifics of the cell line, but may include testing for differentiated cells, and in the case of gene-edited lines, should include residual testing of gene editing components (e.g., nuclease, donor template). Safety testing by far is the most exhaustive, and consequently most expensive, battery of assays for the MCB. Safety testing of an MCB must include standard compendial testing (sterility, mycoplasma, endotoxin), *in vitro*/*in vivo* adventitious agent testing, a large panel of relevant communicable disease agents or diseases (RCDADs), much of which overlaps with the testing required of donor eligibility determination, as well as any necessary human/animal viral testing to account for human- or animal-derived/contacting materials used during cell line derivation or banking. Similarly, WCB testing should adhere to the regulatory guidances listed above and at a minimum should include tests for identity, sterility, mycoplasma, and *in vitro* adventitious agents. In addition, the continued

suitability of MCBs and WCBs should be confirmed via routine stability testing that includes measurements of sterility, identity, purity, quality, and activity or potency.

The exhaustive nature of MCB/WCB testing is important, not only because it ensures suitability of the banks as starting material for drug product manufacturing, but also because it can provide an opportunity to address any potential gaps in donor testing or donor medical history information. Indeed, this concept largely accounts for the advancement of ESC-based therapeutics to clinical trials, virtually all of which utilize ESC lines that either do not meet 21 CFR 1271 Subpart C donor eligibility criteria (given inability to perform donor testing within the required 30-day window [38]), have limited donor medical history, have gaps in traceability of their initial derivation and subsequent provenance, or some combination of all of these deficiencies. In each case, comprehensive safety testing of the downstream MCB served as a critical de-risking approach to enable their clinical use.

Despite an exciting plethora of iPSC advances/applications in recent years, ESCs remain a highly desirable starting material for allogeneic cell therapies as they offer the same scalability advantages as iPSCs without the risk of reprograming-associated genomic instabilities. Accordingly, within the last decade, EUTCD-compliant human ESC lines began to be derived and deposited in repositories, such as the UK Stem Cell Bank [94–96] and evaluated in academic settings [97–98]. The UK Stem Cell Bank has released numerous human ESC lines which meet the requirements of the Human Tissue (Quality and Safety for Human Application) Regulations 2007 (as amended) and are suitable as starting materials for clinical applications in the UK and EU. Matched research variants of these lines are also available.

However, cellular therapies, including those derived from ESCs, that are intended for implantation/transplantation/infusion/transfer into a human recipient in the US should be compliant with 21 CFR 1271 [38], and sponsors presenting a non-compliant PSC line to the FDA (even if derived prior to the effective date for Subpart C, May 25, 2005) are likely to receive a strong recommendation to select an alternative, compliant cell line. Considering the requirements of 21 CFR 1271, many ESC lines derived in the UK (due to donors being UK citizens/residents, or from BSE affected countries) would not be considered suitable by the FDA due to the risk of transmission of TSE. The primary reason for this is that to date, an FDA accepted test for evaluating BSE/TSE contamination does not exist.

Advances in biotechnology have led to the development of screening methodologies for the presence of BSE/vCJD. For example, the protein misfolding cyclic amplification (PMCA) assay has been developed to emulate the process of accelerated prion replication in a manner that is conceptually analogous to DNA amplification by PCR, and results in prion signal amplification and detection. Currently, the PMCA assay (together with a variation referred as RT-QuIC) is being used in the US and Europe to support the diagnosis of CJD. Several publications have established that these techniques can detect vCJD and BSE in both infected human and bovine samples [99–101]. Moreover, it has been demonstrated that PMCA has the sensitivity to detect a single cell infected with prions within a pool of millions of non-infected cells [102]. Thus, while it has long been considered that UK-derived ESCs would be deemed categorically unsuitable by the FDA (without amendments to 21 CFR 1271), development of more reliable and sensitive BSE/TSE detection methods, such as the PMCA assay may offer a path forward into the US market for cell-based therapeutics with theoretical risk of TSE/BSE exposure. However, as of yet there has been no clear guidance issued from the FDA on this topic.

A final rapidly evolving area of cell line testing is evaluation of genome integrity. Even in the absence of viral vector-based genetic

engineering or gene editing, extended culture of PSCs has been shown to result in accumulation of genetic abnormalities, including within known oncogenes [103,104]. Accordingly, it is becoming increasingly important to include in-depth evaluation of genome integrity for PSC lines, both during initial derivation and for the resulting MCB/WCBs. The importance of genome safety testing is significantly greater for genetically engineered cell lines. In an attempt to keep stride with this fast-paced field, the FDA recently finalized its Human Genome Editing guidance [7]. This guidance includes a general overview off-target editing analysis and regulatory expectations, as well as some newly added statements acknowledging the potential for reduced documentation/testing of one-time gene editing of cell lines (as noted above) and that comprehensive genome safety analysis of the MCB and/or WCB can be used to reduce analytical testing burden of drug product. While the guidance is an important resource for developers, there are some important aspects of genome safety testing that are not addressed in the guidance and remain up to the sponsor, including selection of appropriate analytical methods for genome testing and development of a scientifically sound method for data interpretation. As the scientific community gains more experience with gene edited cell lines and how their given genomic profile impacts product safety, it will be important for the industry and health authorities to continue to build upon and refine a set of best practices for genome safety testing.

FINAL REMARKS/TRANSLATION INSIGHT

Development of a PSC-based therapeutics is highly specialized and complex, making it challenging to navigate the wide multiplicity of available options. Decisions pertaining to donor sourcing and cell line production are impactful to the resulting efficacy and safety of the product, in addition to the cost and complexity of manufacturing.

Whilst the regulatory guidelines pertaining to PSC line sourcing and development discussed in this article are fairly well defined, there remain differences between regulatory jurisdictions. Moreover, regulatory guidelines are routinely amended and updated. In April of 2024 the FDA issued new Draft Guidance for Industry documents relating to:

1. The collection and testing of donated source material, reducing risk of TSE in human-derived materials, and use of human-derived feeders and cell-derived particles [91]; and

2. The testing of MCBs and WCBs of highly expanded cells, including iPSCs [90].

Furthermore, readers should take note that FDA CBER announced in January a list of guidance documents it is planning to issue in 2024 [105]; many of which pertain to sourcing, testing and eligibility of human cells.

Accordingly, it can be both valuable and appropriate to obtain regulator alignment on the suitability of a chosen cellular starting material. For PSC-based products, the importance of early regulatory buy-in cannot be overstated given that the chosen cell line typically is intended to serve as the starting point for the entire product lifecycle.

For cell-based therapies in the USA, an INTERACT meeting represents the first opportunity to obtain nonbinding advice from FDA regarding CMC, pharmacology and toxicology, and clinical aspects of their development program. Similar early engagement mechanisms exist in other jurisdictions, as listed in Table 2. If concerns exist regarding the intended cell line, it is highly beneficial to ask specific questions that correctly flag and describe issues of concern during an initial regulatory engagement. In the case of INTERACT, a meeting only will be granted to sponsors at the appropriate stage of development, specifically once there is an understanding of the drug product concept and target indication, and pilot proof-of-concept (PoC) data has been obtained in the

TABLE 2

Regulatory engagement opportunities with different global health authorities.

Regulator (jurisdiction)	Regulatory advice meeting opportunities	Approximate timeline (meeting request to proposed meeting date)
FDA (USA)	INTERACT[1]; Pre-IND[2]	75 days; 60 days
Health Canada (Canada)	Pre-CTA	60 days
EMA (Europe)	National Scientific Advice meeting[3]; EMA Scientific Advice meeting	Approximately 8 weeks[4]; approximately 7 weeks
MHRA (UK)	MHRA Innovation Office meeting; MHRA Scientific Advice meeting	Innovation Office: 20 days; Scientific Advice: >30 days[6]
PMDA (Japan)	Pre-consultation meeting (Jizen-mendan)[5] and Formal PMDA Consultation Meeting (Taimen-jogen)	Pre-consultation: 2–5 weeks; Consultation: 2–3 months[7]
TGA (Australia)	Pre-submission meeting	Approximately 30–60 days
NMPA/CDE (China)	Category III Pre-consultation meeting[8]; Category II Pre-consultation meeting	CAT3: 75 days; CAT2: 60 days
HSA (Singapore)	Pre-submission meeting	≥40 days
MFDS (Republic of Korea)	Pre-IND	Approximately 30–60 days[9]

[1]Early interaction opportunity.
[2]Advice prior to conducting pivotal studies.
[3]Held with a National Competent Authority.
[4]The process by which an NCA should be approached to request Scientific Advice is well defined by each individual agency. Typically, a request for Scientific Advice should be submitted at least eight weeks in advance of the preferred meeting date.
[5]This meeting is to confirm the materials that are to be submitted and the sponsor's questions that will be discussed at the subsequent *taimen-jogen* ('full consultation meeting').
[6]When this article was written, lead times for non-COVID-19 related scientific advice meetings were reported to be much longer, ranging 6–9 months from the time of initial meeting request.
[7]Starting from the next first working day of a month.
[8]Held for innovative drugs for topics not covered by a category I or II meeting—i.e., major issues encountered during development.
[9]MFDS schedules meetings for the first Wednesday of each month. Sponsors will be given the next available slot.

proposed nonclinical model(s). Therefore, it is imperative that sponsors appropriately time their initial regulatory engagements to ensure alignment with regulatory expectations and to maximize the opportunity to get clear and actionable regulatory feedback.

Early regulatory engagement also helps ensure that cell line production can align seamlessly with other aspects of product development. For example, it typically is acceptable both for initial nonclinical PoC efficacy and safety studies and early process development studies to utilize a research version of the intended cell-based therapeutic, or potentially even a different a PSC line or clone (recognizing that there may be some degree of business risk associated with this approach). In this case, early regulatory engagements represent a key opportunity to ensure that materials are used in a phase-appropriate manner. Importantly, prior to the initiation of pivotal nonclinical safety and toxicology studies, developers should ensure that the PSC line, clinical starting material (e.g., MCB or WCB) and drug product manufacturing process all are fully representative of what will be used clinically.

As the cell and gene therapy field continues to grow and further biotechnology advancements are made, it is imperative that health authorities, the biotech industry, and the scientific community continue to work together to refine the way in which PSC-based products are developed and regulated. Alignment between these groups on critical early product development activities such as sourcing of donor material, PSC line derivation, and MCB/WCB bank production will help ensure that PSC-based products are developed in manner that is both commercially forward compatible and maximized for patient safety.

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AFFILIATIONS

Samuel JI Blackford
Dark Horse Consulting Group, Inc.

Nathan C Manley
Dark Horse Consulting Group, Inc.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

G-Rex bioreactors incubated in Heracell™ Vios™ CR CO_2 Incubators help prevent cross contamination and bacterial contamination in cell and gene modified cell therapy production

Mary Kay Bates, Dan Fick, Jürgen Schneider, Dan Welch, Josh Ludwig, and Lindsey Low

Abstract: for cell and gene modified cell therapy (GMCT) manufacturing, a robust contamination control strategy (CCS) is necessary to ensure compliance with current good manufacturing practices (cGMP). A successful CCS helps ensure safety, purity, efficacy, and quality of a cell therapy product. Cell therapy manufacturers should implement closed systems (CS) wherever possible and employ equipment that maintains a stable, clean environment with advanced monitoring. We show that CS G-Rex® bioreactors combined with Thermo Scientific™ Heracell™ Vios™ CR carbon dioxide (CO_2) incubators enable simplified parallel processing capabilities and significantly reduced contamination risks in a highly efficient production. Producing T cells, NK cells and others in a CS G-Rex bioreactor (Wilson Wolf Manufacturing, LLC) reduces the risk of contamination. The G-Rex approach eliminates most manual handling, a leading source of contamination. G-Rex bioreactors include weldable tubing for simple CS sterile connections and do not require interventions for feeding. Importantly, CS G-Rex bioreactors feature a validated sterile fluid path that reliably maintains integrity throughout manufacturing as shown by microbial ingress testing based on

ASTM E-3251. The bioreactors withstand full immersion in a challenge media solution containing bacteria for at least 14 days. G-Rex bioreactors also passed Viral Penetration testing based on ASTM Method F1671, demonstrating suitability for use in CAR-T and other CGT applications requiring use of virus. Heracell Vios CR CO_2 Incubators provide recovery of all parameters in 10 minutes or less, and uniformity of ±0.3 ˚C per DIN 12880, which defines how incubators and other laboratory heating equipment should be measured. These parameters are maintained even when performing high volume production with ten G-Rex 500M-CS bioreactors simultaneously. Thermo Scientific™ CultiMaxx™ shelving maximizes production capacity per footprint. Incubating multiple G-Rex bioreactors in a single chamber means the incubator must offer proven contamination control features, to protect individual donor/patient product, if applicable. An on-demand cycle delivers 12-log sterility assurance level (SAL). In-chamber HEPA filtration generates air cleanliness in the chamber equal to ISO Class 5. Data show the Heracell Vios CR CO_2 incubators are certified for use in ISO Class 5, GMP Grade A/B cleanrooms. Together, G-Rex bioreactors incubated in Heracell Vios CR CO_2 incubators offer a high yield, parallel processing method with reduced contamination risk in a highly efficient footprint enabling robust CCS in a cGMP environment.

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MATERIALS AND METHODS

G-Rex microbial ingress test

Tests were performed by a third-party test lab, Wuxi AppTec (Atlanta GA USA) in accordance with ASTM E-3251 [1]. Briefly, a 1×10^6 CFU (colony forming unit) challenge solution was prepared using *Brevundimonas diminuta*. The test sample G-Rex bioreactors were aseptically filled with Nutrient Broth Media through each of the tubing lines, including the sample port, the reduction lines, and the harvest lines to sufficiently wet all internal surfaces. 5,000 mL of Nutrient Broth Media was introduced into each test sample G-Rex bioreactor. 12 L of challenge solution was filled into a lined container (Figure 1A). The G-Rex bioreactors were weighted to ensure full immersion in the challenge solution during the entire 14-day incubation test period (Figure 1B). The vent filter was unclamped and in an upright position to ensure that it did not make contact with the challenge solution.

To confirm no growth, the test bioreactors were carefully removed from the challenge solution and placed inside a new sterile bag. The bioreactors were then gently swirled to mix the contents, and a 1.0 mL aliquot of Nutrient Broth was removed and added to a 10 cm Petri dish in triplicate per bioreactor and incubated 2–3 days then counted. For positive controls, the *B. diminuta* challenge solution was incubated in parallel with the samples, and growth was confirmed.

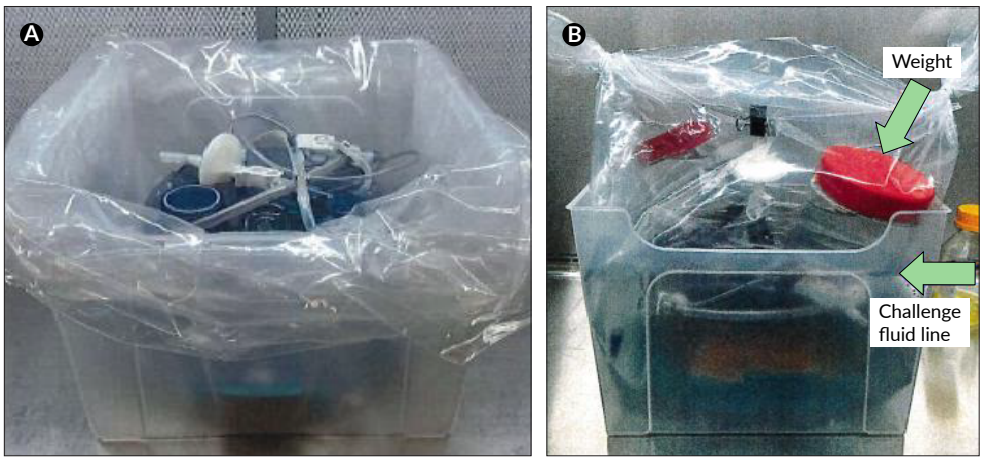
G-Rex membrane viral penetration test

Viral penetration testing was performed by a third-party test lab (Nelson Laboratories, Salt Lake City, UT, USA) according to their internal protocols. Tests were performed on the G-Rex membrane material according to ASTM F16712 [2]. Briefly, a challenge suspension was prepared using bacteriophage Phi-X174 maintained at a concentration of at least 1.0×10^6/mL. A total of 32 test articles (G-Rex membrane material) were prepared by loading each test article membrane into a test apparatus as shown in Figure 2.

The bolts around the test frame were torqued to create a perimeter seal. Each test

FIGURE 1

G-Rex bioreactors immersed for the microbial ingress test.



(A) Loading the bioreactors: 12 L of challenge solution were filled into the lined container and the bioreactor completely immersed except the vent filter. (B) During the test: the bioreactors were weighted to ensure full immersion for the complete 14-day test.

reservoir containing the filter sample was then filled with 60 mL of PhiX174 bacteriophage challenge suspension and pressured to 2.0 psig (~103 mm Hg) for 1 minute. Air pressure was then vented, and the test articles were allowed to sit for 54 minutes with no applied pressure while the surface of the membranes was observed for liquid penetration. As confirmation, an assay titer was performed for each test article in addition to positive and negative controls. For this test, the observed side of the membranes was rinsed with a sterile medium and assayed for the presence of Phi-X174 bacteriophage.

CO_2 incubator temperature uniformity mapping

Validated type K, Class 2 nickel chromium-nickel (NiCr-Ni) thermocouple temperature probes (Gantner, Germany) were placed in 27 locations in the incubator chamber, with 9 equidistant probes on each of three shelves, placed according to DIN 12880 [3]. The thermocouples were used in conjunction with a TV A4-32 MG data logger system (Gantner). The temperature was set to 37 °C, the humidity reservoir was filled to the maximum 3 L and the humidity was set to maximum (93% or higher). The incubator operated at these conditions undisturbed for 12 hours before commencing the test measurement. Each measurement had a 10-second duration and the test continued for 22 hours, in an ambient temperature of 22.8 °C. Uniformity equals the difference between the highest and lowest recorded temperatures.

CO_2 incubator recovery tests

For temperature, the tests were performed similarly to the uniformity mapping, except the incubator 'low humidity' setting was used and a validated PT100 temperature probe (KMP, Germany) was placed in the center of the center shelf. 10 G-Rex 500M-CS bioreactors were each filled with 5 L of water pre-heated to 37 °C. Both the inner and outer doors of the incubator were opened for 60 seconds, then closed. Recovery is defined as returning to 98% of the set value.

For CO_2 gas recovery testing, the incubator was set to the most commonly used concentration of 5%, temperature was set to 37 °C, and the 'low humidity' setting was used, which reduces humidity to 90%. CO_2

FIGURE 2

Membrane viral penetration test set-up.

The test membrane was loaded into the test apparatus, then the bolts tightened to seal the perimeter. The test reservoir was filled with the bacteriophage challenge suspension and pressurized. Following the venting, the membrane surface was visually inspected for liquid penetration. Then each test reservoir was tested for the bacteriophage.

was measured using a GMM221 infrared sensor (Vaisala, Finland) used without the protective cover and placed in the center of the middle shelf between the 4 G-Rex bioreactors (10 G-Rex vessels total in the incubator chamber). Recovery is defined as returning to 98% of the set value.

For humidity, the 'low humidity' setting was used. Humidity was measured using a FHAD 462 relative humidity (RH) sensor (AHLBORN, Germany) positioned in the center of the middle shelf between the 4 G-Rex bioreactors (10 total in the incubator chamber). Recovery is defined as returning to 98% of the set value.

HEPA filtration tests

Tests were performed in a room at 22 °C, 50% RH. Ambient particles greater than or equal to 0.5 µm were counted, and an average of 4,269,400 particles/m^3 was recorded (ISO Class 8) [4]. The incubator chamber and glass door were wiped with 70% ethanol to remove any surface residual particles. A calibrated particle counter, ACS Plus 328 (KM Optoelectronik GmbH, Germany) was used with an airflow setting of 1.0 cubic foot per minute (cfm). Particles were generated to boost to ISO Class 8–9 levels using an aerosol generator, CFG290 LMT (Topaz Gmbh, Germany). The sample tubing for the particle counter was located in the center of the empty chamber and the return air located in the top left rear of the chamber. The return air velocity was set to 2.1 m/sec. The test sample tubing and the return air tubing were run through the access port in the left upper rear wall. The space around the tubing was sealed, and the incubator water drain was sealed inside the chamber to ensure no passive air. A new HEPA filter was installed in the incubator chamber according to the user manual. Samples were collected for 30 seconds and purge time was 2 seconds between samples. Particles of 0.5 µm and larger were counted. The particle counter was operated in 'Automatic' mode for a minimum of 10 minutes.

Cleanroom compatibility tests

Tests were performed by an industry specialist, TŰV SŰD (Munich, Germany). Briefly, working in an ISO Class 4 cleanroom, the entire incubator was manually wiped and analyzed for surface particle shedding. The entire incubator exterior was then sampled using a particle counter to determine the areas of highest emission. This area was then sampled for 100 minutes with a sample taken every minute during the approximately 12-hour sterilization cycle at 180 °C. Particles of 0.5 µm and larger were counted.

RESULTS

Closed-System G-Rex Bioreactors prevent bacterial contamination and viral penetration

G-Rex Bioreactor validations enable fully closed system manufacturing

G-Rex CS Bioreactor validations include Shipping Simulation (ASTM D 4169)

[5], Environmental Conditioning (ISTA Procedure 3A) [6], Accelerated and Real-Time Shelf-Life Studies (3 year per Q10 Theory principles and ASTMF 1980-16) [7], and Sterilization Validation (Sterile Fluid Path) per Method VDmax25 ANSI/AAMI/ISO 11137 [8] 10^6 SAL Dose Substantiation and Max Dose validations. This validation testing supports the shelf life and sterile fluid path claims listed on G-Rex Certificates of Compliance. G-Rex is manufactured by Wilson Wolf Manufacturing LLC, in accordance with cGMP, and is 100% leak tested prior to release. To further substantiate G-Rex CS and sterile fluid path integrity claims during use, third-party microbial ingress testing, and viral penetration testing were performed.

Incubating multiple G-Rex bioreactors simultaneously in Heracell Vios CR CO_2 incubators represents minimal risk due to in-chamber protections

Heracell Vios CR CO_2 incubators offer proven contamination control

Based on a history of incubation innovation, Heracell Vios CR CO_2 incubators are the first-to-market certified cleanroom compatible CO_2 incubators [9]. In this way, the Heracell Vios CR models protect the cleanroom environment as an extension of the proven protection for cells incubated in the incubator chamber. This in-chamber protection includes a HEPA filtration system to capture airborne viable and non-viable particles, a 180 °C 12-log sterilization cycle which has been proven effective by a third-party test lab [10]. Humidity is provided by a covered, protected water reservoir which is easily drained and fully opened for easy cleaning and disinfection. All interior surfaces are electropolished to reduce microscopic structures where microorganisms could attach and to provide enhanced chemical resistance. The exterior casing is sealed, brushed stainless steel with ingress protection (IP) 54 rated electronics. A unique Active Particle Control system captures particles that would otherwise be emitted to the cleanroom.

Microbial ingress testing verifies sterile fluid path integrity after rigorous microbial challenge

For microbial ingress testing, a very small bacterium, *B. diminuta*, is considered an ideal challenge. *B. diminuta* is a highly motile, gram-negative bacterium. Due to its small size (0.3–0.6 µm), it is the preferred indicator organism for testing filter integrity and pore size.

As described in the 'Methods' section, three test G-Rex bioreactors, three negative control bioreactors, and three positive control bioreactors were prepared. All bioreactors were immersed in the challenge solution and placed in an incubator for 14 days. After incubation, the bioreactors were examined for the presence or absence of bacterial growth. All three test bioreactors showed no bacterial growth after 14 days of incubation under test conditions.

After the completion of the 14-day test, no growth was observed in the nutrient broth tested from the test bioreactors (Table 1), confirming the results of the microbial ingress testing, and verifying sterile fluid path integrity despite full immersion in the bacterial challenge solution.

Similar microbial ingress testing has been performed according to the protocol described above for all available sterile Closed System G-Rex models resulting in no growth in any of the test bioreactors. These results confirm that the G-Rex bioreactors are a closed system.

Membrane viral penetration testing confirms non-porous membrane structure and no viral penetration

G-Rex bioreactors include a highly gas-permeable membrane comprised of thin

TABLE 1

Microbial tests show no growth.

	Replicate 1	Replicate 2	Replicate 3
Test bioreactor: pre-subculture	No growth	No growth	No growth
Test bioreactor: post-subculture	No growth	No growth	No growth
Positive control	Growth	Growth	Growth
Negative control	No growth	No growth	No growth
Growth promotion	Growth	Growth	Growth

None of the tested bioreactors showed any growth, indicating that they passed the microbial ingress test challenge and demonstrating that the G-Rex bioreactors operate as a closed system.

TABLE 2

Viral penetration tests show no penetration.

Test article number	Pre-challenge concentration (PFU/mL)	Post-challenge concentration (PFU/mL)	Assay titer (PFU/mL)	Visual penetration	Test result
1–32	2.5×10^8	3.0×10^8	<1[a]	None seen	PASS
Negative control	2.5×10^8	3.0×10^8	<1[a]	None seen	Acceptable
Positive control	2.5×10^8	3.0×10^8	TNTC1[b]	Yes	Acceptable

No bacteriophage was found in any of the 32 test samples, providing further evidence that the G-Rex bioreactors are a closed system.
[a]A value of <1 plaque forming unit (PFU)/mL is reported for assay plates showing no plaques.
[b]TNTC = PFUs were too numerous to count.

silicone rubber. According to Fick's law of diffusion [11], gas molecules diffuse through the non-porous membrane's molecular structure into the liquid medium inside the bioreactor. The oxygen consumption rate of cells at the bottom of a typical non gas permeable cell culture vessel such as a T flask easily exceeds the diffusion rate of oxygen through the overlying culture medium [12]. With the G-Rex bioreactor, gas diffusion through the membrane surface at the bottom of the bioreactor negates reliance on oxygen diffusion at the gas-liquid interface above the medium inside the bioreactor for sufficient oxygen delivery to cells. Unconstrained by height, enough media can be present in the device at onset of culture to eliminate the need for medium exchanges.

The membrane material at the bottom of the bioreactor was tested for viral penetration according to ASTM F1671 (Figure 2). This test method is intended to evaluate blood-borne pathogens of major concern, including hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. The Phi-X174 bacteriophage has the following attributes: it is a non-enveloped 15–27 nm virus with an icosahedral or nearly spherical morphology, excellent environmental stability, a limit of detection which approaches a single virus particle, and it grows rapidly.

As shown in Table 2, all 32 test articles passed viral penetration testing, no liquid penetration was observed, and no (<1) plaque-forming units (PFU/mL) were reported for each test sample matching the negative control assays and confirming no viral penetration of the membrane.

These test results support closed-system G-Rex bioreactors in cell therapy and GMCT manufacturing processes in low-cost and lower-grade cleanrooms. Additionally, G-Rex bioreactors are structured with sufficient wall heights to contain medium

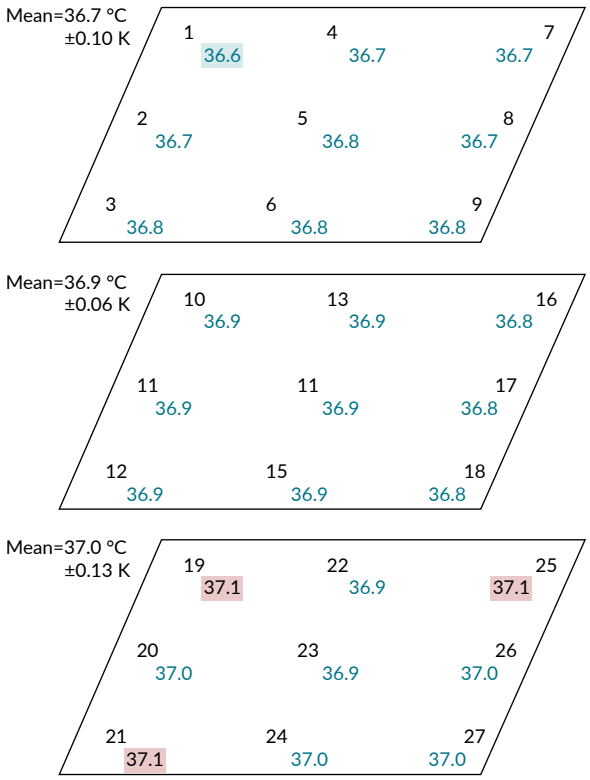
at unconventional volumes. The G-Rex structure also ensures the medium surface remains at a uniform distance of 10 cm above the gas-permeable cell growth surface. Together, these structural elements allow immune cells to expand from a minimum cell density per square centimeter of gas-permeable cell growth surface area to a maximum cell density per square centimeter of gas permeable cell growth surface area without medium change. An optimal ratio of 10 mL of medium per square centimeter of cell growth surface area eliminates all interventions during expansion, including medium exchanges or cytokine spikes, further reducing contamination risks and simplifying cell manufacturing processes [13].

CultiMaxx shelving increases G-Rex capacity without compromising incubator performance

CultiMaxx Shelving is specifically designed to increase the incubator chamber capacity from 4 to 10 G-Rex 500M-CS units. Like the rest of the chamber, these shelves are electropolished to increase cleanability and reduce areas where microorganisms could attach. Due to the modified shelving configuration, we wanted to determine effects on environmental uniformity throughout the incubator chamber, because reactive T cells and NK cells are demonstrably affected by culturing conditions [14]. For the G-Rex specialized CultiMaxx shelving, Heracell™ Vios™ CR incubator shelves have been extended and the lowest shelf sits lower in the incubator chamber compared to the standard shelving. Conceivably these changes could negatively affect the uniformity of culturing conditions. When testing the new shelving in an empty Heracell VIOS incubator at 27 points according to DIN 12880, results show that the uniformity specification of ±0.3 °C is maintained (Figure 3), similar to the standard shelving (results not shown).

FIGURE 3

Temperature uniformity with CultiMaxx™ shelving for G-Rex® units remains within specifications of ±0.3 °C.



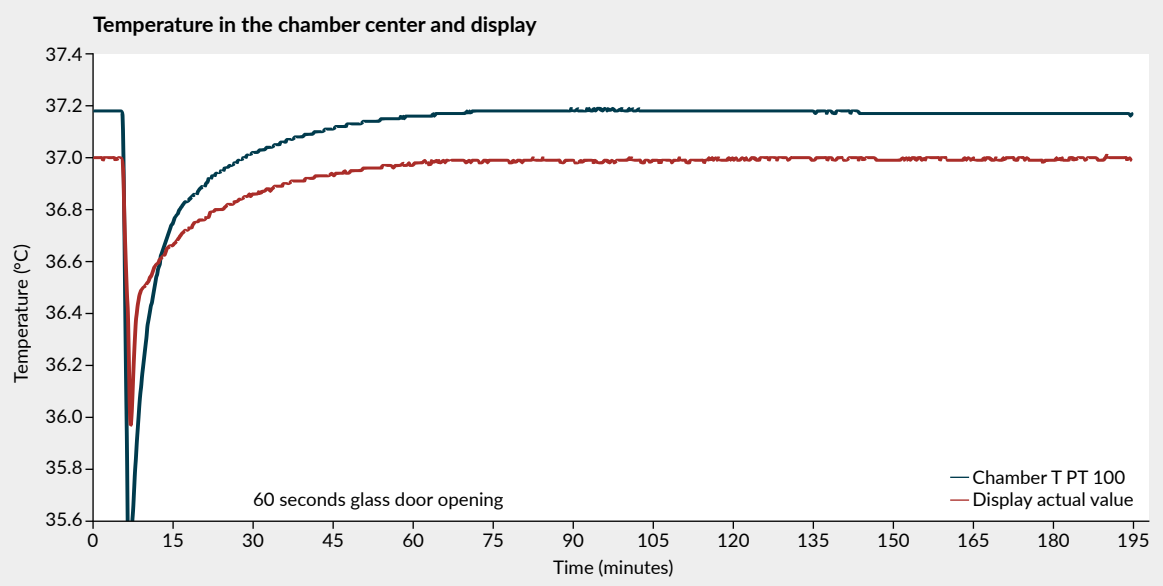
The temperature uniformity is shown to be ±0.25 °C and the temperature fluctuation (temporal deviation) was ±0.03 to ± 0.05 °C during the uniformity measurement.

Conditions recover quickly following a long door opening, even with 10 G-Rex bioreactors in the chamber simultaneously

Especially for primary patient immune cells, it is important that cultured cells spend their maximum time at their ideal conditions [14]. Because incubator recovery to set conditions following a door opening could be affected by size, type, and placement of large culture vessels, we tested recovery of each parameter following a sixty second door opening with ten filled G-Rex 500M-CS in place in the incubator chamber. Results show that all parameters recover quickly (see Figures 4–6). Recovery is similar to the 10-minute or less performance specification for the standard Heracell Vios

FIGURE 4

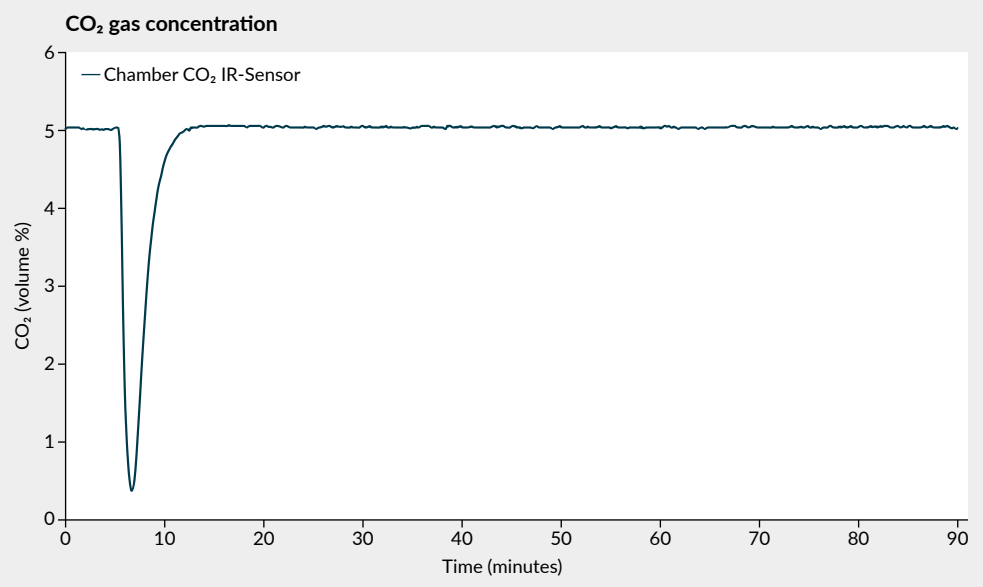
Temperature recovers in 5.3 minutes from a 60-second door opening.



With the Heracell Vios CR CO_2 incubator and CultiMaxx shelving filled with ten G-Rex 500M-CS units, each containing 5 L of water, both incubator doors were opened for sixty seconds. The temperature recovered to the set conditions of 37 ˚C in 5.3 minutes.

FIGURE 5

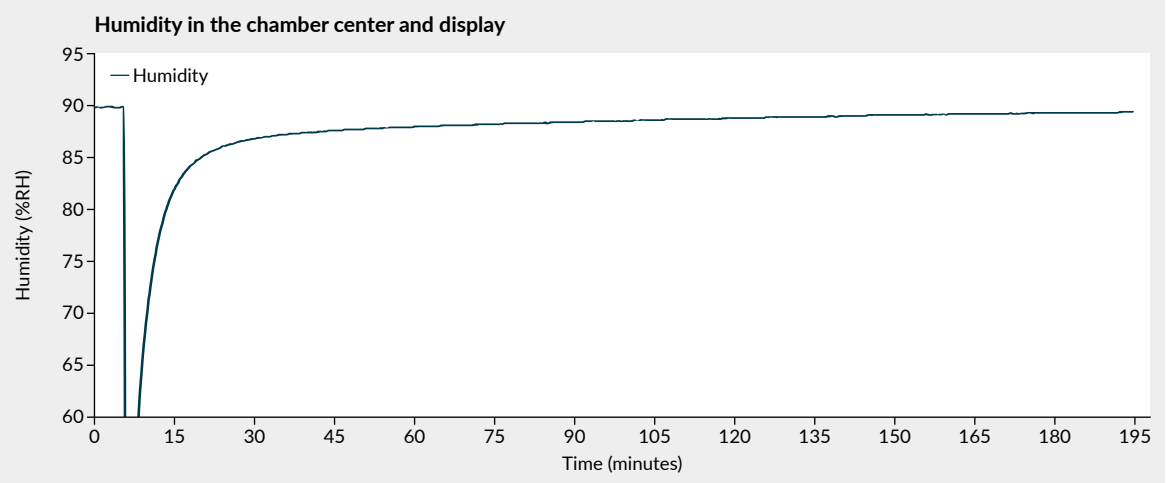
CO_2 gas recovers in 5 minutes from a 60-second door opening.



With the Heracell Vios CR CO_2 incubator and CultiMaxx shelving filled with ten G-Rex 500M-CS units, each containing 5 L of water, both incubator doors were opened for sixty seconds. Carbon dioxide recovered to the set 5% CO_2 in 5.0 minutes.

FIGURE 6

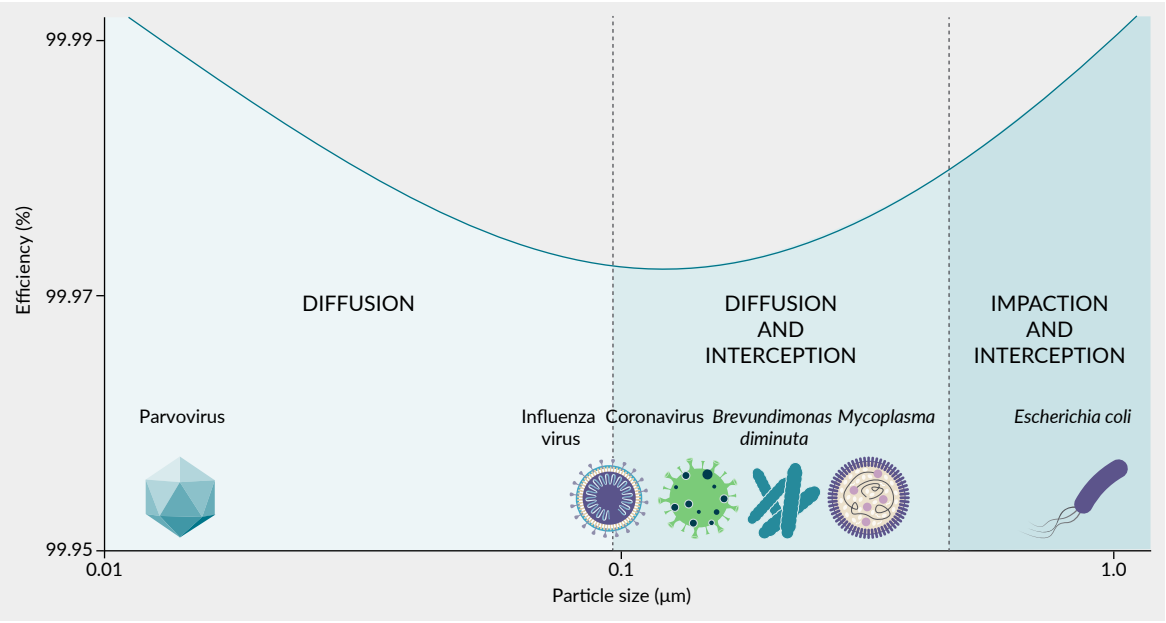
Humidity recovers in 15 minutes from a 60-second door opening.



With the Heracell Vios CR CO_2 incubator and CultiMaxx shelving filled with ten G-Rex 500M-CS units, each containing 5 L of water, both incubator doors were opened for 60 seconds. Relative humidity recovery following a 60-second door opening was 15 minutes, longer than our standard specification of 10-minute recovery from a 30-second door opening. Here, in 10 minutes, humidity recovery has reached 95% of the 'low humidity' parameter of 90%. The slower recovery in this case is due to the 'Low humidity' setting being switched on, rather than the standard high humidity with faster recovery. A shorter door opening or the standard high humidity setting would speed humidity recovery.

FIGURE 7

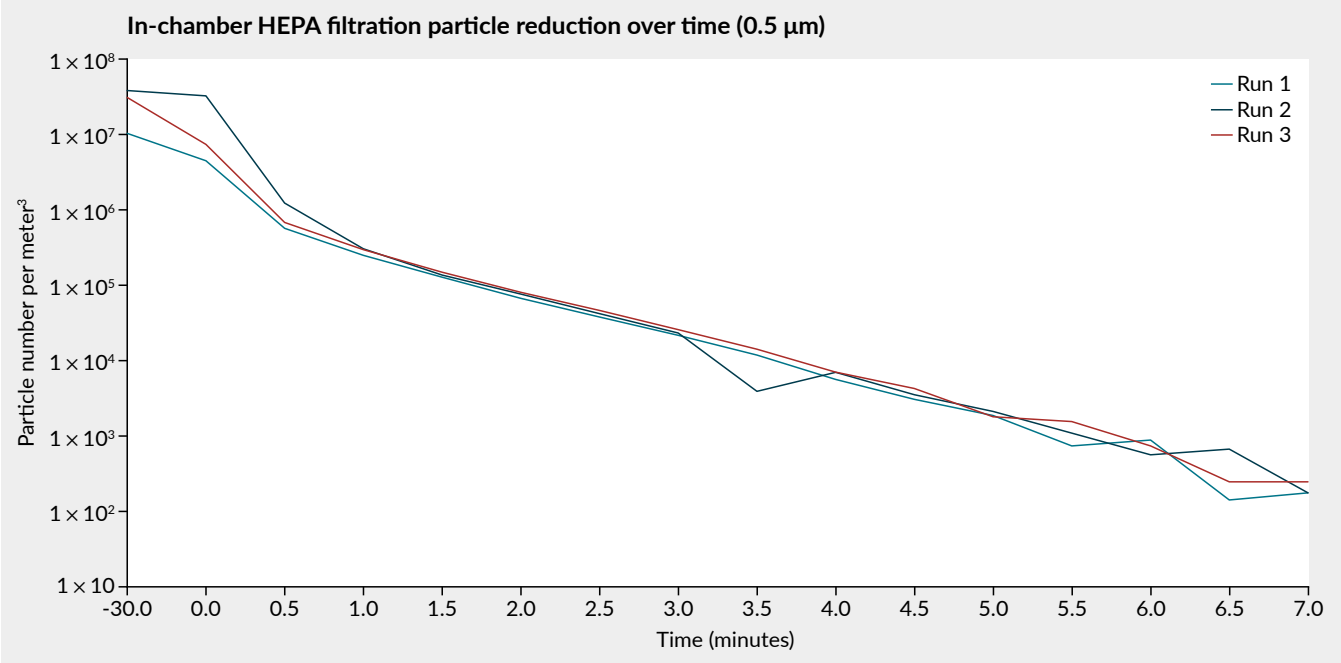
Heracell Vios CR CO_2 Incubator in-chamber HEPA filtration captures all particles regardless of size.



In an H13 HEPA filtration system, all particles are captured regardless of size. The MPPS is 99.97% at 0.3 µm, meaning smaller particles including the smallest viruses and mycoplasma bacteria are also captured with even greater efficiency.

FIGURE 8

In-chamber HEPA filtration provides ISO Class 5 conditions in 5-minutes after a door opening.



Representative test results showing that in the Heracell Vios 250i CR CO2 incubator, the in-chamber HEPA filtration combined with the THRIVE Active Airflow generates ISO Class 5 conditions in 5 minutes following a 30 second incubator door opening (both doors) and the air continues to get cleaner over time, reaching less than ISO Class 4 conditions in 7 minutes.

CR incubator, which is based on a 30-second door opening (results not shown). We have defined recovery as 98% of the set value.

Vios CR incubator protects cells with proven contamination control technologies

Many CO_2 incubators today offer features to help limit contamination inside. However, there is a wide range of efficacy of these technologies. For cell therapy manufacturing, proven technologies should be employed.

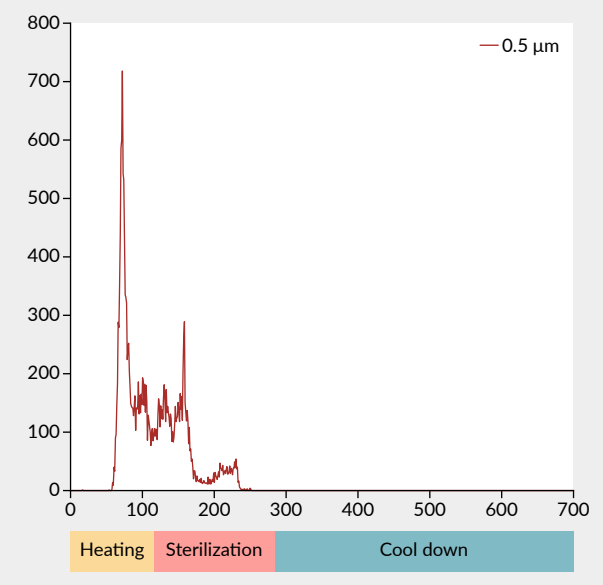
HEPA filtration

It is a common misconception that HEPA filtration only captures particles 0.3 µm or larger. This stems from the Most Penetrating Particle Size (MPPS) classification. Different physical processes are involved including impaction, interception and diffusion [15] such that for an H13 HEPA filter, the MPPS is 99.97% efficient at particles of 0.3 µm but as shown in Figure 7, smaller and larger particles are captured with higher efficiency approaching 100%.

A HEPA filtration system is driven by a circulating fan. Thermo Scientific™ THRIVE™ Active Airflow system works with the H13 HEPA filter to clean the air over time, as a dilution, where the entire chamber air volume is passed through the HEPA filter every 60 seconds. We wanted to ensure that the Heracell Vios 250i CR CO_2 incubator chamber reaches ISO Class 5 cleanroom conditions in 5 minutes after a 30 second door opening. Normal indoor room air is ISO Class 8–9. ISO Class 8 = 3.5×10^6 particles of 0.5 µm or larger per cubic meter of air. A Grade B cleanroom is equal to ISO Class 5 when at rest, ISO Class 7 in operation. These tests were conducted in an ISO Class 7 room at 23 °C. Particles were injected in the chamber to

FIGURE 9

The unique Active Particle Control filtration system in the Heracell Vios CR CO_2 Incubator limits particle emissions in a cleanroom even during sterilization.



Representative test results show particles 0.5 µm or larger released from the Heracell Vios CR CO_2 incubator during the Steri-Run 180 ˚C sterilization cycle. Results show that at all times during the 12-hour sterilization cycle, the device is certified for use in an ISO Class 5 environment. Tests were repeated three times by an independent industry specialist, TÜV SÜD (Munich, Germany).

FIGURE 10

Stacked Heracell Vios CR Incubators allow the production of 400 billion cells in about 3 square feet.

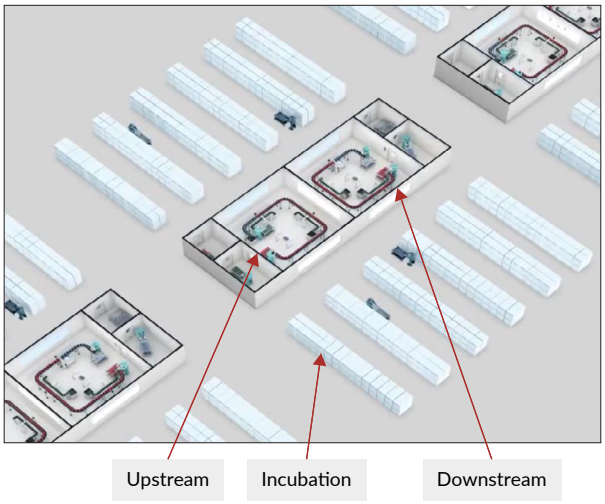
equal approximately ISO Class 8–9. Samples were taken according to ISO 14644-1 [4,16]. The results show that conditions inside the chamber reach ISO Class 5 conditions in about 5 minutes and continue to get cleaner over time (Figure 8). This system helps to protect cultures from any microorganisms which could enter the incubator when the doors are opened.

Cleanroom compatibility certification

An estimated 70% of particulates in a cleanroom come from the staff, and an estimated 15% comes from the process equipment [17]. As that process equipment is heated, more particles are shed into the air. For this reason, a CO_2 incubator with a high temperature sterilization cycle represents a greater risk and should be certified for use in a cleanroom. Heracell Vios CR CO_2 incubators include an exhaust filtration system that protects the cleanroom even during sterilization. They are certified by an industry specialist to be compatible with EU Grade A/B, ISO Class 5 cleanrooms [8]. As shown in Figure 9, the particles sized 0.5 µm or larger given off during the 12-hour (720 minutes) sterilization cycle fall below ISO Class 5, which has a limit of 3,520 particles/m^3 sized 0.5 µm or larger.

FIGURE 11

Novel facility layout enabled by combined technologies.



During normal operation at 37 °C, the incubator is also certified for use in ISO Class 5 conditions.

Closed system G-Rex bioreactors in combination with Heracell Vios CR CO_2 incubators enable simplified and efficient cGMP cell therapy manufacturing

The results of this testing tangibly demonstrate the possibility of high-throughput parallel processing of cell and gene-modified cell therapies. The modular approach is easily automated and scaled in a highly efficient facility layout. Without intervention, G-Rex bioreactors regularly achieve 40 million cells per square centimeter of gas-permeable cell growth surface area [18]. Each G-Rex 500M-CS can produce 20 billion cells, and each Heracell Vios CR CO_2 Incubator can hold 10 G-Rex 500M-CS (Figure 10). Thus, when the incubators are stacked, up to 400 billion cells can be produced in just over 3 square feet of floor space. This predictability enables repeatable, robust, and low-risk cell production for large-scale allogeneic and/or autologous processes.
A scalable, low-cost, and high-throughput manufacturing facility layout is now possible. Production set-up can occur in a small cleanroom, which houses upstream cellular processing equipment and reagents for high throughput closed system cell production set-up (i.e., apheresis wash, media fill, activation, etc.) and closed system inoculation of G-Rex bioreactors, followed by transfer of G-Rex bioreactors to a separate incubation room, which houses numerous incubators for parallel expansion processes with no risk of bacterial or cross-contamination (Figure 11).

Upon completion of the expansion phase, closed system G-Rex bioreactors can be removed from the incubators and moved to a cleanroom dedicated to downstream processing. The downstream cleanroom houses downstream cell processing equipment and reagents for cell harvest, final formulation, and fill processes. The resulting facility will produce significantly more doses in a smaller space than conventional facility designs. In addition, this combination has already been demonstrated in a fully automated system [19].

CONCLUSIONS

- G-Rex bioreactors are shown to operate as a closed system using multiple tests including microbial ingress testing and viral penetration testing.
- Heracell Vios CR CO_2 incubators are shown to retain their specified uniformity and recovery specifications even when filled with 10 G-Rex CS bioreactors. They also protect cells with ISO Class 5 conditions inside the chamber, and are certified for use in ISO Class 5, EU GMP Grade A/B cleanrooms environments.
- Heracell Vios CR CO_2 incubators with CultiMaxx shelving can hold up to 10 G-Rex 500M-CS bioreactors, producing up to 400 billion cells in a small footprint. G-Rex bioreactors in combination with Heracell Vios CR incubators enable novel facility design for high-throughput and easily automated cell therapy manufacturing.

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AFFILIATIONS

Mary Kay Bates
Senior Global Applications Scientist,
Thermo Fisher Scientific,
Waltham, MA, USA

Dan Fick
Director of Quality,
Wilson Wolf Manufacturing LLC,
St Paul, MN, USA

Jürgen Schneider
R&D System Engineer,
Thermo Fisher Scientific,
Waltham, MA, USA

Dan Welch
Director of Product Development,
Wilson Wolf Manufacturing LLC,
St Paul, MN, USA

Josh Ludwig
Global Commercial Director,
ScaleReady USA LLC,
St Paul, MN, USA

Lindsey Low
Senior Global Product Manager,
Thermo Fisher Scientific,
Waltham, MA, USA

ThermoFisher SCIENTIFIC

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

An automated 24-hour CAR-T manufacturing process

Mina Ahmadi

As the cell therapy field progresses, manufacturing challenges like patient safety, cost, automation, closed operation, and scalability must be addressed with improved capabilities and workflows. Leveraging new cell therapy technologies and tools to develop innovative end-to-end workflows can enable more cost-effective cell therapy processes and workflows. This article presents an automated and shortened lentiviral-based CAR-T workflow using the Gibco™ CTS™ Detachable Dynabeads™ CD3/CD28 magnetic beads, Gibco™ CTS™ Detachable Dynabeads™ Release Buffer, and Gibco™ CTS™ DynaCellect™ Magnetic Separation System.

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AUTOLOGOUS CAR-T WORKFLOW CHALLENGES AND SOLUTIONS

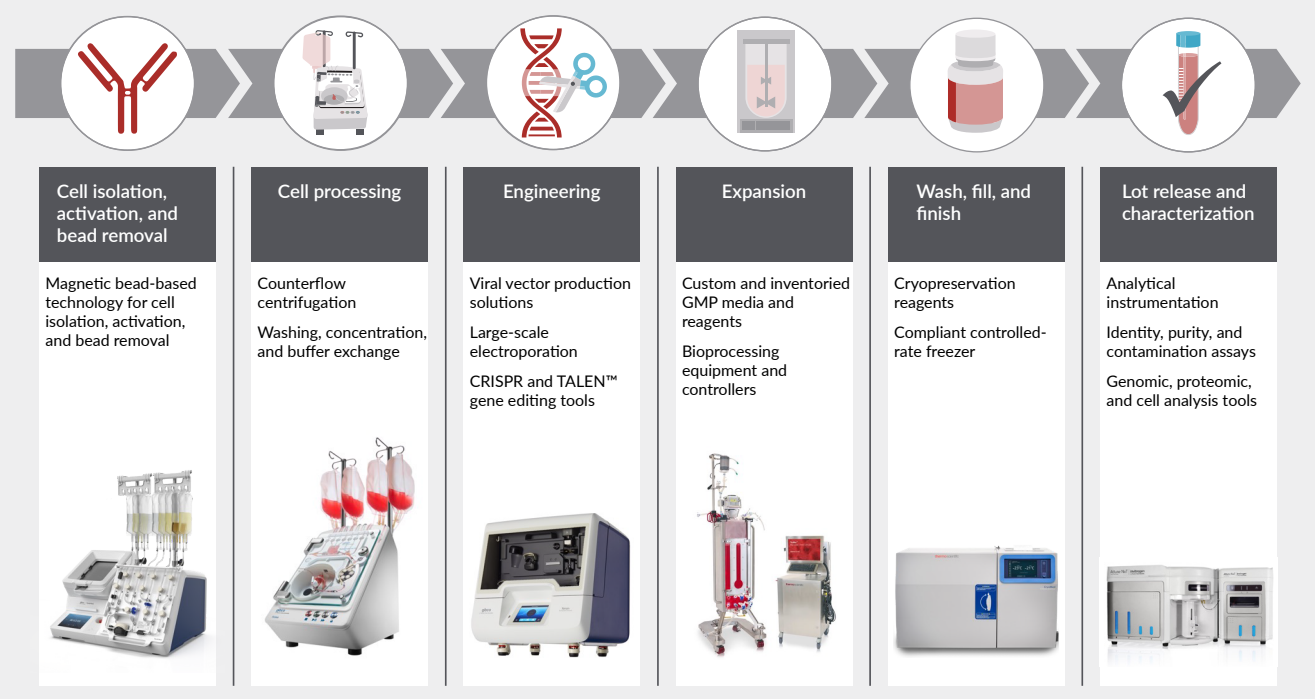
A typical autologous CAR-T workflow is a complex, multi-day GMP manufacturing process, which is often characterized by several labor-intensive and open steps that are prone to errors. These lengthy processes also require costly and extensive QC steps prior to product release. The need for CAR-T cell therapies, however, continues to grow, meaning there is a crucial need to address these challenges so that life-saving therapies can reach patients in a timely manner.

Several of these challenges could be addressed by scaling up the process, closing and automating the manufacturing process, and including in-line analytics. Figure 1 shows Cell Therapy Systems (CTS) solutions developed by Thermo Fisher Scientific, which help provide manufacturers with closed, automated, and modular technologies that can be combined with reagents specially formulated for cell therapy manufacturing to enable end-to-end cell therapy workflows.

The cell therapy instruments in Figure 1 are flexible, fast, and have intuitive touchscreen interfaces that enable users to easily scale their

FIGURE 1

Closed, modular CAR-T cell therapy manufacturing process workflow.



cell therapy processes from research through to clinical manufacturing. Additionally, manual touchpoints in this workflow can be reduced with the use of CTS Cellmation™ software, an off-the-shelf automation solution powered by Emerson's DeltaV™ Distributed Control System (DCS). Each of the CTS instruments comes equipped with an open platform communication unified architecture (OPC-UA) that is compatible with Emerson's DeltaV software.

CTS Cellmation software allows manufacturers and users to digitally connect these closed and modular systems, allowing for data traceability and consistency (using batch recipes), while enabling increased efficiency and scalability.

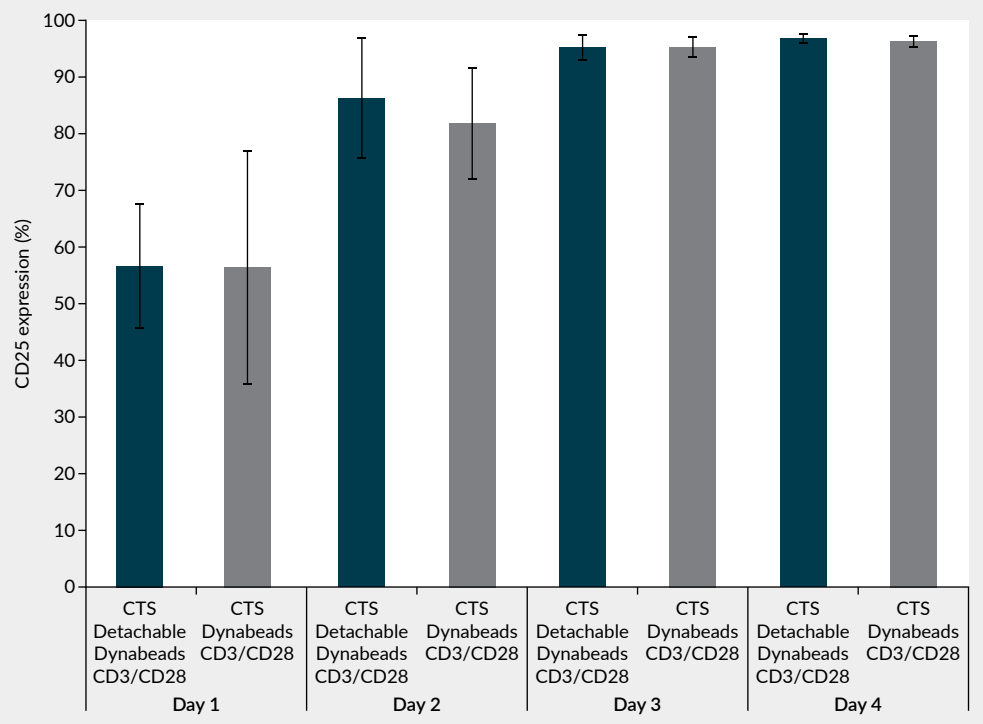
CTS DETACHABLE DYNABEADS CD3/CD28 MAGNETIC BEADS

In addition to the closed and modular instruments, significant efforts have been made to create reagents that can offer increased flexibility and that can be customized and leveraged for different cell therapy workflows. One example is the CTS Detachable Dynabeads CD3/CD28 beads, which combines CaptureSelect™ and Dynabeads™ technology. The CaptureSelect ligands are variable domain of heavy chain (VHH) fragments. The VHH antibodies are 12–15 kDa in size with tunable specificity and affinity to efficiently isolate or activate target cells of interest. These VHH antibodies are free of animal-derived components, highly stable, and suitable for use in GMP environments.

When used with the CTS Detachable Dynabeads Release Buffer, the CTS Detachable Dynabeads CD3/CD28 magnetic beads are actively released, enabling greater control of the T cell activation signal. The same Dynabead magnetic core is currently being used in over 200 active clinical trials, as well as several approved cell therapy drugs. In combination with the CTS DynaCellect system, the Detachable Dynabeads provide a powerful tool that allows for customization,

FIGURE 2

Average CD25 expression for active- and passive-release Dynabeads CD3/CD28 magnetic beads.



which can contribute to high drug efficacy, safety, and cost efficiency downstream of cell isolation.

With an emphasis on automation, flexibility, and scalability, the CTS DynaCellect system includes fit-for-purpose, single-use consumables for cell isolation. It has a touchscreen user interface that allows for the customization of protocols for cell isolation, cell activation, depletion, and magnetic separation.

CTS DETACHABLE DYNABEADS CD3/CD28 VERSUS CTS DYNABEADS CD3/CD28 BEADS

While the passive release Gibco CTS Dynabeads CD3/CD28 (SKU: 40203D) magnetic beads are a robust product which has been used in over 200 active clinical trials and several commercialized drugs, the active release CTS Detachable Dynabeads CD3/CD28 beads help provide manufacturers with better control of the T cell activation signal. Unlike the passive release Dynabeads magnetic beads, which rely on passive dissociation of beads from target cells over time, the CTS Detachable Dynabeads magnetic beads are designed so that users can actively detach at any point, post-isolation, using the CTS Detachable Dynabeads Release Buffer. Furthermore, because inefficient bead removal may result in hyper-activated and exhausted T cells, the ability for users to actively remove the CTS Detachable Dynabeads has the potential to reduce these effects while delivering comparable or improved results compared to the passive release beads.

Figure 2 shows data from a recent study that measured the average expression of T cell activation marker, CD25, for active- and passive-release Dynabeads magnetic beads. T cell expression of CD25 was comparable for both Dynabeads magnetic beads across the time points studied.

CAR-T cells with a less differentiated T memory cell phenotype show higher effectiveness in treating patients with blood

FIGURE 3

T cell memory phenotypes for active-and passive-release CTS Dynabeads magnetic beads.

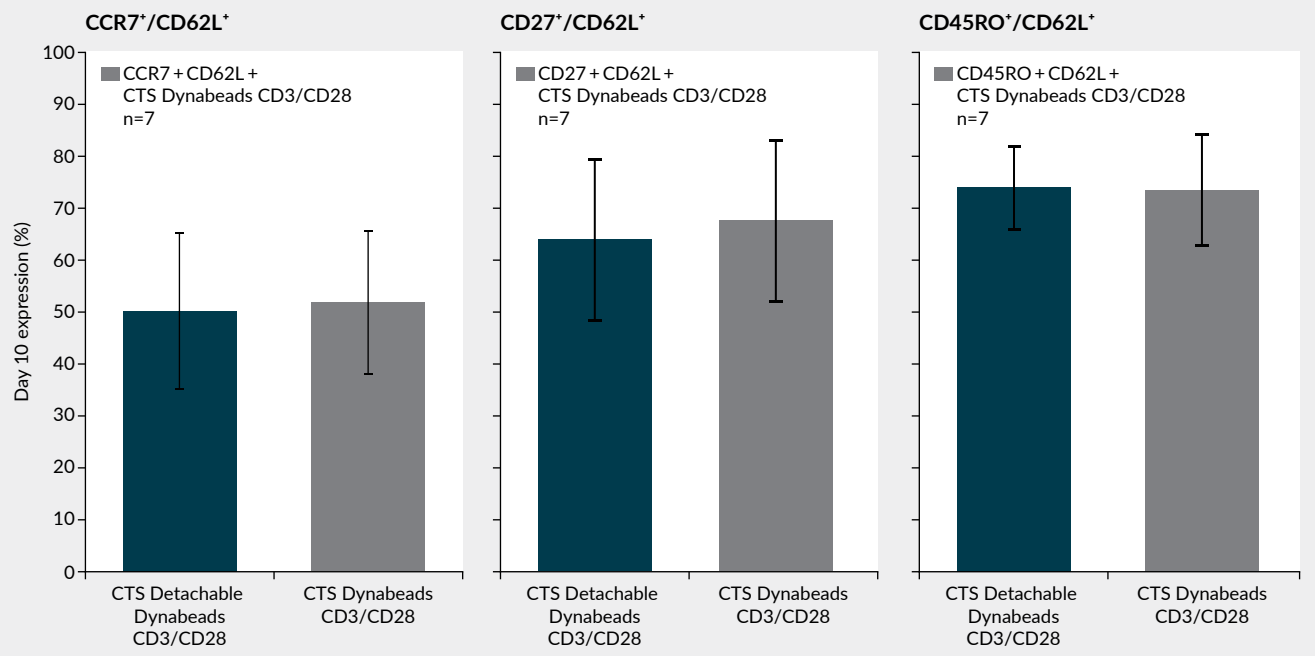
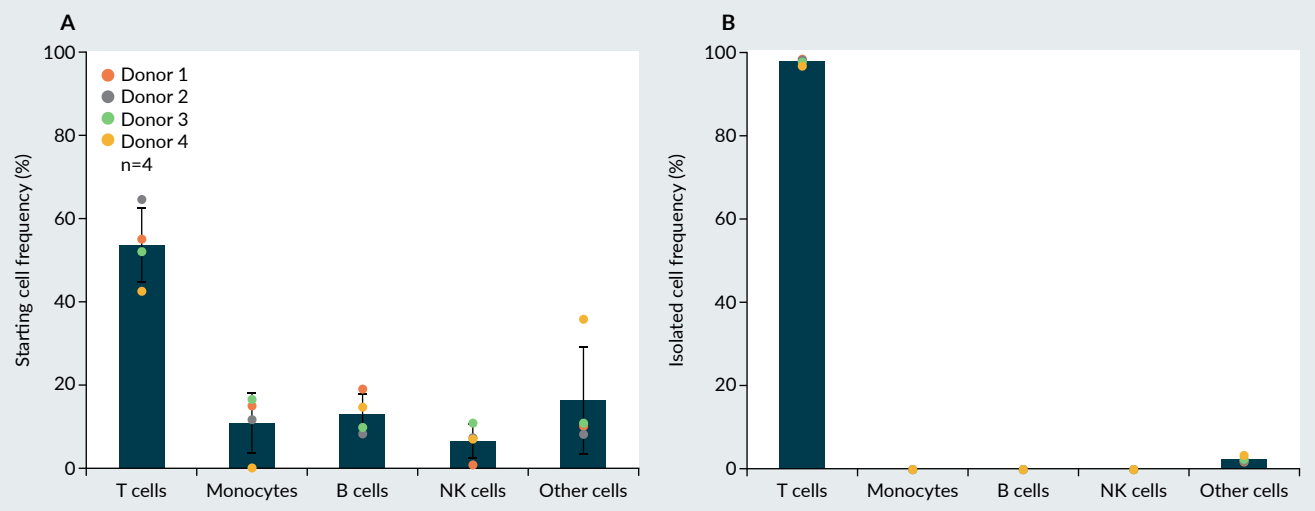


FIGURE 4

T cell purity with CTS Detachable Dynabeads CD3/CD28 beads.



malignancies. Therefore, this attribute was also investigated with both active- and passive-release Dynabeads magnetic beads. CCR7, CD62L, and CD27 were examined to assess the phenotype of early memory cells. Figure 3 shows comparable levels of naïve central memory phenotypes observed on day 10 post-isolation for both passive release and active release CTS Detachable Dynabeads.

Figure 4B shows that T cell purity levels following isolation using CTS Detachable CD3/CD28 beads of >98% was achieved with

samples from four donors. This consistent performance is necessary when starting material is characterized by great biological variability, as shown in Figure 4A. Apheresis profiles differ from one individual to another and fluctuate further based on the patient's indication and stage of disease. CTS Detachable Dynabeads CD3/CD28 magnetic beads can help overcome that challenge by consistently delivering high T cell purity post-isolation.

CASE STUDY: ADDRESSING THE CURRENT CHALLENGES IN CELL THERAPY MANUFACTURING USING A SHORTENED LENTIVIRAL-BASED CAR-T CELL WORKFLOW

The high cost of commercially available CAR-T cell products creates a major access barrier and limits its broad application for

FIGURE 5

Workflow for a 24-hour CAR-T process.

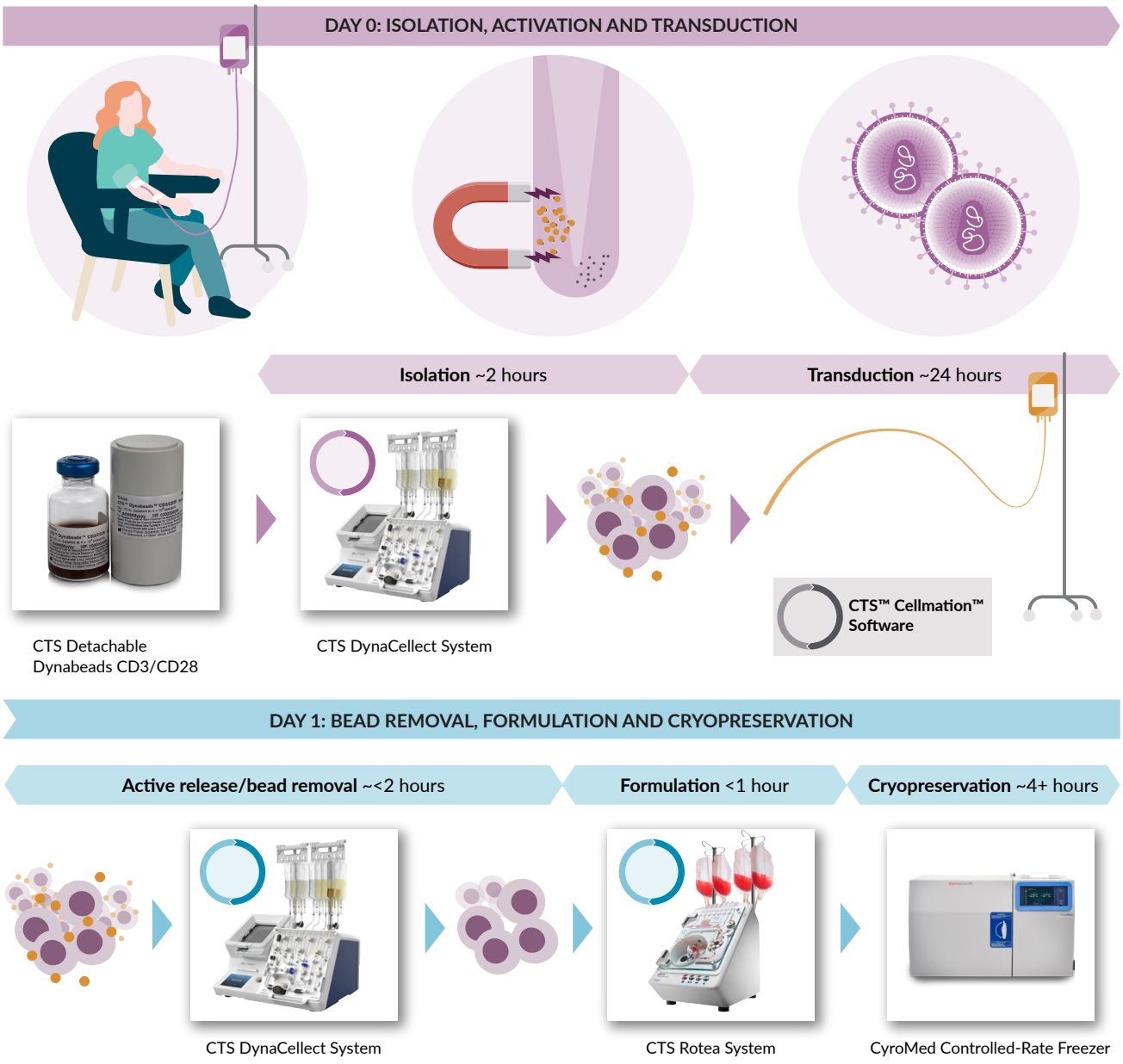


FIGURE 6

LV-CAR expression and total anti-CD19 CAR positive T cells.

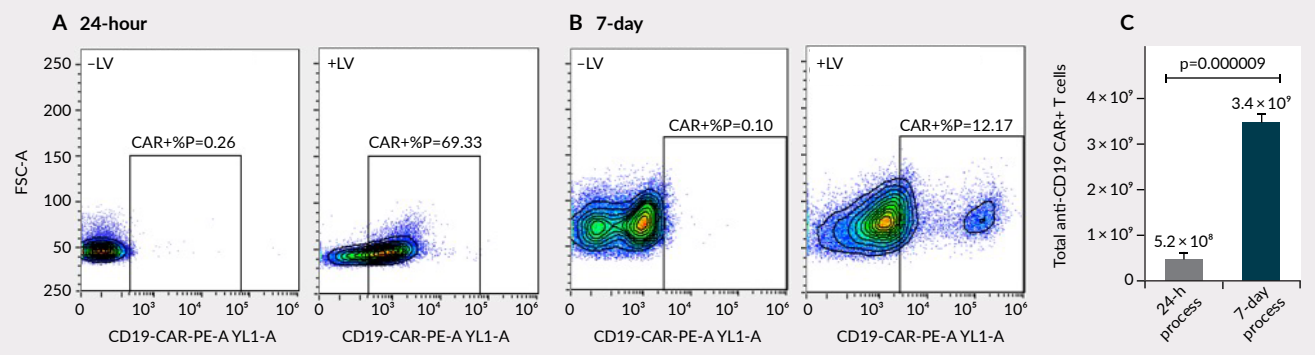
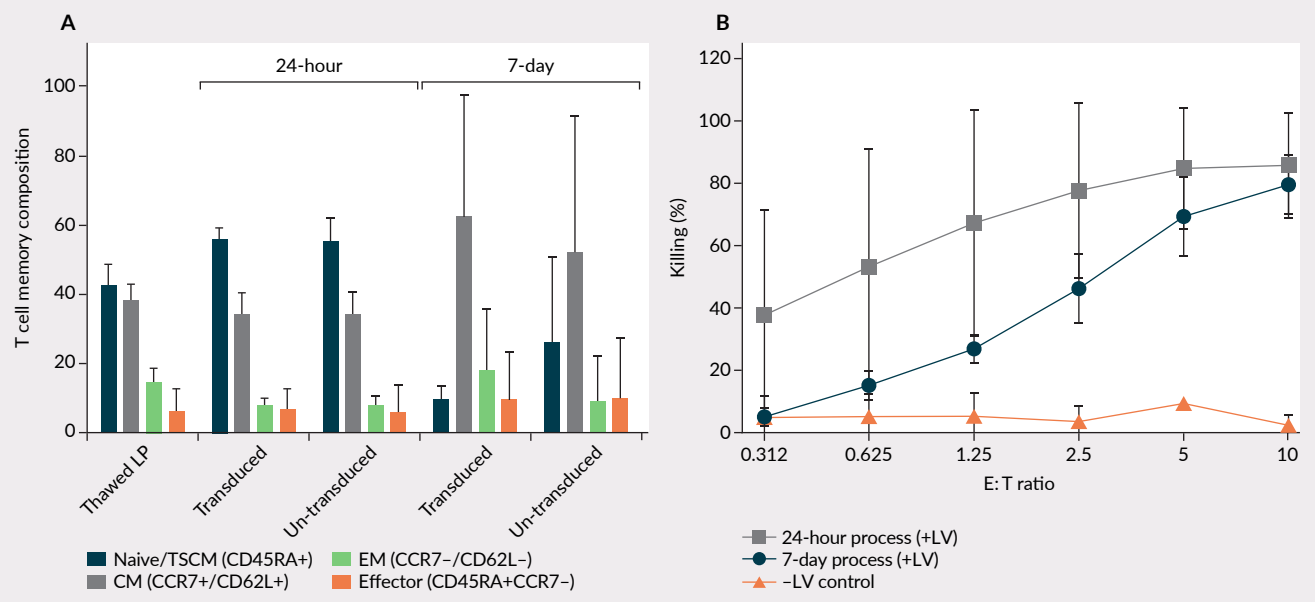


FIGURE 7

CAR-T cell memory phenotypes and cytotoxic activity.



patients who could benefit from these modalities. Recent reports indicate that a significant portion (20%–30%) of patients with B-cell acute lymphoblastic leukemia (B-ALL) who were scheduled to receive CAR-T cell therapy did not end up receiving it due to either rapid disease progression or manufacturing failure [1,2]. Therefore, speeding up the time between apheresis and CAR-T cell therapy infusion is crucial for patients with relapsed refractory B-ALL, as well as other fast-progressing cancers. Additionally, streamlining and automating these processes can help reduce the risk of treatment delays.

Furthermore, traditional CAR-T cell manufacturing often involves a cell expansion step, which can lead to a more differentiated CAR-T cell profile. These highly differentiated cells may exhibit lower cytotoxic activity and cytokine activity compared to less differentiated T cell phenotypes, such as naïve central memory cells or stem cell-like memory T cells.

Shortening these manufacturing workflows not only results in a cost-effective process that helps provide patients with quicker access to CAR-T cell therapies, but importantly, it could result in a product with higher potency [1,2].

Recently, CTS Detachable Dynabeads CD3/CD28 beads were used with the CTS DynaCellect system for one-step isolation and activation of T cells in a 24-hour lentiviral (LV)-based CAR-T workflow. The goal of this study was to eliminate the expansion step in regular autologous CAR-T workflows and shorten the process from a standard 7- to 14-day process to a 24-hour process. Figure 5 highlights a general overview of this workflow.

Under the control of CTS Cellmation software, T cells were isolated with the CTS Detachable Dynabeads CD3/CD28 and the CTS DynaCellect system from quarter Leukopaks. Post-isolation, cells were transduced with a lentiviral vector encoding a CD19-targeted CAR gene with a multiplicity of infection (MOI) of 2. 24 hours later, the CAR-T cells were separated from the CTS Detachable Dynabeads using the CTS DynaCellect system and the CTS Detachable Dynabeads Release Buffer. These CAR-T cells were then washed and concentrated using the Gibco CTS Rotea™ Counterflow Centrifugation System, while the separated CTS Detachable Dynabeads CD3/CD28 beads were captured by the CTS DynaCellect system.

One portion of the washed and concentrated CAR-T cells was cryopreserved using the Thermo Scientific CryoMed™ Controlled-Rate Freezer while a second was cultured for an additional seven days for comparison.

This method resulted in CAR-expressing T cells just 24 hours after lentiviral transduction, as shown in Figure 6.

Figures 6A and 6B illustrate anti-CD19-CAR expression levels on transduced cells at 24 hours and following culture for 7 days.

While a high CAR expression 24 hours post-transduction was observed, there was a drop in CAR-expressing T cell percentage by day 7 post-transduction. This is likely due to high levels of pseudotransduction at earlier time points post-transduction. This observation is not surprising, as it is well known that membrane proteins expressed in packaging cells could be incorporated into an HIV envelope, which can then passively transfer to both activated and naïve T cell membranes.

The lower-than-expected levels of transduction efficiency on day 7 may also be a result of using an MOI of 2 for these experiments, which is on the lower end of what has been routinely used for these types of processes. The percentage of CAR-expressing T cells remained above 40% on day 7 when higher MOIs, (e.g. MOI=5) were used in small scale experiments (data not shown).

Figure 6C shows that the total number of CAR-expressing T cells increased from day 1 to day 7. Although there was a lower percentage of CAR-expressing T cells on day 7 (Figure 6B), there was still effective expansion of CAR T cells resulting in approximately 3.4 billion CAR-T cells by day 7 post-transduction.

As mentioned previously, a key factor in determining the ability of CAR-T cells to engraft following adoptive transfer is their state of differentiation. Preclinical studies show that less differentiated and naïve stem cell-like memory T cell (TSCM) populations show greater potency [1–2].

As shown in Figure 7A, a higher naïve TSCM memory phenotype (CD45RA+/CCR7+) was observed in the 24-hour CAR-T cells, while the 7-day CAR-T cells had an increased number of the more differentiated central memory phenotype (CD45RA-/CCR7+). Both CAR-T cell products had lower numbers of the more differentiated T cell phenotypes-effector memory cells and effector T cells.

The CAR-T cell potency for each process was measured by exposing the two different CAR-T cell products to CD19-expressing NALM6 cells. 24-hour CAR-T cells exhibited higher cytotoxicity, especially at the lower effector-to-target (E:T) ratios (Figure 7B).

Overall, this data suggests that the 24-hour process results in a highly functional CAR-T product with an enrichment of early T memory phenotype cells.

SUMMARY AND FUTURE DIRECTIONS

In this study, a simplified and automated 24-hour LV-based CAR-T cell manufacturing workflow produced CAR-T cells with improved cytotoxicity compared to a 7-day process.

Future efforts will be made to expand optimization for these shorter workflows. It is also key that the potency of such products is tested with animal models. There are important ongoing efforts to implement proper assays to better characterize cell therapy products generated through shorter workflows such as the 24-hour LV-based workflow. For example, one concern regarding these shorter LV-based workflows is the pseudotransduction that appears to happen at earlier time points post-transduction.

Besides using flow cytometry to check the CAR expression on the cell surface, a more direct approach must be implemented to allow more input regarding CAR integration at earlier time points. This can help the users of such workflows to better determine the dosage of the final drug product.

Q&A

MINA AHMADI AND FABIO FACHIN

Q Have you observed any difference in CD4 versus CD8 transduction efficiency in the shorter process?

MA: We did not see any differences between the transduction efficiency in our CD4 and CD8 populations. The transduction efficiency was similar in both early time points as well as later time points.

Q Does this shorter CAR-T workflow fit a centralized or a decentralized model?

FF: Both of these models can and will coexist. This is an area that will generate a lot of excitement in terms of the future and some of these new approaches. When it comes to start-ups, biotechs, and academic centers, I do feel that the decentralized approach represents a novel manufacturing space where these players can now demonstrate their drugs.

That approach was not available at the beginning of the industry. The ability to be able to manufacture close to the clinical centers and be able to leverage this broader network, especially with some of these 'GMP on demand' and movable GMP pods that are starting to become available, is quite exciting. It also fits nicely into this shorter manufacturing span where there is more turnaround of the plots.

At the same time though, as I said, these models will have to coexist. It is naïve to think that manufacturing infrastructure will be available everywhere. That balance will need to play out, especially as products move towards the commercial side.

That is perhaps where the more centralized approach can be leveraged. There are benefits there in terms of economies of scale, which is something to consider. I am very curious to see how it pans out.

So, to answer the question: I do believe that this rapid process does lend itself more to a decentralized approach than the more traditional CAR-T cell therapy manufacturing processes.

Q What are the pros and cons of all-in-one versus modular automation?

FF: It depends on the maturity of your process and product. Simplistically, the more you move towards, for example, Phase II clinical trials or commercialization, the more your process and product become concrete.

In that regard, there are many benefits of an all-in-one solution because you have a locked-in process to pursue. The downside of having an all-in-one device is that you are allocating that instrument to your lot for the entire duration of the manufacturing. That is where I see, again, the rapid manufacturing approaches being a little more amenable to the solution.

They provide a shorter timeframe, so you are not utilizing an instrument for 12 days, but rather you are dedicating only 3 days or so to that instrument. However, there are also the downsides of being locked in to one partner as opposed to having the ability to derisk your supply chain. To rectify this, there are solutions in progress with a little bit more flexibility. On the other side, the modular approach is more dynamic. It gives you the ability to mix and match, to some extent.

New instrumentation is coming all the time, so another downside to an all-in-one approach is that if a great technology comes out, you would need to essentially get out of your all-in-one and do a process in this other modular component and then go back, which could introduce error. The modular approach is easier to use, especially earlier in your development, but this approach also has more potential failure points. There are different connections, different disposables, and different data systems that need to be considered. There are also more vendors that you need to manage and support.

The balance between both approaches will continue to evolve, and it is important that each continues to be implemented and pursued.

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BIOGRAPHIES

MINA AHMADI is a R&D lead scientist at Thermo Fisher Scientific where she focuses on developing end-to-end closed and automated viral and non-viral cell therapy workflows. Ahmadi received her PhD in Molecular Genetics from McGill University, Canada. After her PhD, she joined as Postdoctoral Fellow at Department of Pharmacy, University of Toronto, Canada.

FABIO FACHIN is the Head of Cell Therapy Process Development, Automation and Digital Technologies at Takeda, a company focusing on off-the-shelf, allogeneic cell therapies targeting both solid tumors and hematological malignancies. Fachin holds a PhD in Aerospace Engineering from the Massachusetts Institute of Technology, and a Postdoctoral Fellowship from Harvard Medical School.

AFFILIATION

Mina Ahmadi PhD
R&D Lead Scientist,
Cell and Gene Therapy,
Thermo Fisher Scientific

Fabio Fachin PhD
Head of Cell Therapy Process Development,
Automation and Digital Technologies,
Takeda

AUTHORSHIP & CONFLICT OF INTEREST

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T cell culture process development in stirred-tank bioreactors

Stefan Schlößer, Scientific Communications Manager, Eppendorf SE
Experiments conducted by **Silvia Tejerina Vargas**, **Vincent Dufey**, **Aurélie Tacheny**, and **Françoise de Longueville**

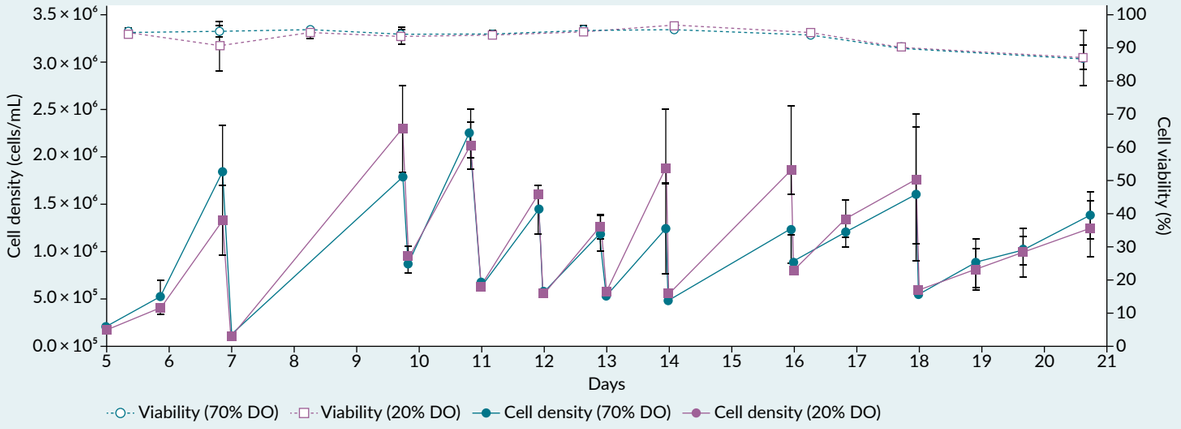
The development of T cell-based therapeutic products requires the production of a large quantity of high-quality viable T cells in a controlled environment. Stirred-tank bioreactors can offer a suitable environment for the culture of T cells by providing a homogeneous distribution of nutrients and gases, along with the maintenance of cells and molecules in suspension with a high process control capability.

ADDRESSING THE CHALLENGES OF T CELL THERAPIES: STIRRED-TANK BIOREACTORS

CAR-T and TCR-T cell therapies are promising strategies for cancer treatment. In CAR-T therapy, a CAR (chimeric antigen receptor) is introduced into T cells, for example, with a viral vector, enhancing their ability to recognize cancer antigens. TCR-T therapy involves replacing the T cell's natural receptor with one designed to target specific cancer antigens. Despite their potential, these therapies face challenges in optimizing T cell engineering to mitigate adverse effects, developing off-the-shelf allogenic treatments, and improving culture conditions to increase cell yield. Stirred-tank bioreactors offer a solution for research and development, since they are readily available in various scales, and provide a controllable and reproducible 3D environment for the cell of choice.

In a recent experiment, $CD4^+$ T cells were cultured in a BioBLU® 0.3c Single-Use Bioreactor where consistent culture conditions were maintained by the DASbox® Mini Bioreactor System. The culture involved seeding T cells in a medium with activating factors and expanding for 5 days in flasks. This was followed by cell transfer to the BioBLU Single-Use Bioreactor for a 16-day expansion period with two additional activation steps on days 7 and 14. Cells were mixed by a pitched-blade impeller and the cell density adjusted to maintain a balanced cell culture, while the level of nutrients and growth-inhitory by-products (such as lactate) was monitored. T cells were grown at 20% and 70% dissolved oxygen (DO), corresponding to atmospheric oxygen levels of about 4% and 15%, respectively.

Figure 1. Cell viability is depicted by the dotted lines, and cell density is depicted by the solid lines, showing the proliferation and viability of a 16-day bioreactor culture. DO: dissolved oxygen.

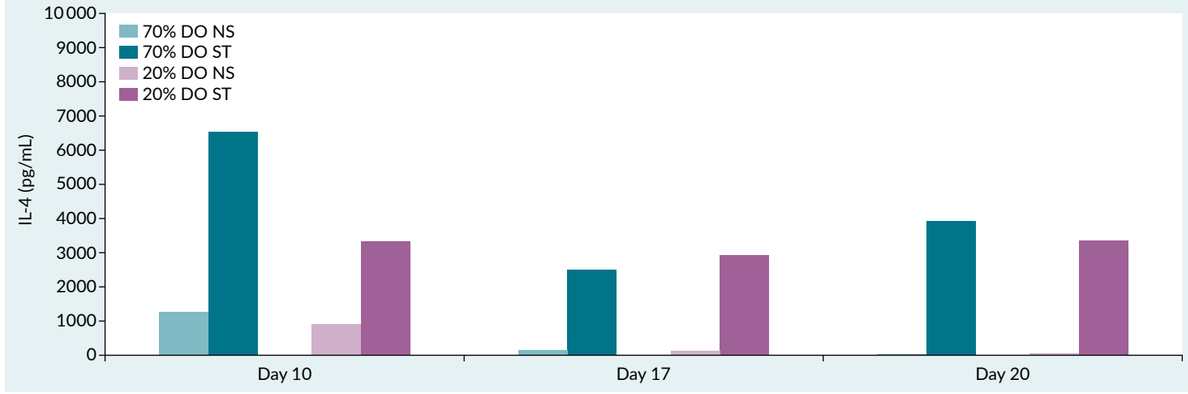


T CELL BIOREACTOR CULTURE: PRECISE CONTROL OF CULTURE CONDITIONS

Cell proliferation and viability were assessed under both DO conditions, as seen in **Figure 1**. Viability was comparable for both DO conditions, ranging from 97 to 87% across the last days of culture. Following each cell density adjustment, rapid increases in cell numbers demonstrated efficient proliferation within the bioreactor.

Glucose and lactate levels were monitored throughout the culture period. Since the accumulation of lactate has the potential to inhibit cell growth, regular dilution adjustments were made to control lactate concentration. This also ensured glucose levels stayed within a narrow range, avoiding fluctuations that could negatively impact cellular metabolism.

Figure 2. IL-4 production in T cell bioreactor culture, detected by ELISA. IL-4 production is increased in stimulated (ST) but not non-stimulated (NS) T cell samples from indicated timepoints (days 10, 17, and 20) at 70 and 20% dissolved oxygen (DO).



After bioreactor culture, T cell quality was evaluated by their ability to produce IL-4. An ELISA analysis of cell supernatant on days 10, 17, and 20 revealed that stimulated T cells produced higher levels of the cytokine IL-4 compared to non-stimulated cells, see **Figure 2**. This indicated functional T cells post-bioreactor culture.

SUMMARY

The integration of stirred-tank bioreactors into T cell culturing enables precise control of culture conditions, laying the foundation for process development to advance the field of cell and gene therapies.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

Enabling affordable access to CAR-T cell and other cellular gene therapy products

Rimas Orentas, Ying Xiong, Ibe Oparaocha, Oxana Slessareva, Megan Forrest, Yangping Xie, Kara Anlauf, Beatrix Ferencz, Matt Addington-Hall, Yang Liu, Russ Lea, Zhonghu Zhu, and Boro Dropulić
Caring Cross

"...global access to CGT like CAR-T cell and stem cell-based gene therapies is a growing problem..."

VIEWPOINT

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Cellular gene therapy (CGT) products such as CAR-T cells (which are priced at multiple hundreds of thousands of dollars in the USA) and more recently, gene-modified stem cells for the treatment of sickle cell disease (exceeds $2 million) and metachromatic leukodystrophy (exceeds $4 million) have proven once more that the approval of new biotherapeutics comes with high costs. Such high costs for advanced technologies represent

a direct reversal of Moore's law—a phenomenon termed Eroom's (reverse of Moore's) law, which was first described by Scanell *et al.* in 2012 [1,2]. In contrast, the cost of breakthrough therapies for hepatitis C has shown a decline over time, demonstrating that for small molecules, an evolving regulatory framework and a competitive market can benefit patients [3]. Equity of availability and access to treatments utilizing CGT products face additional hurdles beyond the high-cost barrier, such as documented racial and geographic disparities within the USA and abroad. Rather than focusing on reducing cost, many in the industry focus upon justifying the high price using pricing models such as value-based pricing to levels they think the market can bear. However, many insurance providers are limiting access to these therapies, with even some government insurers presently not willing to pay the high prices. In the future, most insurance providers, including government-based national health systems, will not be able to afford these therapies for the majority of their constituencies, particularly when they become mainstream and treat tens of thousands of patients. Leaders of the field are acutely aware of the imminent access crisis for an expanding range of therapeutically efficacious CGT products due to their high price. In a recent Economist Impact meeting in Brussels (April 2024), the Director of CBER at the US Food and Drug Administration (FDA), Dr Peter Marks, stated that the cost of cell and gene therapies needs to be reduced by a magnitude to ensure their sustainability.

The ability to drive down the price of CGT products rests upon challenging the current industry model, which features centrally manufactured CGT products. Centralized manufacture of CGT products utilizes a complex logistical system between the hospital and the central manufacturing facility, which is extremely expensive to establish and maintain. Furthermore, since each company wants to own and maintain its own manufacturing facility, this investment must be recovered in the price of the product, dramatically increasing its cost. One solution is the manufacture of CGT products at the point of care. Academic Medical Centers (AMC) that are presently manufacturing hematopoietic stem cell transplantation products could, with a modest investment, manufacture chimeric antigen receptor (CAR)-T cells by leveraging the manufacturing and clinical infrastructure already resident at their facilities. AMC have been manufacturing transplantation products for decades, even FDA approved products (e.g., cord blood). AMC have the knowledge and infrastructure to manufacture CGT products safely and cost-effectively. For example, an AMC network in Spain manufactures an anti-CD19 CAR-T cell product for approximately €89,000 (US$95,000) [4], demonstrating that these products can be manufactured in a hospital environment for a price that is fully reimbursable under a national insurance system. Such pricing would be affordable and sustainable in the USA, especially since a product price of less than $100,000 would leave sufficient funds for the cost of clinical care and would be fully reimbursable at the $257,958 CAR-T cell therapy product limit set by Medicare and Medicaid in 2024. In a low- and middle-income country such as India, an AMC manufactured an anti-CD19 CAR-T cell product for approximately $35,000, leveraging a highly skilled but less expensive technical labor force to reach a more affordable cost in that country [5]. Therefore, AMC have the potential to significantly reduce the cost and improve access of cellular gene therapies for patients located in their region, which is of particular importance for patients in low- and middle-income countries, where current access is either extremely limited or does not exist.

As with all manufacturing, simplifying the cell manufacturing process improves the economics, and there are many unit operations that can be improved and optimized. For example, omitting the cumbersome and expensive bead selection step for the isolation of cells used to manufacture the final product

is one approach that substantially simplifies the process and reduces cost. But omitting this step without T cell purification leads to a product with variable growth potential, due to the inhibitory effects of non-T cells in the starting material. One solution is negative depletion of unwanted cell types from the peripheral blood or apheresis starting material using an antibody cocktail through a well-established, Ficoll-based density gradient centrifugation process [6]. This process results in a highly purified T cell product that is now optimal for transduction with vector and robust CAR-T cell expansion to the target cell dose. In addition, utilizing cost-effective bioreactors such as the G-Rex for cell expansion considerably improves the cost of goods over more complex bioreactors used in automated systems. Automation should only be used when it significantly lowers the cost of the production process while maintaining the safety and efficacy of the product. Otherwise, low-cost manual methods of production are more suitable, particularly in low- and middle-income countries where skilled technical labor is less expensive, and where the need to absolutely drive down cost as much as possible is critically important for patient access.

Another key aspect to creating a highly active CAR-T cell product while lowering the overall cost is to minimize the vein-to-vein time. This is achieved by minimizing the time from obtaining the initial blood product starting material until the time the final CAR-T cell product is produced and ready for infusion back to the patient. While single-day manufacturing of CAR-T cells has been demonstrated [7], the product still must be frozen prior to infusion, adding both process steps and material costs, as opposed to a 'fresh-in fresh-out' approach, where the starting blood product can be obtained, the product manufactured over 7 days, and then non-frozen material returned to the patient. A fresh product with point-of-care manufacturing saves cost by removing or limiting shipping, freezing/storage, and associated quality assurance costs. The use of a fresh product necessitates real-time testing for its release, which can be challenging. However, most of these assays such as digital PCR and flow cytometry can be done in a short period of time. Potency testing is a challenge, but surrogate markers, such as evidence for antigen-mediated cytokine production, are particularly useful for rapid release of a fresh CAR-T cell product. A number of assays, such as long-term sterility, are sent out for testing after infusion, as the timing does not comport with a fresh-in fresh-out manufacturing and treatment protocol. Moreover, to date, all CAR-T therapies are accompanied by lymphodepleting chemotherapy (cyclophosphamide and fludarabine as standard). It takes several days post-administration for these drugs to be washed out of the system. Thus, decreasing manufacturing times to under a week would not decrease the vein-to-vein time.

It is widely accepted that lentiviral vector (LV) manufacturing is a key cost driver and bottleneck for the development and commercialization of cellular gene therapy products. Improving the efficiency and scalability of the manufacturing process is critical for timely access to affordable, high-quality GMP LV for clinical development. For example, Vector BioMed, a public benefit (B) corporation, was formed to provide the industry with rapid access to affordable, custom GMP LV manufacturing services, where the higher LV manufacturing efficiency results in pricing that is about half of that of competing CDMOs. The highly efficient, serum-free, chemically defined, suspension HEK293 cell-based LV manufacturing process produces high harvest titers and entails a downstream process that needs less concentration. It is therefore less prone to particle aggregation, resulting in higher yields of LV in the final sterile-filled product.

AMC have been at the forefront of CGT research and development. This has resulted in novel product candidates being evaluated in clinical trials, resulting in improved patient outcomes. It is important that these innovations reach patients in a timely manner, with pathways created to accelerate their safety

and efficacy validation in the clinic prior to subsequent regulatory approval. This acceleration is particularly true for AMC, which can drive research and development iteratively with high efficiency and are not affected by factors such as high profitability and market share. Further investment is needed in such non-profit structures so that patients have access to the best possible therapies as soon as they are validated for safety and efficacy in the clinic. For example, Caring Cross, a 501 (c)(3) non-profit, is developing a pipeline of clinical candidates for its AMC partners for clinical development, regulatory approval, and market authorization in multiple countries around the world. One such candidate is a novel TriCAR for the treatment of leukemia and lymphoma that addresses CD19-negative relapse in patients receiving a single anti-CD19 CAR-T cell therapy. By targeting three surface antigens on the malignant B cells simultaneously (CD19, CD20, and CD22), tumor escape and relapse due to loss of any single antigen becomes unlikely, and could result in significantly improved long-term outcomes for patients. Other candidates in the pipeline include an anti-HIV CAR-T cell therapy that is presently in clinical trials, and a globin-expressing stem cell gene therapy for the treatment of beta thalassemia and sickle cell disease.

One of the next steps in decreasing cost of and increasing access to CAR-T cell therapies is to develop the use of wearable sensor technologies for patient monitoring. The main concern for patient safety is cytokine release syndrome (CRS) and the more serious immune effector cell-associated neurotoxicity syndrome (ICANS) [8]. The hallmark of these syndromes is a rise in serum cytokine levels for IL-6, IFN-γ, IL-1, IL-18, and accompanying rise in c-reactive protein (CRP), among others. The initial physiological readout is fever, and changes in fever and cardiac performance can rapidly and sensitively be reported using wearable devices. These devices are currently being piloted in early-stage clinical trials at the NIH and elsewhere (personal communication, Dr James Gulley).

In conclusion, global access to CGT like CAR-T cell and stem cell-based gene therapies is a growing problem, and particularly acute for underserved patient populations and those in low- and middle-income countries. Access can be improved by lowering their price, which is driven by reducing the cost of the materials and unit operations used to manufacture these products. It is also driven by the logistical systems utilized to deliver these personalized therapies, where point-of-care manufacturing will be important to alleviate unnecessary costs and decrease the vein-to-vein delivery time. AMC have been at the forefront of the development of these therapies and can play an increasing important role in enabling access by creating a CGT manufacturing network to support rapid clinical development, manufacturing, and product provision to patients, much as bone marrow transplantation is done today. An AMC network with the support of similarly aligned organizations can play a vital role in an accelerated cycle of innovation, clinical development, point-of-care manufacturing, regulatory approval, product provision, and equitable reimbursement at a cost that is affordable and sustainable for insurance and health systems around the world.

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AFFILIATIONS

All authors are affiliated with Caring Cross, Gaithersburg, Maryland, USA

Rimas Orentas

Ying Xiong

Ibe Oparaocha

Oxana Slessareva

Megan Forrest

Yangping Xie

Kara Anlauf

Beatrix Ferencz

Matt Addington-Hall

Yang Liu

Russ Lea

Zhonghu Zhu

Boro Dropulić

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

INTERVIEW

Planning for success with an allogeneic Treg cell sourcing strategy

Autologous Treg cell therapy products have shown promise in early clinical studies, but obstacles related to scalability and adoption have hindered their commercial viability. **David McCall**, Senior Editor, BioInsights, speaks with **Matthew Breton**, Director, Process Development and Manufacturing, Tr1X, about overcoming these challenges by following the allogeneic pathway.

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Q What are you working on right now?

MB: Currently, I am the Head of Process Development and Manufacturing for Tr1X, a clinical-stage cellular therapy company. We are developing a suite of novel allogeneic off-the-shelf Treg and CAR-Treg cell therapies that target various autoimmune and inflammatory diseases.

"Presently, the industry is focusing on developing therapies from multiple lineages within the Treg cell population including FOXP3s, CD8s and Tr1s."

I have more than 11 years of experience in the cell and gene therapy space, primarily in allogeneic cell therapy technical operations, spanning process development, CMC, GMP manufacturing, and manufacturing science and technology.

Q Can you give us more details of the Tr1X platform/approach and pipeline—what differentiates them?

MB: Tr1X's technology emerged from the work of our scientific founder, Dr Maria Grazia Roncarolo. Initially, she identified a subset of regulatory T cells (Tregs) that she termed type 1 regulatory cell, or Tr1. This discovery occurred during her investigation into the role of these cells in immune system balance and tolerance, particularly in understanding why certain early transplanted severe combined immunodeficiency (SCID) patients exhibited full tolerance despite having two active immune systems.

Presently, the industry is focusing on developing therapies from multiple lineages within the Treg cell population including FOXP3s, CD8s and Tr1s. While autologous Tr1 cell products have shown promise in early clinical studies sponsored by both industry and academia, challenges related to scalability and adoption have hindered their commercial viability.

Tr1X aims to overcome these limitations by employing cellular engineering to produce a range of fully allogeneic Tr1-based cellular therapies. This approach represents a departure from autologous Tr1 therapy and offers multiple potential solutions for autoimmune disorders, with the goal of creating a diverse portfolio of products. By essentially mimicking the fundamental functions and properties of Tr1 cells through genetic engineering, Tr1X has achieved considerable success. While other companies predominantly focus on autologous FOXP3 applications, Tr1X stands out as one of the few companies focused on developing Tr1-based therapies in a fully allogeneic manner.

In terms of manufacturing, Tr1X's processes allow for the production of multiple doses per manufacturing batch, contrasting with autologous methods that yield one custom-specific dose per patient. Allogeneic therapies mitigate variability inherent in autologous approaches, where patient-derived cells are subject to individual variability and potential impacts from underlying diseases.

The allogeneic approach employed by Tr1X enhances manufacturing effectiveness and reliability, resulting in consistent and reliable product outputs. This eliminates treatment delays on the backend, as ready-to-use products are available for immediate administration upon diagnosis, bypassing the need for individualized manufacturing processes.

Q What are the considerations around cell sourcing for Tr1X Bio's product candidates?

MB: Tr1X, like other allogeneic companies, relies on healthy donors for starting materials. Thus, we must maintain a robust network of reliable vendors who can provide material meeting our predefined specifications and quality standards, which is crucial for us. As a result, rigorous screening and testing are essential for donors before their material can be used in our manufacturing processes. We meticulously screen for communicable diseases and adventitious viral pathogens to ensure the safety and quality of our materials.

Our focus is on creating a substantial bank of healthy donor cells. These cells undergo thorough verification and pre-qualification to align with our quality standards. We select the best donor material to input into our manufacturing processes, ensuring the highest quality outcomes.

Q Are there any specific considerations for Treg cells as the cell target of choice versus other immune cells?

MB: Currently, cell therapy guidance is somewhat ambiguous. The FDA often issues general statements, but specific application requirements can vary. This variability is also true for Tr1X, as additional FDA requirements depend on factors like the physiological context of the cells and other variables.

In our case, we had to meet some additional FDA requirements based on their feedback. However, our focus remains on screening for adventitious viral agents and additional cell characteristics that we've identified as optimal to ensure donors will respond favorably and reliably in our manufacturing processes.

Q What for you are the key current challenges in enabling the sourcing of allogeneic donor-derived cellular starting material, and can you expand on Tr1X Bio's approach to addressing them?

MB: There are four main challenges. Firstly, there is a limited availability of qualified vendors capable of producing material that meets the standards for GMP manufacturing. Finding and qualifying these vendors poses a significant challenge. Secondly, there is a scarcity of healthy donor pools—particularly as requirements become more stringent, narrowing down the available sources of material. Thirdly, the complex logistics involved in cell sourcing, from donor screening to procurement and transfer to manufacturing sites, present a formidable challenge. Each step requires careful coordination and execution. Additionally, there are extensive

testing requirements at every stage, adding complexity to the workflow and necessitating attention to detail. Lastly, as I mentioned, regulatory guidance is moving quickly as the field evolves, making it difficult to anticipate FDA expectations until engaging directly with them. This uncertainty can lead to delays and challenges in advancing strategies.

To address these challenges, Tr1X has developed a comprehensive strategy approved by the FDA through multiple rounds of feedback sessions. Our recent announcement of our first IND approval underscores our commitment to tackling these issues effectively. While specifics cannot be divulged, our approach addresses these challenges in a multifaceted manner to ensure success.

Q How do you approach the optimization of this aspect of the upstream supply chain with both quality and logistics in mind?

MB: When initially developing a donor screening strategy, it is imperative to thoroughly examine and identify the biological characteristics relevant to your process and product. This upfront investment in research is key to predicting both manufacturing and quality outcomes accurately, and informs the strategy employed by logistics and procurement teams. By gathering sufficient data, you can confidently screen donors from a wide pool, eliminating those unlikely to meet your manufacturing targets. Such groundwork becomes essential as you progress to late-stage development and commercialization phases, particularly for deployment in GMP settings or at scale. An iterative approach is necessary to refine the process over time, preparing it for transition to procurement teams. This approach holds particular importance in allo-derived cellular therapy, where consistency and reproducibility are paramount. Achieving these requires consistent inputs to generate expected outputs consistently.

Overall, starting early is crucial as it ensures readiness for launch and competitiveness in the allogeneic space. It is no longer a luxury but a necessity for success of an advanced therapy product. I cannot stress enough—initiating and executing your strategy early on is vital to the long-term success of your program

Q As you look ahead to clinical development and the marketplace beyond, how are you preparing to meet barriers in scale-up?

MB: Scaling is a critical consideration that necessitates a long-term vision. While we are starting small, both in terms of donor screening requirements and throughput, scaling is inevitable. Building a team of dedicated specialists to support the program's growth across various logistical aspects is essential. This preparation ideally begins in the preclinical phase or even earlier, a step often overlooked by companies. Investing in the right expertise and skill sets is crucial, yet challenging, given the novelty and complexity of the task.

"We must ensure that any potential negative consequences of donor material are outweighed by the therapeutic benefits intended."

Companies will need to devise innovative business and scientific solutions to navigate this uncharted territory effectively. Communication, collaboration, and strategic thinking will be pivotal in determining the most effective approaches to scaling, much like the ongoing efforts in manufacturing process scalability.

Q How about meeting barriers regarding regulatory divergence across key regulatory jurisdictions—for instance, around donor eligibility?

MB: From Tr1X's perspective, we recognize the evolving nature of regulatory thinking regarding donor selection and criteria, particularly as more clinical experience is gained. It is crucial for companies in the allogeneic space to grasp and adapt to these changes in real time. To stay ahead, we closely monitor regulatory communications and evolving perspectives, acknowledging the nascent nature of this field of study.

Our strategy revolves around understanding regulatory expectations and taking a proactive approach to meet them. Regardless of the regulatory body, scientific and clinical justification of our strategy is paramount. Safety is of utmost importance, with a focus on conducting risk–benefit assessments, especially in autoimmune applications. We must ensure that any potential negative consequences of donor material are outweighed by the therapeutic benefits intended. Conducting thorough risk profile analyses and considering the physiological reactions of cells in clinical settings are essential aspects. Additionally, developing appropriate CMC strategies is vital to navigate the regulatory approval process effectively.

Q How do you view the potential of induced pluripotent stem cells (iPSCs) as a viable alternative Treg cell source?

MB: From a donor sourcing and criteria standpoint, iPSCs are subject to a lot of the same things that we are in the engineered Treg space. Since we rely on healthy donors contributing a subset of their cells, ensuring these cells are free from safety concerns remains paramount. iPSCs offer a unique advantage in that a master cell bank or an identified optimal cell bank can yield a substantial amount of product, requiring only a few cycles once characterized and established. However, differentiation into commercial scale, highly functional Tregs remains an obstacle for iPSC-reliant platforms.

For companies like us not utilizing iPSCs, the process may involve more extensive and routine banking and cycling through donors more frequently to maintain a continuous supply. While I have not worked directly with iPSCs, it is plausible that the main trade-off lies in the ability to establish a small number of banks providing the majority of the product versus the need for ongoing cycling through donors to sustain manufacturing and supply continuity.

Q Lastly, what are some key goals and priorities, both for yourself in your own role and for Tr1X Bio as a whole, over the foreseeable future?

MB: Personally, as someone deeply involved in process development and operations, I recognize the challenges facing allogeneic cell therapy, especially in comparison to autologous approaches. My focus lies in devising innovative processes aimed at scaling production to deliver hundreds, and eventually thousands, of high-quality cell doses from a single healthy donor. Achieving this goal could significantly reduce patient costs and improve access, which are crucial tenets of the allogeneic approach.

For Tr1X, one of our top initiatives, which I'm spearheading, is the development of a robust cell sourcing program, crucial for competing effectively in the allogeneic space. This strategic element is highly sought after by both the market and investors, and we are dedicated to establishing leadership in this area. Based on our work and the progress we have made to date; we are pushing that agenda forward at a record pace. This is a testament to the team of best-in-class scientists and engineers we have assembled in La Jolla to help realize our vision of universally available, novel off the shelf cellular therapies to cure disease.

With our collective focus on autoimmune science and advancement, we are committed to delivering much-needed products to patients. I am honored to be part of Tr1X and eagerly anticipate the future of allogeneic cell therapy, as well as the broader cell and gene therapy and biotech landscapes.

BIOGRAPHY

MATTHEW BRETON has over 11 years of experience in cell and gene therapy technical operations, spanning process development, CMC, GMP manufacturing, and MS&T. He has helped companies develop a broad array of cell types for therapeutic purposes—including MSCs, HSCs, CPCs, CAR-T, and Tregs–or both 'patient-specific' and 'off-the-shelf' applications with varying levels of cell engineering *ex vivo* (e.g., single gene, multiplexed gene editing), utilizing both viral and non-viral modes for gene delivery, to elicit the intended therapeutic effect. Currently, he is the Director of Process Development and Manufacturing at Tr1X, San Diego, CA, USA—a clinical-stage company developing novel 'off-the-shelf' T-reg based cellular therapies for the treatment of autoimmune and inflammatory disease. At Tr1X, he led process development and manufacturing functions to take the first allo, polyclonal, Tr1 cellular therapy from concept to IND approval in a short 18-month period. Prior to that, he held positions of increasing responsibility at Poseida Therapeutics, SanBio and Sangamo

Therapeutics where he worked on CMC development and strategy initiatives to support numerous IND filings, information amendments, and licensing applications to enable the advancement of innovative, engineered cell therapy products into clinical development, FIH clinical trials, late-stage studies, and beyond. Mr Breton holds a BS in Biology from University of Maryland College Park, College Park, MD, USA and master's degrees from both the Johns Hopkins University, Baltimore, MD, USA and the University of Illinois, Champaign, IL, USA.

AFFILIATION

Matthew Breton
Director,
Process Development and Manufacturing,
Tr1X,
San Diego, CA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

INTERVIEW

Weighing up the pros and cons of adopting a piecemeal versus a full automation approach to cell therapy manufacture

While the menu of automated cell therapy processing solutions continues to grow apace, the difficulty in deciding precisely when and what to automate remains frustratingly consistent. **David McCall**, Senior Editor, BioInsights, speaks to **Raymond Luke**, Head of CMC, Director of Manufacturing Science and Technology at Verismo Therapeutics, about the critical considerations for early-stage biotechs in shaping an automation strategy that will stand the test of time.

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Q What are you working on right now?

RL: Verismo Therapeutics is a cutting-edge, next-generation CAR-T cell therapy company. My responsibility is to make sure that the cells are manufactured and delivered to the patient in the cheapest, fastest, and easiest way possible, which involves a lot of supply chain work and a lot of making sure that our contract manufacturers do their job.

"There is a lot of processing equipment, either newly available or coming soon, that is aimed at both automating and simplifying/improving a single unit operation."

At the same time, I need to know what will be the next innovations coming through that will allow us to deliver better cells to patients more economically and efficiently. We are currently in an early-phase clinical trial for our lead product, SynKIR-110, and we have just received IND clearance for our second product, SynKIR-310. We are planning for a home run with both of these products, so despite the fact we are still in the early phases of development, we think it's important to start looking at the next stage. I am therefore looking for the solutions we will need to be able to deliver these products to as many patients as possible in the future.

Q Can you frame for us the current state-of-the-art in cell therapy upstream processing as you see it—what have been some key recent advances for you, particularly in terms of enabling reductions in manufacturing costs and timeframes?

RL: Cell therapy is a very exciting space because it is in the phase of exponential technological growth. When I started in cell therapy a decade ago, it was a very different world.

There is a lot of processing equipment, either newly available or coming soon, that is aimed at both automating and simplifying/improving a single unit operation. Then there are other tools that aim to innovate the entire end-to-end process.

One of the things that is starting to grab a lot of attention on the single unit operation side is the ability to close the fill-finish step. A lot of the fill-finish work done previously in cell therapy was open, utilizing syringes and biosafety cabinets—an approach that is both dangerous from a contamination perspective and highly manual, which drives the need for extensive training. Several of the emerging closed-fill finish devices alleviate this burden whilst also removing much of the guesswork traditionally involved in this unit operation—you simply program a machine, it does all of the fill-finish for you, and you then you just take the product off and freeze it.

Over the past couple of years, we have seen much more advanced automation arriving in the cell therapy manufacturing field. This has enabled a rapid increase in end-to-end solutions. Obviously, some end-to-end solutions have existed for quite some time—the Miltenyi Prodigy®, for instance—and these are still widely used. However, the likes of the Prodigy and the Cocoon® are now somewhat the elder statesman of the space, and new solutions are appearing all the time to fill gaps in the market. Cellares, for instance, has a very exciting full end-to-end automation solution—a closed, robotically-enabled trailer, essentially, that takes the entire process and puts it into a single space. MicrofluidX does something similar with a microfluidics device.

I am also very excited by Multiply Labs approach: rather than seeking to replace your standard, single unit operation instruments, they instead work to automate those instruments for you. If you have a specific cell washer that works well for your process, for example, they will program their robotic arm to be able to utilize that cell washer (or that cell selection device, or that bioreactor) in a closed, automated environment. I think this particular approach allows you to retain a lot of flexibility while automating—there are a lot of great innovations coming out of the instrument and technology space to support cell therapy, and if one emerges that you think could improve your product or its manufacturing process, you can simply adopt it then look to program the automation around it.

Q Where specifically is further innovation in upstream processing tools most pressingly needed?

RL: I think it is especially important for innovation to offer enhanced flexibility. As I mentioned earlier, there is a growing menu of end-to-end solutions out there, but often, a one-size-fits-all approach is difficult to utilize because of the variability between cell therapy products. For example, some products, such as TCR-T cell therapies, require a much higher dose than others. A lot of the instruments currently on the market are largely based and designed around the commercial cell therapy products that are out there. However, if there is a single area of the biologics world where a one-size-fits-all approach is least likely to work, it would have to be cell therapy, and especially, autologous cell therapy. Furthermore, we are still learning about some fundamental characteristics of these living medicines. We have all seen the research suggesting that shorter manufacturing timeframes lead to more potent cell therapy products, for instance, which is not something that you would necessarily see in other modalities such as monoclonal antibodies or small molecule drugs.

Having some flexibility allows you to 'plug and play' depending upon the necessities of both your individual product and your optimal dose. And of course, with autologous cell products, the natural variability in cellular starting material from one patient to another is another key consideration. I think flexibility is a big part of what is necessary as we look to mature the sector.

Q Is the cell therapy industry ready for full automation yet? If not, why not?

RL: I think that the cell therapy industry is ready, but I don't think it is prepared.

What I mean by that is that I think the industry wants it—as a manufacturing person who has worked in cell therapy for quite some time now, I would love to be able to push a button and a couple of days later, a machine spits out my frozen drug product. That would be

fantastic. However, I don't think the industry is prepared for this eventuality because there are several aspects in which we just aren't ready yet.

One is that we don't understand the biology of the cells well enough. Another is that when you think about full automation, it generally involves an irreversible commitment—you are effectively setting your path forward for the entire lifespan of the product in question. If you put full automation in place for your Phase 1 clinical trial, for example, you are going to be stuck with it moving forward, and you won't have the flexibility to harness the improvements that will inevitably happen in an exponentially growing cell therapy industry.

It is also difficult to invest in automation technologies early on in autologous cell therapy development because you will often be in dose escalation trials where you are treating perhaps just one or two patients a month. Putting in the sort of capital investment required for full automation technology is a big ask for start-ups that have yet to prove that their product is something that can be taken to Phase 2, let alone to the pivotal trial and commercial stages. Again, this is why enhanced flexibility is such a prerequisite.

I think that the necessary preparedness is coming as the industry matures, but there will need to be a lot of change in the way automation is currently done because, again, it is hard to do at small-scale. At the present time, I don't believe there is a genuine pilot-scale fully automated solution out there.

Full automation that can process multiple patient batches simultaneously is something that will potentially cost millions of dollars. In other words, it is not an investment that a small biotech company that is just starting its clinical trials can make. I think attention needs to be paid either to how we ensure that those early-phase product processes are ready to be automated once they get into later-phase clinical trials, or to designing an automated system that is much more easily scalable, allowing for early-phase adoption without bankrupting the cell therapy developer.

Q How do you weigh up the pros and cons of taking a piecemeal approach to automating a call therapy process and what should be some specific priorities in this regard, particularly for early-stage developers?

RL: Obviously, the piecemeal approach is the one that has been most frequently adopted so far. It's certainly one that I have done in the past. It is the easiest option to pursue, and I think it is the one that is the most appetizing for a lot of early-phase companies because you can implement it a little bit at the time without exposing your company to too much risk. However, while I fully agree that a piecemeal approach is the way to go for many companies, there are a lot of downsides to consider.

For example, compared to a full automation approach, there is potentially going to be more capital expenditure in the long-term. You are going to have to do a lot more process development. There is also still a lot of labor that will need to go into a piecemeal approach

"The best thing about automation in cell therapy isn't the scale-up and the ability to operate with additional capacity, it is the consistency..."

to automation. Taking a cell selection instrument as an example, you will need someone to manually insert the cells, to operates the equipment, and to then take the selected cells from the device and move them to the next unit operation, whether that be a bioreactor, an electroporator, etc. That labor requirement obviously leads to additional costs.

In terms of the advantages of a piecemeal approach, first and foremost, you can identify and focus on only the step or steps that most pressingly require automation. This allows you to generate the highest possible ROI on a relatively minimal capital expenditure. It allows you to take small bites, which is much better from a financing perspective.

Regarding priorities for early-stage companies, I think the most important thing that will both make your process easier and lower your cost of goods is to make sure that whatever you are doing closes the process. That for me should be the first step. It doesn't always have to involve automation, but it often will do—for example, with something like a fill-finish device. I think that closing that end of the process really helps because it means you can potentially avoid the requirement for a full clean room, or at least, that you can operate in an environment with a lower biosafety classification. This can increase your throughput as well as reduce potential contamination risks.

The next priority or consideration would be to identify those unit operations that are most likely to be impacted by operator-to-operator variability. The best thing about automation in cell therapy isn't the scale-up and the ability to operate with additional capacity, it is the consistency—the fact that the process step will be carried out in exactly the same way every single time. While training will still be important around any such unit operations, it does make it much less critical and burdensome.

Q What is Verismo Therapeutics' approach to this particular conundrum, and how does it fit with the longer-term objectives of the company and its R&D pipeline and strategy?

RL: Because we are in early-phase clinical trials, we are not investing in full automation just yet. We are focused instead on developing a process that is easily automatable.

Firstly, we want to make sure that the manual process we have is as simple and easy to perform as possible, so that we don't necessarily have to worry as much about the level of expertise or training of the operators at our contract manufacturer. Secondly, we are focused on staying abreast of the field. We are making sure that we understand the entire landscape in terms of what kinds of automation technology are available and what they can achieve, whether that be

from the piecemeal instrument-by-instrument or a full automation perspective. We make sure that we go out there and speak with everybody involved, so that as we grow our understanding of what our product needs to be, we will be able to ascertain which technological solution is the best fit for us.

That segues directly into the third element of the strategy, which is that we are making sure we capture as much data on, and understand as much as we can about, our product, our process, our supply chain, and our patients' needs. We can then turn this knowledge into a really strong automation strategy, so that we understand that our chosen automated solution needs to be able to achieve a certain dose, for example, or that it needs to deliver a certain kind of cell enrichment or cell depletion based on a particular clinical indication. We want to know both our product and the automated tools landscape well, so that when we are ready to automate, we can marry the two together to make good, informed decisions, as opposed to committing to a particular solution early on and creating the potential for that to come back to bite us in the end.

The way that this approach works in with our pipeline strategy is that we are making sure that we remain flexible, so that we can easily pivot, if needed—for instance, to a larger disease indication where automation may be more critical. Again, that is why we are adopting a piecemeal approach—so that we can remain nimble and adaptable for when we reach later phases of development, and we come to fully understand our product and target patient population, and therefore, what our optimal approach to automation should be.

Q Can you distil for us some key areas of focus and next steps towards ensuring manufacturability of cell therapies moving forward?

RL: Again, I think one of the most important aspects is that we need to understand more about the products themselves. Obviously, this will vary in degree from product to product, but I think having a strong product and process characterization in place will provide you with a strong basis to understand what your product requires in order to be safe and efficacious in a patient. Do your cells need to be highly stem-like, for instance, or do you need to have a lot of cells per dose? Do your cells need to have a high transduction efficiency?

Another important element is that, as we move forward, we need to understand what are the needs of the supply chain and the patients, and where are the corresponding bottlenecks. Then, when we understand what those bottlenecks are, how do we alleviate them so that we can reduce the vein-to-vein timeframe and simplify delivery to the patient as much as possible?

Additionally, one of the things that is often not adequately considered when automating a full process is the question of whether or not the analytics are automatable as well. Cell therapies differ from most other modalities in that the time available for testing is finite—and in many cases, severely limited. What we have seen to date in the cell therapy space is a great deal of focus on automating the processing side of things. However, we would be wrong to neglect

the analytical side. Maybe you automate your process perfectly well, with significantly reduced vein-to-vein time and enhanced reproducibility, but then you hand the final product over to a fully manual QC process on the backend—all of a sudden, you are back with long vein-to-vein times and high variability, plus you have the major costs associated with skilled QC labor to consider.

Finally, I would highlight the importance of remembering that it is not just the basic upstream and downstream processes that can be automated, but the full vein-to-vein process. That can include things like chain of identity/chain of custody software to secure the cellular starting material and final drug product. While I think it is important to primarily focus on the production process, since that is likely the single component of the vein-to-vein process that will have the greatest impact on lowering cost of goods, we would be remiss not to retain a focus on the entire lifecycle of the product. You don't want solve an issue just to have another one pop up; you want to make sure you take a holistic approach.

Q Lastly, can you sum up one or two key goals and priorities for the future, both for yourself in your own role and for Verismo Therapeutics as a whole?

RL: One of the most important things for everyone at Verismo over the foreseeable future is dosing patients—I think that is probably the key goal of every early-phase biotech company. For me personally, my first priority is to make sure the patients receive those products in as efficient and compliant a manner as possible.

The second priority for me is to continue our work in designing new processes and evaluating new process and analytical instruments and assays (including automation), so that we can be ready in the event that we experience 'catastrophic success'. If we do, we need to be able to pivot, step on the gas, and move as quickly as possible. For instance, we are currently working to identify long-term contract manufacturing partners for both our vector and our cell therapy manufacturing. At the same time, we have a lab here at Verismo to study process and analytical development in order to make sure that what we are doing is ideal for our product and for our patients.

Again, we are making sure that we capture that data as well as we can, so that we can have the possibility in future to study and leverage what happened in the past, whether it relates to the product and its characteristics as observed and measured with flow cytometry and PCR, or if it is from the perspective of how well the vein-to-vein supply chain worked. We will also work to make sure that the QC/release process and documentation go as smoothly as possible until we are ready to implement an automated solution.

BIOGRAPHY

RAYMOND LUKE is Director of Manufacturing Science and Technology at Verismo Therapeutics, Philadelphia, PA, USA and has more than 10 years of experience in CMC for biotechs. Prior to joining Verismo Therapeutics, Mr Luke spent 6 years at Adaptimmune developing TCR-T and next generation cell therapies targeting NY-ESO-1, MAGE-A4, MAGE-A10, and AFP against solid tumor indications He held leadership roles within CMC and was responsible for GMP manufacturing and technical oversight at CDMOs, technology transfer (sending and receiving) into cGMP manufacturing and technical oversight of manufacturing operations. He also led teams responsible for developing automated processing solutions, analytical development of commercial-ready assays and late-stage process characterization for afamitresgene autoleucel. Raymond worked in a variety of roles at WuXi AppTec, University of Pennsylvania, Cancer Institute of New Jersey, LifeCell, and Chromocell. Mr Luke holds a BA in Genetics from Rutgers University, The State University of New Jersey, New Brunswick, NJ, USA.

AFFILIATION

Raymond Luke
Director of Manufacturing Science and Technology,
Verismo Therapeutics,
Philadelphia, PA, USA

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

EXPERT ROUNDTABLE

iPSC-based therapy dilemmas explored: overcoming hurdles for future success

Melissa Carpenter, Lise Munsie, Kim Raineri, and Bruno Marques




As the number of iPSC-based therapies in clinical development is increasing, the industry is seeing significant advancement following decades of the industry's iPSC development efforts. Despite these noteworthy strides, there are still many existing dilemmas to address for sustained progress and commercial viability. In this article, four esteemed industry experts delve into the critical aspects of reshaping the future landscape of iPSC-based therapies, exploring key areas such as material access and readiness, operational intricacies, technological innovations, and standardization.

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Q How do you perceive the market direction concerning off-the-shelf induced pluripotent stem cells (iPSCs) compared to custom and solely owned iPSC lines considering regulatory frameworks and licensing requirements?

MC: Several groups are struggling with the build versus buy question for iPSCs. Thinking about the criteria for how we select our cell lines, one question to ask is whether the cell line works inside of your manufacturing process. Is there sufficient yield in your manufacturing process? Is genetic stability maintained through the expansion and differentiation of cells? Another criterion is regulatory compliance, which can be complex depending on your goal. Are you looking for clinical entry or for market authorization? Are you looking to enter the clinic in one jurisdiction or multiple? Think about what the end product will look like and work back from there when selecting an iPSC line.

People may not spend enough time considering freedom to operate and securing licenses necessary for the entire manufacturing process, from reprogramming to expansion. If you can find a clinical off-the-shelf cell line, the advantages may include shortening your timelines. Further, if the cell line is already being used in the clinic being, there might even be some human safety data for the iPSC line, which could provide some peace of mind and decrease the perceived risk for product development.

On the other hand, there are not many clinical iPSC lines available right now. We know that different cell lines may perform quite differently in manufacturing processes. If you end up having to alter your manufacturing process to fit an off-the-shelf cell line, it may not save you time overall. In some cases, the licenses for accessing these cell lines can be cost-prohibitive. Some people are concerned if a group uses the same cell line as you and they experience a safety event that is traced back to the iPSC line, it may put your program at risk of clinical hold. To my knowledge, this has never happened, but it is something that folks worry about. The selection of starting material is one of the most important decisions to make in product development.

The advantage of producing your own cell line is that you have ultimate control. If you have proprietary technology to make the cell line, you might even have fewer licensing costs. However, the challenges in doing so can involve long timelines. Going to a CDMO to make a cell line usually involves waiting in a queue, and making these cell lines is still, for the most part, an artful process. Choosing a CDMO that has skilled and experienced operators can be challenging. A cell line can be made in-house, but setting up GMP infrastructure or controlled lab operations infrastructure is a big challenge. Getting the quality systems in place and all the documentation can take time.

Ultimately, there are pros and cons to both off-the-shelf and building a cell line yourself. It depends on the product being developed, the timelines, and the budget. The way that most groups that I work with approach this is to realize that the selection of your iPSC line is entirely imperfect. You pick the path that you can navigate given your budget and timelines and try to risk mitigate.

BM: From my perspective at Century Therapeutics, a developer of iPSC-derived allogeneic products, I would emphasize that the decision around which iPSC line to use for clinical development is probably the most critical decision that we make. Once we make that decision, we are locked in with a particular cell line. This is critical, especially for an allogeneic framework of drug development.

"...the selection of your iPSC line is entirely imperfect. You pick the path that you can navigate given your budget and timelines and try to risk mitigate." — Melissa Carpenter

Century Therapeutics is pushing the boundaries of iPSC-derived therapies, not only in the reprogramming and expansion of naive iPSCs, but also in the genomic modifications that we make before we differentiate into an immune effector product. This means there are scientific considerations in the selection of the cell line. Different donors and resulting cell lines may or may not be able to handle the genomic manipulations required for a given clinical program. A single donor and naïve IPSC bank can support multiple clinical programs since bespoke drug products are created through the genetic engineering process.

LM: If there was a perfect off-the-shelf cell line, there would be no question that that would be the way to go. Currently, there is no perfect off-the-shelf cell line that allows the testing of multiple clones in a process. Other key needs are transparency and easy access to documentation to make a good choice from a regulatory perspective. The groups making the off-the-shelf lines may need to put more work into that to make that more feasible. In the meantime, we believe having high-quality operators and excellence teams at CDMOs to make the bespoke lines is the way to go.

Q What iPSC GMP material and information are available for iPSC-based therapy development, and how does this affect iPSC-based therapy development and clinical development progress?

MC: iPSC products seem to be rapidly increasing, and there is a lot of excitement in the space. However, we are early on in this process. There are several groups in Phase 1 and Phase 2 trials, but no one has reached commercialization yet. Given the novelty of these types of products, the path to commercialization is currently unclear. It is important that we work closely with regulators to educate each other and determine the best path to commercialization.

In terms of starting material, there are a handful of off-the-shelf clinical iPSC lines, and many groups are making their own lines both for allogeneic and autologous therapies. For the iPSC starting material, developers need to know all the information possible, including everything about the provenance of the cell line, the reagents used, and all the processes performed. Developers will need a complete history of the cell line. Making the cell line yourself, while difficult, is feasible, and this type of information will be more straightforward to obtain.

When using off-the-shelf iPSC lines, some of that information might not be available, especially if the cell line was generated some time ago. If there are gaps in the information, a risk mitigation plan will be necessary to navigate any risks associated with any holes in the history. In some cases, when you in-license a cell line, the manufacturer will not want to share all of the information in order to protect their IP. In this case, in the US, the cell line manufacturer can file a drug master file (DMF) and give the FDA access to that file so that at the point of IND filing, authorization for the FDA to review the DMF is granted. I should note that DMFs are not automatically reviewed by FDA, they are reviewed when an IND includes a letter of authorization.

"Genomic characterization is an area of the iPSC field that we are only starting to explore and gain consensus around as we discover how to characterize genomic manipulations." — Bruno Marques

Furthermore, some groups are using iPSC lines that are 'research-grade', due to aggressive timelines. To get to the clinic, they may start with a research-grade cell line, and then swap this for an 'improved' clinical iPSC line later in clinical development. Swapping out starting material can make it incredibly difficult to demonstrate comparability of your final product. Different cell lines can perform differently in the same manufacturing process. If you swap your iPSC line, you may need to modify your manufacturing process to get to the same product. While you may reach Phase 1 more quickly with a research iPSC, you may end up with large delays for commercialization. I strongly encourage everybody to think carefully about choosing their iPSC line. If your goal is to get to commercialization, use a cell line that you can take all the way down that path.

BM: One of the themes here is the criticality of making a good decision around the iPSC line for clinical and commercial development. Genomic characterization is an area of the iPSC field that we are only starting to explore and gain consensus around as we discover how to characterize genomic manipulations. Some problems that come up downstream may require you to have a deep understanding of the genomic profile of your donor line. In the DMF framework, that may or may not be possible, possibly causing problems down the line.

Q What is the current licensing strategy for iPSC technologies, and could this paradigm be reshaped going forward?

LM: From my perspective as someone who has tried to navigate licenses for off-the-shelf lines, the current licensing strategy is the Wild West. Finding and understanding your freedom to operate is important for any product, but the iPSC landscape is busy, fragmented, and difficult to navigate. For the iPSC starting material alone, multiple licenses are needed, including for reprogramming technology and the delivery vehicle. If you are engineering the line, licenses to the editing technology are needed, and potentially even the genes you are editing. This means that for a starting material, one can be subject to three to five different licenses, which, for an early-stage company or product, becomes untenable with often unreasonable royalty payments. Developers must also check the end field and the application for every one of those licenses.

For some technologies, there are well-established and public licensing paradigms, whereas others are more nuanced usually held in confidence with the license or patent holder, which makes seeking out information expensive and time-consuming. This is keeping products from reaching the market. There is no simple answer, as without lucrative licensing prospects the technology and tool-driven innovation will not happen. On the other hand, if the licensing is too restrictive, products simply will not go to market.

For many, the simple act of pursuing licenses and freedom to operate is a no-go point. My recommendations are that companies should consider this early when thinking about their

product and approach. This involves starting conversations, forming collaborations, and thinking about creative deals. It is our responsibility to start educating some of the tool and technology holders on the promise of iPSC therapies and how they might differ from other therapies like gene therapies, where the license leads to the product instead of being a small piece of a starting material.

We can perhaps reshape how licenses in this modality are granted. For the companies providing licenses, having more attainable license costs and a standardized structure with consideration for royalty stacking may be needed. This will increase the likelihood of getting to that commercial stage. The payoff of this is weighted long term and license holders must be able to accommodate that. Additionally, groups who are trying to make off-the-shelf lines need to work with companies holding IP to establish a set of licenses and consider royalty stacking. At a minimum, set up relationships that can be passed down to customers who are trying to remove roadblocks so the therapeutic pipeline can move quicker. At the end of the day, we cannot lose sight of our end goal, which is getting these therapies with so much promise into patients.

MC: It takes a long time to get from discovery to commercialization. As you navigate this, be mindful of when different technologies will come off patent.

Q Are standardized iPSC characterization practices feasible and established industry-wide? What areas are still being evaluated and are still open for debate?

BM: When I began working with these novel and highly complex iPSC-derived therapeutic modalities circa 2019, establishing an analytical strategy felt overwhelming. At Century, we broke this process down into a few buckets. The first bucket of our analytical strategy encompasses safety testing, which has been required across the biopharmaceutical industry for many years. Several of those standards apply to iPSC-derived therapeutics, such as sterility, mycoplasma, bioburden, and endotoxin testing which are all well established.

The next bucket is product-specific and exists around establishing and testing the phenotype of each product, as well as its potency and mechanism. There are typical techniques that people use, such as flow cytometry and cytotoxicity. For potency assays, most iPSC-derived drug developers are still in the very early phase and assay development/validation is not expected until later stages of clinical development.

The third and final bucket is around genomic characterization. This is particularly interesting with iPSC-derived therapies because the pluripotency of iPSCs is necessary for their function, but there is an inherent risk associated with that. On top of that, several genomic manipulations are made. These factors lead to uncertainty and risk that must be managed.

At Century, we used the draft genome editing guidance from the FDA a few years ago, which focused on *in vivo* gene therapies, and *ex vivo* autologous and donor-derived cell therapies as those were what was available at the time. We started to apply and adapt that to the iPSC-derived therapies we are developing. We focused on how the DNA sequence change would be achieved, the type of genomic modifications needed for therapeutic effects, and the delivery method for each of the human genome editing components. With that, we were able to create a systematic risk assessment, which involved identifying each one of the variants, filtering the risk of each variant, and making conclusions. That has led us to develop the genomic

"Manufacturing teams often fail to take the flexibility required for cell therapies into consideration. In the future, hopefully, automation using AI will be able to control these processes better." — Lise Munsie

characterization approach that we filed with our first clinical program, which was well received by the FDA.

One of the main concepts for us was to test what we could along the manufacturing process. Donor considerations can be tested and de-risked early on. Once reprogramming is complete, one can test and de-risk aspects around the naïve iPSCs. Once genomic manipulations are complete and a master cell bank is established, one should fully characterize and de-risk that master cell bank. The key is to minimize what needs to be tested in each batch of drug product, thus reducing overall cost and risk.

KR: At Aspen Neuroscience, we are leaning into a bioinformatics approach and deeper characterization of our cells, utilizing whole genome sequencing to get a full picture of the cell's genetic identity and integrity whilst looking at any concerning genetic alterations or oncogenic markers. We do this for every autologous product that we make, including for both the iPSCs and the final product. We also use whole transcriptome analysis to correlate and predict the cell's capabilities for pluripotency in our PluriTest. On the final product side, in our lead program, we use transcriptome analysis to evaluate key profiles that could predict efficacy. This bioinformatics approach gives us important information on each patient's cells at each stage in the manufacturing process. We think that this will become the industry standard for these highly important iPSCs and cell therapy-derived products from iPSCs.

Q What are the key challenges and benefits of using clean rooms in iPSC manufacturing?

LM: For the last few years, I have been working on moving reprogramming and other iPSC manufacturing processes from Parkinson's Disease into clean rooms. One of the main challenges is that iPSC derivation is still a highly manual process under strict control. Much of the manufacturing expertise comes from fields where hundreds of runs worth of data have been collected, such as in antibody production and vaccines. Manufacturing teams often fail to take the flexibility required for cell therapies into consideration. In the future, hopefully, automation using AI will be able to control these processes better.

Managing variability in a clean room requires having flexible batch records that allow some decision-making outside of what is normally expected in manufacturing. It also requires trained operators who are skilled and able to use flexibility to produce a high quality product while staying within GMP standards. It is doable, but it is not easy. Groups are approaching making their iPSCs in a controlled lab space, but those look different. This would allow non-GMP qualified staff with strong expertise to perform technical steps to have higher levels of success and potentially a higher quality product. To take this approach, you must think through your strategy with a regulator ahead of time. The level of documentation should never

be compromised. All lots should be GMP compliant, and risk assessments, justification, and testing on the product should be completed. From my perspective, for reprogramming, many different groups have been able to perform an end-to-end process in a full clean room under GMP. However, for gene editing, some of the processes and equipment may not even be compatible with the clean room. It is a balancing act of deciding what you perform in and outside of the clean room space.

KR: The key challenge of clean rooms is that they are expensive to run and maintain. This includes the costs of staff, cleaning, gowning, environmental monitoring, and utilities. The higher the class of the clean room, the higher the expense. For small-scale processes, for example, producing a cell line, this can be manageable, but ongoing manufacturing inside high-class clean rooms is ultimately not scalable. We need scalability to make products suited for large therapeutic indications. However, we can make good, clean, safe cells inside clean rooms, which is their utility today.

BM: iPSC-derived therapeutics are still a nascent field. For us to be successful and deliver their promise to patients, we need to take as many shots on goal as possible. A big impediment to taking more shots on goal is cost. Minimizing the amount of operations in a dedicated GMP environment by applying principles of GMP in a risk-based manner is a way to reduce cost.

I pose a question to the panel: What parts of the process need to be in a GMP facility versus others? And does this differ in an autologous environment? For us, one of the big differentiators is the allogeneic nature of what we do, as gene editing only happens once per product. That enables us to perform certain parts of our manufacturing process in a controlled environment outside of a GMP facility.

KR: The calculation is different in the allogeneic case, where sterility assurance and patient segregation are required each time. What one can do with one cell line, and what the agency will be lenient on may not be the same in an autologous setting.

Q Are closed systems needed for our industry's future commercial success?

KR: We want to commercialize large indications, so we need to move the process out of the clean room and into closed systems. Closed systems will help facilitate that move from higher class clean rooms to lower class or even no clean rooms, which will ultimately impact the cost of goods. Closed systems can come in a variety of forms, including closed culture bags and bioreactors.

Isolation systems are usually combined with some level of automation. Many of the closed systems seen today have some automation built in, such as the Prodigy or the Cocoon. I have had experience closing cell factory systems and manufacturing up to 50 closed cell factory systems on bench tops, using things like tube welders and sealers. This is still a highly manual process, but it is closed. This provides some of the benefits of moving onto the bench, but ultimately, automation is favorable and will help quicken the transition out of aseptic manufacturing. In areas like CAR-T manufacturing, we are seeing more maturity where more processes are closed and automated.

We must begin to look at these types of systems and leverage them as we think about adapting iPSC manufacturing into closed systems, which will provide a higher level of sterility assurance, better patient segregation, reduced cost burdens, reduced aseptic process simulations burdens, and will ultimately be more scalable.

MC: Right now, we are manufacturing for individual patients or tens of patients. Hopefully, one day we will need to perform manufacturing runs of 1,000 or 10,000 doses. To do so, we need automation and closed systems, or treating large populations of patients will remain extremely challenging.

BM: In the development of iPSC-derived products, we are essentially replicating what happens in the thymus inside a bioreactor, making cell culture processes lengthy which leads to higher sterility risk. Therefore, closed systems become imperative.

Q How effective are existing tools and equipment for iPSC-derived therapy manufacturing, and where would you say are the major gaps?

KR: When I joined Aspen Neuroscience, I spent my first year with my team looking to answer the question of what tools were available for iPSC-derived cell therapy. This can be split into two categories: tools for the generation of iPSCs and platforms for differentiation. As an autologous cell therapy company, we need to deal with both and take an end-to-end approach.

There is not a lot out there for end-to-end manufacturing. For iPSC generation, many systems are being used in cell line development that are adapted for clone selection and screening, but this does not help the downstream cell culture portion. There are platforms geared specifically to iPSC clonal selection, such as the CellCelector, although these only perform one part of the manufacturing process.

The field is still nascent, but there are some promising early developments. Companies are tackling this problem, but for now, there is still a big gap in having an end-to-end solution for iPSCs. On the differentiation side, equipment is typically geared either towards allogeneic or autologous.

On the allogeneic side, there is bioprocessing to leverage, such as bioreactor-type platforms for large-scale cell therapy that create culture conditions to promote the growth of sensitive iPSC-derived cells. However, these are suited for cells in suspension. On the autologous side, there are many single-batch platforms used in CAR-T that could be deployed for iPSC differentiation. The key issue is the economics of some of these platforms, which becomes difficult when the residence time in the platform is long. The other caveat is most of those single-batch platforms are suited for suspension-based cultures. There is a big gap on the adherent cell culture side, which has also been the case for all other cell therapies. There are still no large-scale, adherent, automated cell culture platforms.

Q What strategies can optimize the iPSC production process?

BM: Right now, we are still stitching together different technologies to handle the different cell types in iPSC-derived manufacturing processes. This involves adherent

"When I joined Aspen Neuroscience, I spent my first year with my team looking to answer the question of what tools were available for iPSC-derived cell therapy. This can be split into two categories: tools for the generation of iPSCs and platforms for differentiation." — Kim Raineri

technology to grow the iPSCs, and then a suspension bioreactor to produce hematopoietic progenitor cells. Then, we need to start activating and differentiating cells, requiring some sort of signaling, which can pose difficulties. Once we have terminally differentiated immune effector cells, then we can start thinking about having a single batch to treat thousands of patients.

My chemical engineering background makes me excited about exploring the different types of reactors that are used elsewhere in the biopharmaceutical industry. My team has been thinking a lot about cell culture media optimization, both in terms of the components and the feeding strategy. A unique factor of iPSC-derived products is ensuring cytokine control throughout these rather lengthy cell culture processes. Finally, it is key to figure out a way to present whatever signaling you need in suspension.

Q When you build your own iPSC cell lines, do you still have to test multiple clones to ensure that they can be differentiated and utilized?

LM: Yes—though this depends on how robust your differentiation process is. In an ideal situation, you would manufacture from one donor, produce multiple clones, and bank them so that they could be tested in your process before making your master cell bank. This would tell you how robust this donor is for your process and how robust the process is in general, whilst also assisting in selecting a clone that works. It would be valuable to do the same thing when searching for off-the-shelf lines, and ideally, you would have multiple clones to test. In general, for iPSC therapeutics, there remains the caveat that it can be both donor- and clone-line dependent.

Q What are the challenges in gene editing of iPSCs at different stages in the process, such as before and/or after differentiation? How is the industry mitigating these issues?

BM: When and where gene edits are performed has a biological implication, because this can lead to cells behaving differently.

Century Therapeutics and many of our allogeneic colleagues are choosing to do the vast majority of gene editing before master cell bank production, giving us the ability to produce however many drug product lots we need from a single bank of fully characterized, homogeneous cells. In the future, we may move away from this for either biological or other reasons.

BIOGRAPHIES

MELISSA CARPENTER has worked on the development of cell therapies using human adult and embryonic stem cells for the last 20 years, in academia and industry, in the US and Canada. She has been involved with human embryonic stem cell (hESC) research since the field was established. Her work involves discovery research and the translation of this research into therapeutics, including developing strategies for preclinical development and navigating the regulatory issues surrounding stem cell therapies. She has held leadership positions at three of the major stem cell companies: CytoTherapeutics, Inc (StemCells, Inc.), Geron, Corp., and Novocell, Inc (Viacyte, Inc). Currently, Carpenter is President of Carpenter Group Consulting and works with early stage companies, academic groups and investors to translate discovery based research into stem cell therapies. She is credited with numerous publications and patents in the stem cell field.

LISE MUNSIE earned her PhD at McMaster University, Hamilton, ON, Canada, focusing on drug discovery in neurodegenerative diseases. Following this, she completed a post-doctoral research fellowship at the Centre for Applied Neurogenetics at the University of British Columbia, Vancouver, BC, Canada, where she focused on the genetic causes of Parkinson's Disease. Lise joined CCRM, Toronto, ON, Canada, in 2015 and is currently the Vice President of the iPSC Technology Platform for CCRM, and its affiliate, OmniaBio Inc. Lise manages iPSC reprogramming, gene editing, cell banking, scale-up, and differentiation projects. Lise's team critical focus is enabling these technologies to be manufactured to produce clinically relevant products.

KIM RAINERI is the Chief Technology Officer for Aspen Neuroscience, Inc., San Diego, CA, USA. He is responsible for the manufacturing, process and analytical development, technology development, and delivery device functions of a leading autologous iPSC derived cell therapy company targeting CNS diseases. Prior to this position he was the Chief Manufacturing and Technology Officer for AVROBIO, Inc., responsible for the CMC, process and analytical development, supply chain, and external manufacturing functions of a leading gene therapy company targeting Lysosomal Storage Disorders through *ex vivo* lentiviral gene therapy. Prior to this role, he held various positions of responsibility in cell and gene therapy CDMO as Vice President of Operations for Nikon CeLL innovation, Business Director for Lonza Bioscience Singapore Pte Ltd., and Director of Operations for Lonza Walkersville. Raineri has a MBA from Kennesaw State University, Kennesaw, GA, USA, and BSc from the University of Miami, Coral Gables, FL, USA.

BRUNO MARQUES is Vice President of Process and Product Development at Century Therapeutics, Philadelphia, PA, USA, with a focus on allogeneic, iPSC-derived therapies for cancer and autoimmune diseases. He is a chemical engineer by training, with a PhD from Carnegie Mellon University, Pittsburgh, PA, USA, and a BS from the Illinois Institute of Technology, Chicago, IL, USA.

AFFILIATIONS

Melissa Carpenter PhD
President,
Carpenter Consulting Corporation

Lise Munsie PhD
Vice President,
iPSC Technology Platform,
CCRM and OmniaBio Inc.,
Toronto, ON, Canada

Kim Raineri
Chief Technology Officer,
Aspen Neuroscience Inc.,
San Diego, CA, USA

Bruno Marques PhD
Vice President of Process
and Product Development,
Century Therapeutics,
Philadelphia, PA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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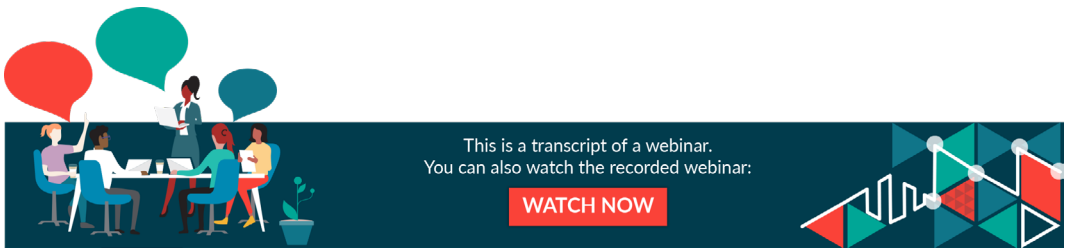

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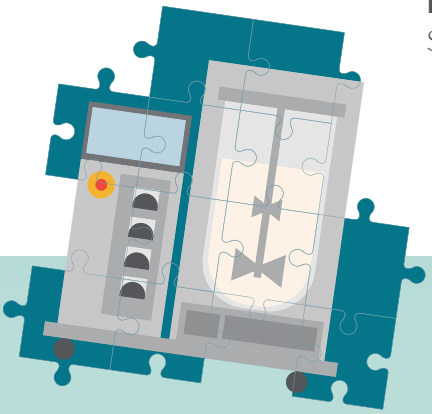

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Accelerating cell therapy production: realizing the advantages of rapid analytical testing solutions

Seth Peterson

Cell therapy is experiencing rapid growth, with an increasing number of treatments receiving marketing approval every year. One notable area is CAR-T cell therapy, which requires a complex *ex vivo* workflow, as illustrated in Figure 1. Many different areas of this workflow could benefit from analytical testing beyond what is required for regulatory compliance.

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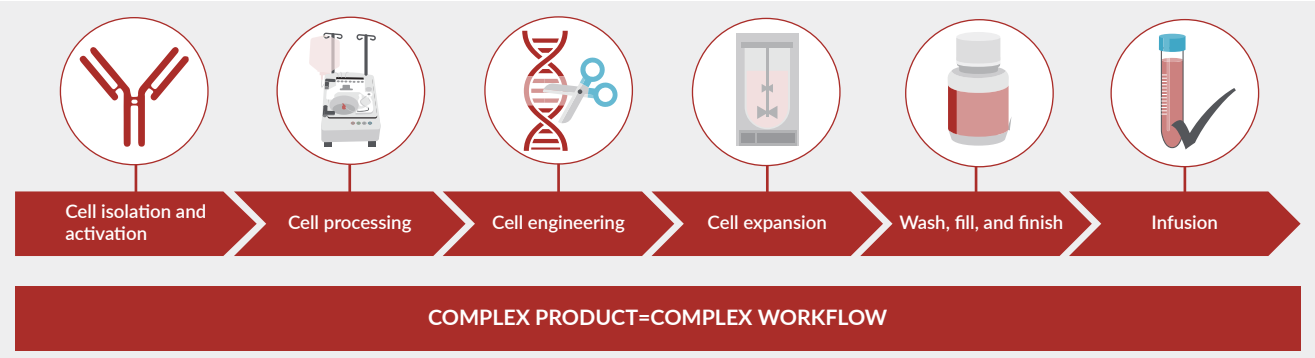
FIGURE 1

CAR-T cell therapy workflow.



CHARACTERIZATION AND TESTING

Every biotherapeutic requires multiple layers of product characterization and testing. As shown in Figure 2, for cell therapies, there are two main categories of testing: identity and purity. Identity testing includes potency, viability, and titer. Purity testing includes sterility testing for bacteria and fungi, mycoplasma, and process residuals such as those listed in Figure 2.

Testing guidance for cell therapies and similar products is developed by a number of groups, including the European Pharmacopoeia and the US FDA. The latest guidance for cell therapy communicates recommendations for CMC, pharmacology and toxicology, and study design. Recommendations specific to autologous or allogeneic CAR-T cell products are noted in this guidance, but certain details and recommendations have relevance to other genetically modified lymphocyte products, including natural killer cells or T cell receptor-modified T cells.

FIGURE 2

Summary of methods used to test the identity and purity of cell therapy products with associated regulatory guidelines.

Cell therapy testing

Identity

- Potency
- Viability
- Titer

The identity of the biologic product must be verified by assay that will identify the product for proper labeling and will distinguish the product from other products being manufactured in the same facility (21 CFR 610.14).

Examples:
Cell surface markers, **gene expression**, secreted molecules, peptide sequences

Purity

- Mycoplasma
- Sterility
- Process residuals

Product purity (21 CFR 610.13, 21 CFR 610.12) testing includes assays for:

- Pyrogenicity/endotoxin—limulus amebocyte lysate (LAL) assay
- Contaminants: unintended cell populations, residual proteins/peptides used to stimulate or pulse cells, and materials used during the manufacturing process (cytokines, growth factors, antibodies, serum, etc.)

Moreover, the FDA provides examples of alternative testing methods and technologies that might be advantageous or even essential for live cell therapies. These encompass rapid alternatives for mycoplasma, sterility, and endotoxin assessments.

RISK-BASED APPROACH: PCR MYCOPLASMA TESTING

There have been rapid methods specifically utilizing nucleic acid amplification included in the regulatory guidelines since as early as 2007, when the European Pharmacopoeia enabled nucleic acid amplification techniques (NAT) as part of regulatory testing practices. In the 2007 document, there is specific guidance to leverage PCR-based mycoplasma testing, including specificity of the kit, limit of detection, and robustness. Current US Pharmacopoeia (USP) guidance only covers culture testing (which takes up to 28 days), so most developers and manufacturers refer to the European Pharmacopeia for reference to the NAT method. One may also utilize USP <1223> for rapid testing, but this is not a method-based chapter. The fundamental point regarding mycoplasma testing using PCR is that it serves as a limit test where specificity, limit of detection, and robustness are assessed.

One rapid mycoplasma testing solution is the Applied Biosystems™ MycoSEQ™ Mycoplasma Detection System. While the MycoSEQ solution has been on the market for over 10 years, it has recently been updated in the form of the Applied Biosystems™ MycoSEQ Plus™ Mycoplasma Detection Kit, which expands the number of detected species to over 200 and leverages the latest Applied Biosystems™ TaqMan™ chemistry to add specificity in more complex bioproduction samples. Furthermore, qualification and validation of lot release with the MycoSEQ Plus kit does not require live mycoplasma or inactivated mycoplasma, which greatly reduces the risk of a contamination event within the facility.

TABLE 1

Regulatory expectations for qPCR rapid sterility testing on ATMPs.

Time to results	Meets regulatory guidelines by delivering results in < 4 hours
Specificity	Probe-based assays target defined sequences providing a high level of specificity
Limit of detection	The acceptable limit of detection for qPCR is 10–100 CFU
Sample size	qPCR does not require a large sample size (< 2 mL)

qPCR is a risk-based rapid sterility method as described in USP <1071>. CFU: colony forming units; qPCR: quantitative real-time PCR

USP <1071> outlines a risk-based approach for rapid sterility tests on limited and short shelf-life products. This guidance acknowledges that existing sterility tests, which are based on growth requiring a minimum incubation period of 14 days, are inappropriate for products with a limited shelf life or those intended for immediate use. Such products are typically administered to patients before the conclusion of the compendial testing period. USP <1071> states that the selection of a rapid sterility test should be driven by a risk-based approach, with stakeholders choosing their preferred technology based on factors such as time to result, specificity, limit of detection, sample size, and product attributes.

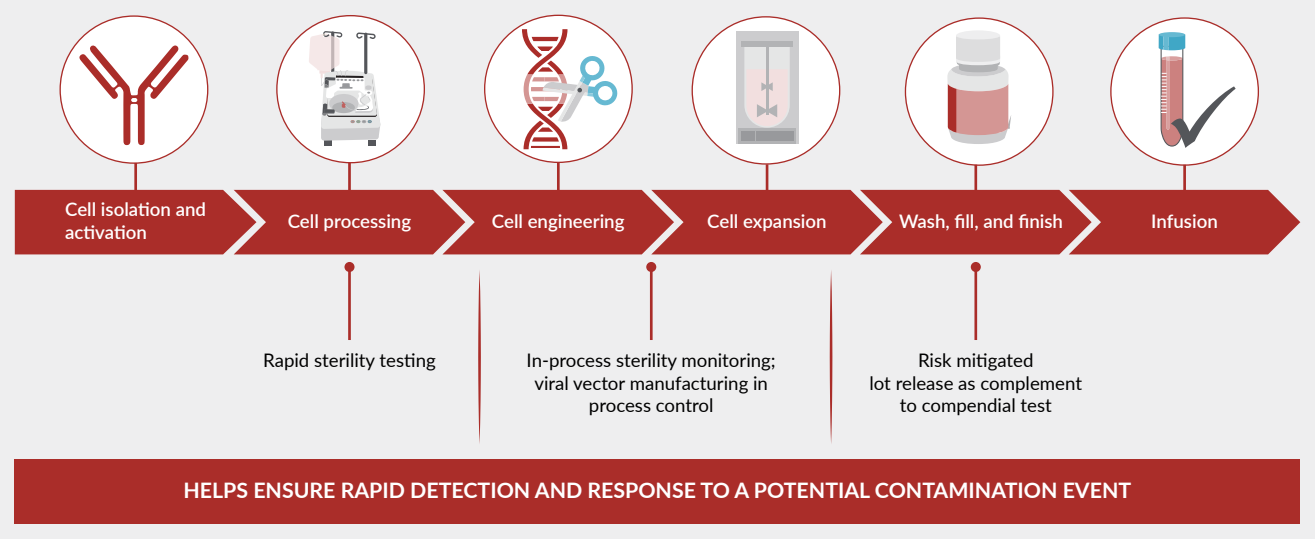
One of the rapid methods outlined in USP <1071> is the Nucleic Acid method, quantitative real-time PCR (qPCR). USP's expectations for this method are listed in Table 1.

Time to result has a recommendation of < 5 hours, but that can be pushed to same-day results. Specificity is defined as the ability to detect a wide range of different bacteria, yeasts, and molds, which helps ensure that no cross-reactivity occurs with surrounding genetic material such as the sample matrix itself. The limit of detection target is 10–100 colony-forming units (CFU), as reported in copies of genomic units as a way to show equivalency or noninferiority. This is specific to the nucleic acid amplification method. Lastly, as far as sample size is concerned, USP <1071> recommends testing a minimum of 1% of the entire product volume for sterility, provided it is justified by a risk assessment. This chapter refers to the 1% sampling plan outlined in the European Pharmacopeia 2.6.27. Specifically, the contamination test sample size for cell preparation with a volume between 10–1,000 mL would be 1% of the total volume. If the cell preparation is < 1 mL, the preparation is not to be tested.

While the regulatory requirement is to test for lot release, which is typically after the cell expansion phase, the guidelines also recommend other test points as part of the risk-based approach. Figure 3 outlines two proposed additional testing points in the cell therapy workflow. Testing the raw materials and additional in-process points, as well as lot release, can help in the detection of potential contamination event earlier in the process. If those tests are negative, confidence in the final product increases. FDA CMC guidance reiterates this point with recommendations for performing in-process sterility testing on samples taken 48–72 hours prior to final harvest. Furthermore, the FDA sterility section in 21 Code for Federal Regulations (CFR) 610.12 states that sterility testing must be performed on the final container material while in-process testing should be representative of that final container material. Therefore, it is important to qualify in-process samples. It is also noted that in-process testing using rapid methods may be used in lieu of final product testing when fully justified. Additional guidance is found in USP <1071>, which suggests that rapid sterility tests or alternative rapid microbiological methods can be employed as in-process controls before the final product release sterility test. This is aimed at offering early detection of significant contamination

FIGURE 3

Proposed testing points in the cell therapy workflow.



or the likelihood of a sterility test failure. In conclusion, rapid testing enables immediate detection in response to a potential contamination event.

STERILITY TESTING: BENEFITS AND CHALLENGES

The cornerstone of any cell therapy manufacturing process is the assurance of product safety. Sterility testing accomplishes this task in several ways.

Firstly, sterility testing safeguards patient safety by serving as a preventative measure against infections, especially in vulnerable patient populations. Sterility testing also helps to maintain the quality and efficacy of the therapy. In-process and lot release testing help to ensure that no harmful microorganisms, such as bacteria or fungi, are introduced during cell processing. In this way, this helps to ensure the intended benefits of the treatment. Lastly, sterility testing supports regulatory compliance. This compliance in turn helps to ensure that the providers and manufacturers follow established protocols to maintain a high level of safety set to global standards.

However, the technical barriers associated with manufacturing cell therapy products, including limited production volume, short product shelf life, and the inability to leverage terminal sterilization techniques for living therapies, contribute to sterility testing challenges. Limited production scale and volume result in minimal material being available for the compendial method described in USP <1071>. The use of destructive test methods is not feasible under these circumstances. In addition, existing compendial methods and most existing rapid methods exceed the 1–3 day shelf-life of cell therapies.

Recent regulatory policies have taken all of these challenges into consideration, allowing a risk-based approach utilizing rapid microbiological methods.

APPLIED BIOSYSTEMS™ STERISEQ™: RAPID STERILITY TESTING KIT

One solution to overcome these challenges is the SteriSEQ rapid sterility testing kit, which is a TaqMan-based qPCR assay kit used to detect the presence or absence of bacteria and fungi. The features of the SteriSEQ solution are listed in Figure 4. On the right of this Figure, a subset of the target species tested

FIGURE 4

Features of the SteriSEQ rapid sterility kit.

Fast—sample-to-answer TAT in less than a day
qPCR run time delivers results in <3 hours

Specific—primers/probes designed specifically for bacteria (16S rRNA) and funghi (18S rRNA)

Sensitive—designed to meet or exceed the regulatory guidelines for sterility testing

Robust—tolerance up to 1M cell background

Specific—microbial coverage based on *in silico* testing: >16K bacterial species; ~2.6K funghi species

No cross reactivity to species below and no sample matrix effect

Species/DNA	
Adenovirus 2	CHO genomic DNA
Kanamycin resistance	Baculovirus expression
Ampicilin resistance	Lentivirus expression
HEK 293 genomic DNA	Bovine genomic DNA

for cross-reactivity is listed. These tests helped confirm the specificity of this assay.

This assay utilizes the full multiplexing capabilities of qPCR by including four targets in a single assay. This results in fewer tubes and less handling during setup and plate preparation. Moreover, the kit features two integrated controls, the internal positive control (IPC) and the discriminatory positive control (DPC). The IPC checks for PCR inhibition and helps ensure consistent performance. The assay-specific DPC is a positive plate control and a sample extraction control. This control also generates a discriminatory signal to distinguish a contamination event from a true positive sample call. These integrated controls result in increased accuracy, minimizing both false positives and false negative calls. Figure 5 demonstrates bacteria, fungi, IPC, and DPC

FIGURE 5

Multiplexing capabilities of the SteriSEQ rapid sterility kit.

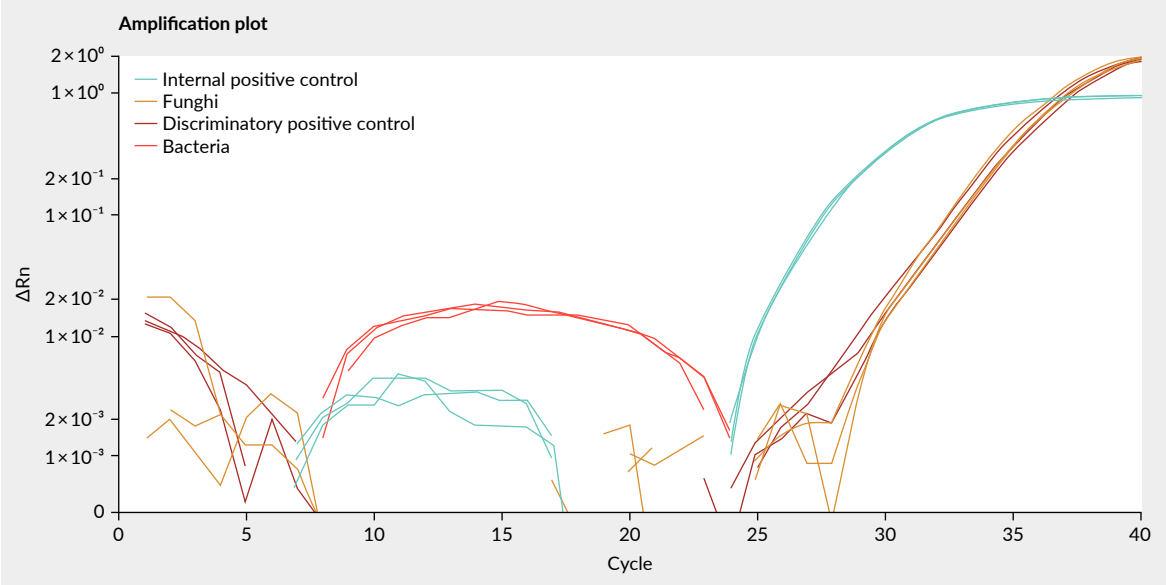


FIGURE 6

SteriSEQ assay workflow.

FIGURE 7

Detecting bacterial species.

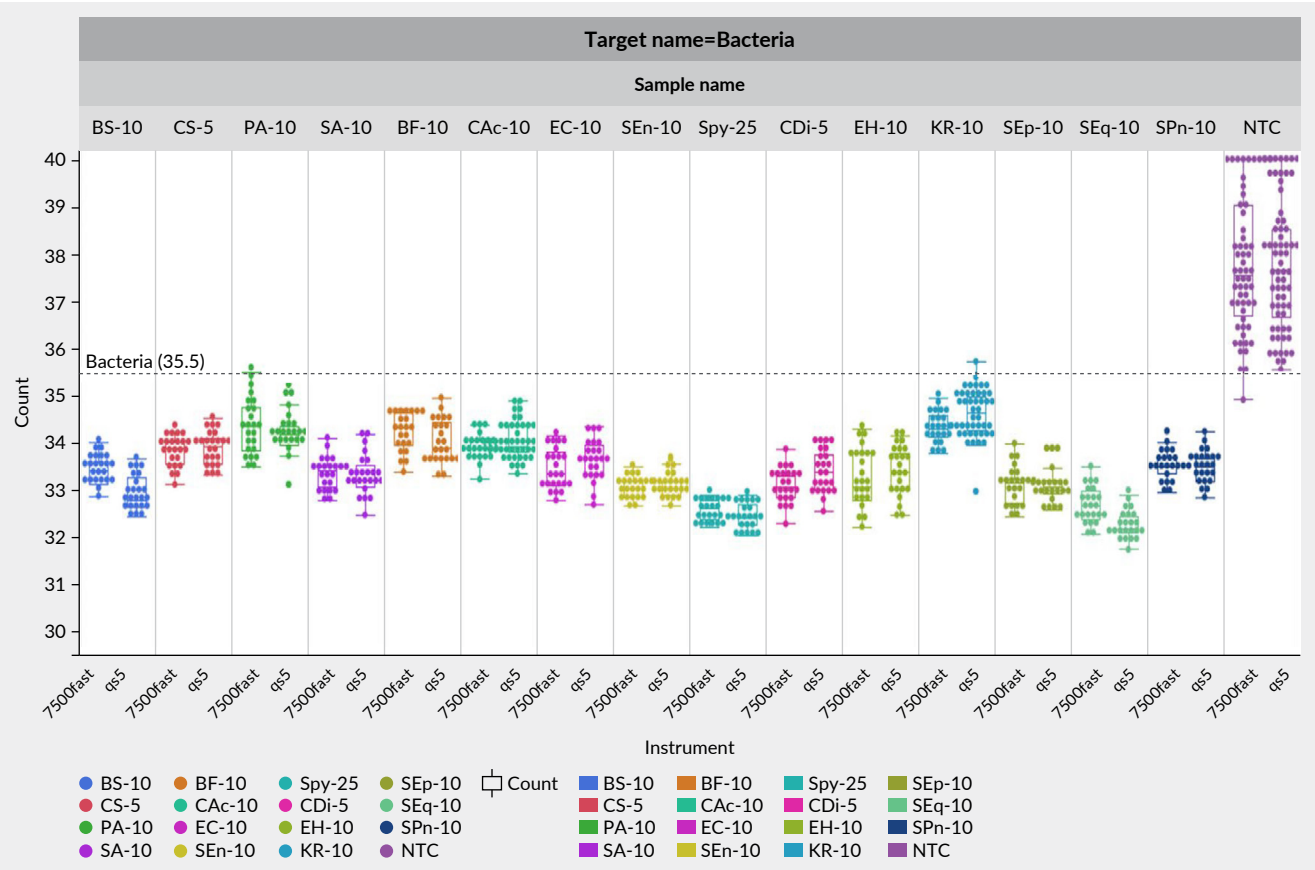
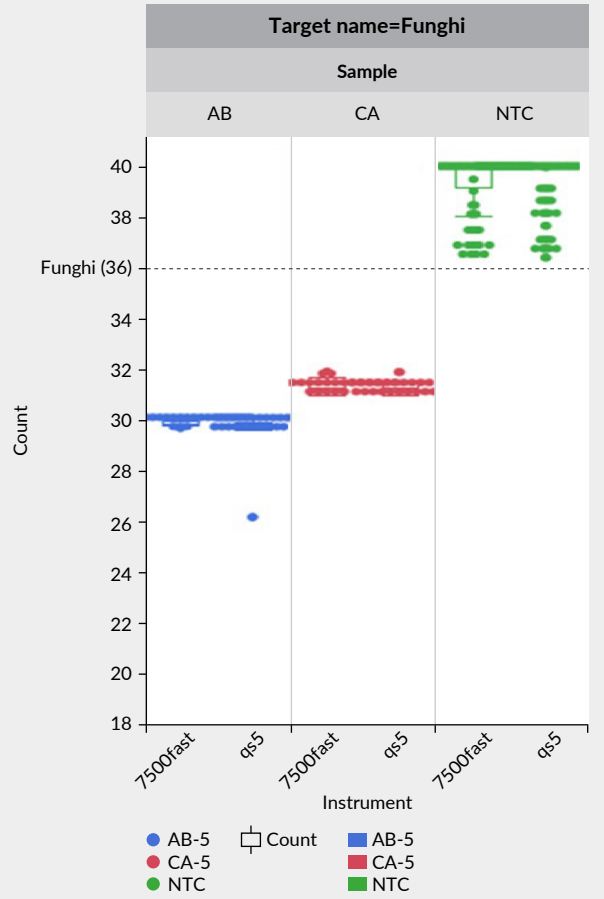


FIGURE 8

Detecting fungi species.



targets all in a single reaction to highlight the full multiplexing capabilities designed into this kit.

As the cell therapy product moves through the manufacturing process, the SteriSEQ system can be leveraged for testing of raw materials, in-process sterility monitoring, viral vector manufacturing, and in-process control.

The SteriSEQ workflow (Figure 6) firstly involves the DNA sample preparation, where the nucleic acid is extracted from the sample. Samples containing up to one million cells can be tested in cell suspension. The reaction is then set up based on samples using the multiplexed PCR kit. The assay can be run using either the Applied Biosystems™ QuantStudio™ 5 or Applied Biosystems™ 7500 Fast Real-Time PCR Systems. Finally, results are analyzed using the Applied Bisosytems™ AccuSEQ™ software to generate a report with the presence/absence calling for the sample(s). The AccuSEQ software package includes a full security audit and e-signature functionality that helps enable users to meet their 21 CFR part 11 compliance requirements. It also has added data integrity features specifically for the GMP environment.

STERISEQ SAMPLE DATA

Figure 7 illustrates data depicting potential contamination, including 6 species listed in the USP <71>, along with additional targets that were added based on customer feedback and literature. The data shown is for genomic bacterial DNA with titers ranging from the equivalent of 5–25 CFU, which meets and exceeds the 10–100 CFU limit in the USP regulatory guidance. This data was generated on both the 7500 Fast and QuantStudio 5 real-time instruments, demonstrating the robustness of the assay and platforms. There are high reproducible outcomes on both platforms across all microorganisms tested.

In terms of detecting fungi, Figure 8 shows that for the fungi species *Aspergillus brasiliensis* and *Candida albicans*, both reactions detect the equivalent of five genome copies with a very low background signal. These tests were also run on both the 7500 Fast and the QuantStudio 5 platforms. Fungi can be challenging to use to isolate the nucleic acid, so it is important to use challenging samples as part of the equivalency and validation efforts.

To summarize, the SteriSEQ workflow delivers reliable results in less than a day, so it fits the requirement for rapid testing. It is a fully multiplexed (4-plex) assay that tests for both fungi and bacteria, and includes two additional controls for increased accuracy. Most importantly, this workflow only requires a very small sample volume, which correlates to the reduced sampling requirements outlined in both USP <1071> and European Pharmacopoeia 2.6.27. This kit is a full sample-to-answer solution with optimized data analysis leading to a simple report.

Q&A

Seth Peterson

Q Do you only need to verify the six organisms mentioned in USP <71> for testing purposes?

SP: Six should be the minimum as part of your method of suitability, but there are a number of reasons to test more than just six. As part of your qualification, I would recommend additional species for enhanced robustness. Those additional species depend on your workflow. If you are expected to produce globally, you should incorporate species from other regional pharmacopeias. I still recommend challenging species in terms of slow growth, difficulty in culturing, etc. I also recommend adding pathogenic or other microorganisms previously found or associated with a particular product.

Q Does this rapid sterility test meet USP <71>?

SP: It is not intended to meet chapter <71>, as that guideline requires inoculation and a 14-day incubation. The goal for rapid testing is to demonstrate similarity to that method. While it does not specifically meet USP <71>, it should be equivalent for this risk-based approach.

Q How do you determine if a positive signal is from viable or non-viable organisms?

SP: It is a nucleic acid test, so as it stands, the workflow does not distinguish between live and non-viable organisms. However, that is not to say that there are not methods that we can adapt to test for viable cells. The sensitivity is such that it can still meet the requirements for this test.

BIOGRAPHY

SETH PETERSON, Senior Manager of Application Support at Thermo Fisher Scientific, is a seasoned professional in the field of pharma analytics. With a dedicated focus on supporting and advancing novel genomic methods, Seth brings a wealth of experience and passion to his role.

In his capacity at Thermo Fisher, Seth oversees application support initiatives, playing a crucial role in developing and implementing innovative genomic methods tailored for both research and regulated environments within the pharmaceutical industry. His commitment to excellence is reflected in his diverse experience, where he has provided support to teams in clinical oncology, pharmaceutical manufacturing, and agricultural biology.

Seth's career spans over a decade in the field with Thermo Fisher Scientific, during which he has directly engaged with customers, leveraging next-generation sequencing (NGS) applications. His expertise particularly shines in the areas of epigenetic profiling and genotyping methods, showcasing a deep understanding of the intricacies of genomic research and its applications in various domains. With a focus on supporting and developing novel genomic methods, Seth Peterson stands as a dedicated leader and advocate for advancements in pharma analytics.

AFFILIATION

Seth Peterson
Application Support,
Bioproduction Group,
Pharma Analytics,
Thermo Fisher Scientific

AUTHORSHIP & CONFLICT OF INTEREST

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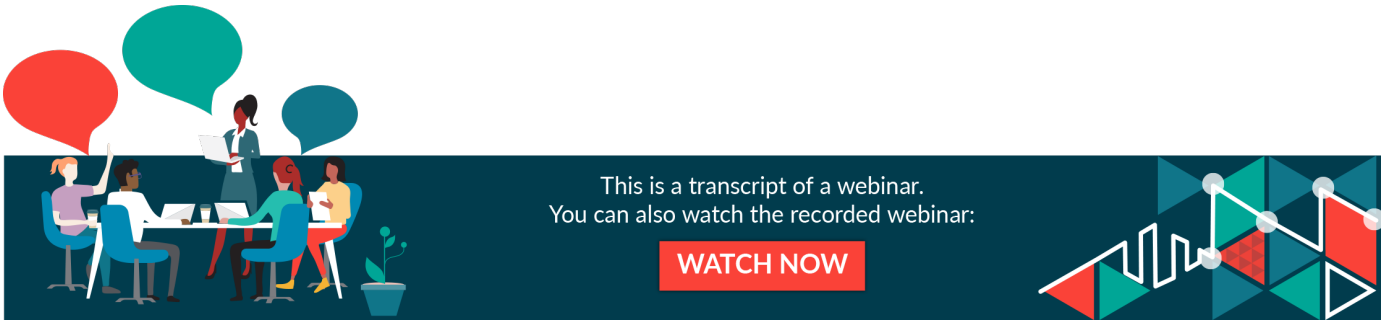

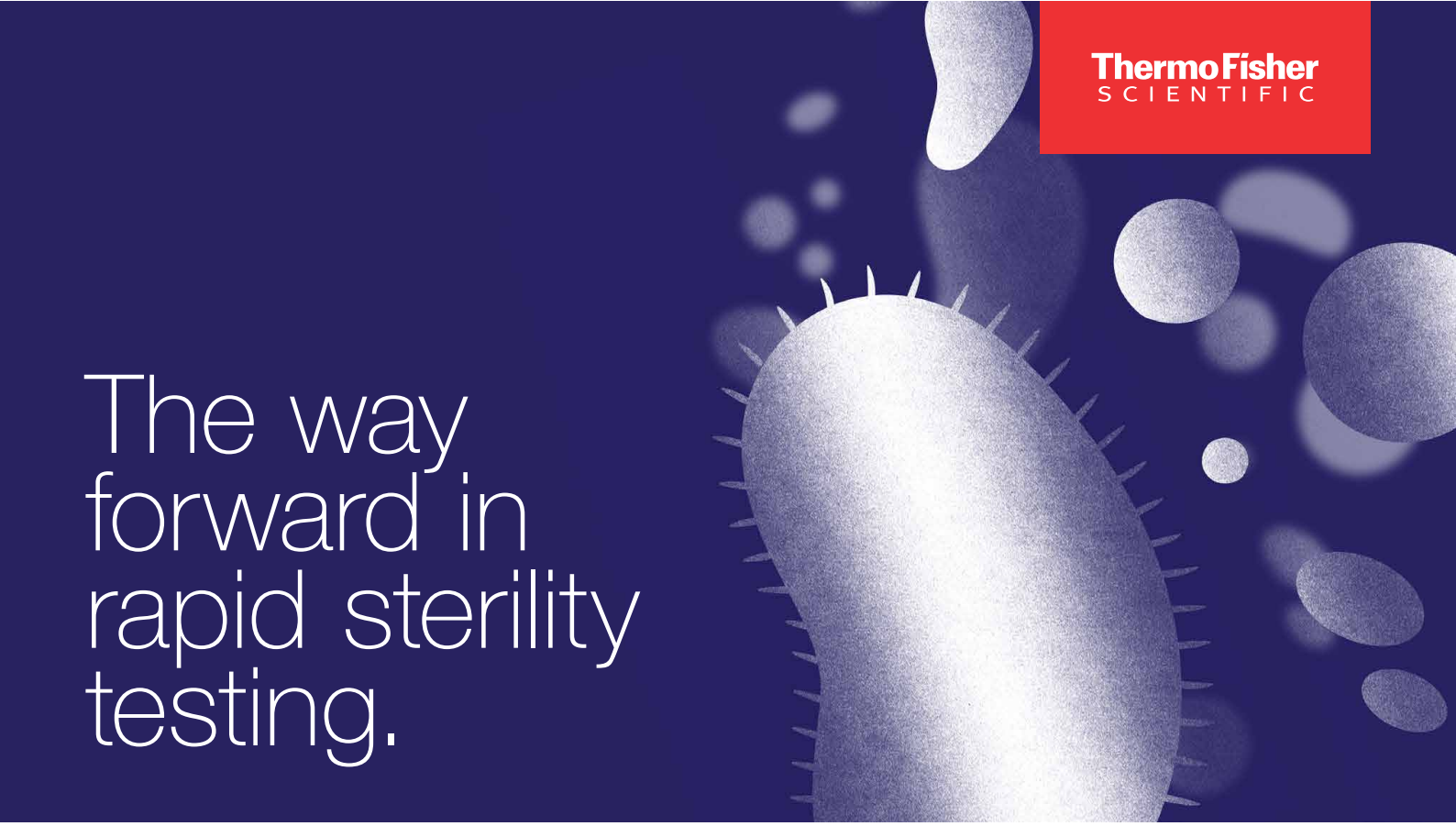

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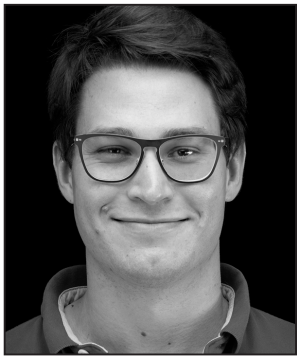
CELL & GENE THERAPY INSIGHTS

INTERVIEW

Fit for function: developing potency assays reflective of the *in vivo* environment

Translating the therapeutic promise of cell and gene therapies into clinical reality relies on robust potency assays. However, designing assays that accurately reflect the complex mechanisms of these therapies can feel like chasing a moving target. Here, **Charlotte Barker**, Editor, *Cell & Gene Therapy Insights*, speaks with **Giorgio Zenere**, CMC technical project lead in the Global QC Technology Innovation Team, Kite Pharma, and **Dirk Windgassen**, Director of Analytical Development, Miltenyi Biotec, to discuss best practices and future trends in developing potency assays for cell and gene therapies.



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Q Can you each start by introducing yourselves and telling us what you are working on right now?

GZ: I have been in cell and gene therapy my entire career. I did a PhD in CAR T-cells against HIV and then went to work for a biotech company, where I was in the R&D and drug discovery departments, looking at novel CAR T-cell strategies against solid and hematological tumors. Through that work, I learned a lot about potency assay development. Now I work

at Kite Pharma, where I develop and validate novel analytical technologies for commercial CAR T-cell therapies, as well as consulting on late-stage clinical products.

DW: I have also been working in the cell therapy field for many years. I started out with a PhD in immunotherapy applications, trained as a biochemical engineer, and worked in assay diagnostics for many years. Now, I'm leading Miltenyi Biotec's assay development team in San Jose, California. We develop assays for clients, including potency assays. We have assays for CAR-T cells, natural killer cells, hematopoietic stem cells, and others currently in development.

Q What are the greatest challenges in developing potency assays for cell and gene therapies?

DW: Bioassays come with several challenges. There is a lot of variability, and the biological system has variability in itself. To enable the qualification and validation of such an assay, we need reference standards, controls, and suitability criteria—and that reference material needs to be produced, maintained, and qualified.

Another challenge is the timeframe needed to measure biological responses. The 24–48 hours needed for some assays could make it challenging to release a freshly made product on time.

Ultimately, what regulators like the US FDA are looking for is a correlation with clinical efficacy and that is also a major challenge—to use an *in vitro* assay to reliably predict *in vivo* response.

GZ: For me, the greatest challenge depends on the purpose of your assay. A lot of potency assays, especially in the early days, were developed with the intention to predict how well your cell and gene therapy perform in patients. As Dirk just mentioned, there is poor correlation between *in vitro* potency and clinical efficacy because the *in vitro* assay cannot accurately model the complex microenvironmental conditions that you would see in a disease, such as a solid tumor or HIV.

However, if your goal is to check that your manufacturing process is giving you a product that's within specifications, there are well-established and well-controlled potency assays available.

DW: I agree. In many cases the goal may not be to develop an assay that is reflective of *in vivo* conditions, but rather one that relates to the mechanism of action and can be used to consistently guarantee the safety of the product. If that is accomplished, I believe the FDA is very open to receiving such an assay for a commercial product.

"To enable the qualification and validation of [bioassays] we need reference standards, controls, and suitability criteria—and that reference material needs to be produced, maintained, and qualified."

Q What is the latest regulatory guidance on potency assays for cell and gene therapy, and are there any gaps or areas of disharmony between regulators?

GZ: I'm by no means a regulatory expert. However, in my field, the ICH Q2(R2) guidelines are coming in June. Those are the latest guidelines that we look at for validation.

In my opinion, sometimes people have unrealistic expectations of the guidelines. The guidelines show you the minimum parameters that your method must pass to be viable. However, they do not tell you about other parameters that are going to be critical depending on where you are implementing your method. An example is instrument reliability. It's a parameter that could make or break your assay depending on where you are implementing it.

If you are implementing it for a commercial method that is seeing thousands of assays and thousands of patients a year, then the wear and tear on that instrument is going to be significant and any failure could hinder your operations because you would not be able to release all those patient samples. In contrast, if you had the same instrument reliability, but the instrument was used in a clinical program that only sees 100 patients a year, where you don't have the same level of wear and tear, you would be less concerned with that parameter. This is where I see a big gap between where the industry is and where the guidelines are.

DW: The FDA released an update to their draft guidelines late last year. What I read from it is that they would like to see potency assays included more in process development for new products, with frequent mention of CQAs, process parameters, and potency assurance strategy. They want applicants for INDs to think about a strategy for potency testing early on.

They expect developers to start with a matrix of assays—multiple assays, capturing multiple modes of actions of your product—and then narrow these down if possible during development. The guidance may still lack some examples, but it is maturing and becoming more formalized, and the industry is evolving.

Q How can assay development be streamlined, while maintaining cost-effectiveness and safety?

DW: This is one of the primary concerns of our clients, who are often working to tight timelines. Nowadays, there is more awareness that they need to think about the assays at the same time as the process. However, some of the assays need a lot of time for development and often developers do not allow enough time. In potency assay development, we are lucky in that we are not required to have a finished potency assay ready for an IND filing.

At a minimum, there should be a plan involving multiple assays that can be used to characterize the process early on. In other words, back multiple horses instead of pinning all your hopes on one. Early on, a lot of the work we do is to look closely at the CQAs in your process and ensure that the assays address those. Some careful thought ahead of time helps a lot with timeline planning afterward.

GZ: I'm a big believer in doing upfront, exhaustive development work. I have seen multiple times in my career that doing bare-bones development gives you an imperfect method at

best. It might be good enough for a Phase 1 clinical trial, but it will not be suitable for Phase 2, Phase 3, or validation of a commercial method.

If you try to move forward with a suboptimal method long term, eventually the FDA or other regulatory agencies will ask you to fix that method. Analysts can end up spending a considerable amount of time trying to fix inherent flaws or even having to start the entire development again with another method.

In addition, post-Biologics License Application method changes are very expensive and time-consuming so saving time upfront by doing the bare minimum in your development will cost you more in the long term. It could also derail your entire implementation plan by halting your clinical or commercial pipeline by a year or more, depending on how long it takes you to develop a robust method afterward.

Q What strategies can we use to address the variability inherent to cell and gene therapies?

GZ: In my opinion, a lot of the variability that we see in cell and gene therapy comes from the fact that most of the methods we currently use are inherently very analyst-intensive, manual methods, with a lot of analyst hands-on time. Whether you look at ELISA or flow cytometry, you could have hundreds of pipetting steps throughout the process over multiple days. That means that the probability of analyst-to-analyst variability is very high.

Given that, one of the strategies that I see in the field to address that variability is automation, because if you can automate certain processes, you reduce some of the variability that's inherent to manual processes.

DW: This analyst-to-analyst variability has been the greatest challenge for us too. Clients with fast timelines, who put all their efforts into one assay, may enter qualification studies and suddenly find out that even trained operators are not able to reproduce the method within the coefficients of variation that we would like to see. As Giorgio points out, going back and re-optimizing the methods is very difficult.

Automation is useful and there are some instruments available in the field that help with that. Miltenyi is putting some effort into exactly this area, trying to automate flow cytometry methods—we see good opportunities in that area.

Another important point is the biological system. All the materials we use in cell therapy are biological materials and inherently variable. Even when using cell lines, you can still see variability. There are some efforts toward replacing those biological systems with more artificial targets (e.g., beads) that mimic cells. These systems do not have the total biological functionality of cell culture, but they can mimic some aspects; for example, they can trigger T-cells to make certain cytokines. There has been some success in that area, and I think that it will evolve. Qualifying new cell lines is a huge effort, so relying on more artificial targets for your cocultures is very beneficial for cost-effectiveness too.

GZ: It is true that automation is not perfect in itself. Dirk mentioned costs, and it has to be addressed that automation is costly, especially in the short term. However, there are long-term benefits if your volumes are high enough to warrant the initial investment.

I also agree that having good processes in place is important to ensure that your target cell lines are very robust across different batches and lots. It also goes back to the question of

"The specification of a product can make or break an operation, so when developing a potency assay, have an eye on your spec."

whether you are using an indirect or direct mechanism of action. An indirect mechanism of action can potentially have more variability because you are measuring the concentration of cytokines versus directly measuring cytotoxic killing with methods such as flow cytometry, bioluminescence, or even the good old chromium-release assay.

Q What technological advances do you see (or would you like to see) coming down the line for CGT assay development?

DW: There are some advanced single-cell technologies that have proven to have value, although we are still exploring what the value is. Sequencing and other techniques have also proven themselves scientifically, but they remain too costly to incorporate routinely. People continue to use very simple methodologies because they are more robust and keep costs down.

I think there should be efforts to be more data-driven—to gain more data from these bioassays and use that data to allow a more detailed response. I'm not seeing that much yet and I feel this is because of the cost and effort it takes to develop those types of assays. I wish there was a bit more data-driven assay development in potency assay development.

GZ: Automation is the low-hanging fruit right now because it does not change the paradigm of how we measure specific parameters; it just reduces variability by removing manual parts from the assay.

My personal opinion is that any new technology that shifts the way we measure something (such as single-cell proteomics), while it may be cutting edge and give you a tremendous amount of information, may not necessarily be the most robust and reliable method. If you are trying to go commercial, I would have some reservations about using new methods versus the tried-and-true methods in use now. We know that the FDA has seen and approved current methods, so they are a little bit more of a safe bet going forward.

However, I do agree that we need to look at new technologies and new ways to measure things. Specifically, going back to predicting clinical efficacy—if you measure direct killing or interferon-gamma alone, it won't tell you how effective your CAR T-cell is in a patient. However, if you were able to access a new type of information that correlates better with clinical efficacy, I think you'd have a game changer.

Q Finally, what best practices would you recommend to cell and gene therapy developers with regard to potency assay development?

GZ: Solid upfront method development is necessary, even crucial. As Dirk said earlier, you probably don't want to put all your eggs in one basket but start by looking at multiple methods and weed them out later. In my opinion, it's really important to look at your

implementation plan and the challenges that you will face wherever you are trying to bring this method, not just in the short term but in the longer term as well. Then understand what parameters are crucial in your longer-term plans for this method and what you need to assess early during your development.

It's also important to consider what your final product is going to be. Is it going to be fresh or frozen cells? Certain methods tend to be more suitable for fresh, while others will be suitable for a frozen final product. It is up to you to understand those factors in advance and choose a method that is suited to you and your process.

The specification of a product can make or break an operation, so when developing a potency assay, have an eye on your spec. Understand what the spec is going to look like and whether it will be wide or narrow. A narrow spec can potentially be problematic simply because you cannot release a lot of the assay or a lot of the patient products that you have if you go outside of that narrow spec.

DW: You should definitely start thinking about assays early. We have often seen people focus on more processes and less on assays. We need to think about all assays, and especially potency, early on.

Another aspect that has worked out well for some of the projects we have done is to have multiple assay ideas in the background. When the clinical trials started, we had one primary candidate for the assay that was run as a release assay, but we also had concomitant research underway testing multiple assays on actual patient samples (not just healthy donors) to see if any were better than the chosen assay. Any opportunity to characterize assays throughout development and clinical trials is very beneficial and should be used.

GZ: Establishing frequent feedback loops is very important. Just as Dirk was saying, test your methods as you go. Challenge it, find the edge of failure, and make different iterations of it as you move forward and gain a better understanding of your product and method. It is crucial that developers continue to improve on methods as they move throughout their entire pipeline process.

BIOGRAPHIES

GIORGIO ZENERE is a dynamic professional known for his commitment to data-driven business innovation. Giorgio has a wealth of expertise in cell and gene therapy spanning both infectious diseases and hematological and solid tumors. Giorgio received a PhD in Cell and Gene Therapy specializing in autologous and allogeneic CAR T-cells against HIV. He subsequently joined the CGTI biotech world, where his focus switched to pre-clinical and clinical drug discovery of CAR T-cells against hematological and solid tumors. Currently, Giorgio leads the CMC development, validation, and specification of novel analytical technologies for commercial CAR T-cell programs at Kite Pharma.

DIRK WINDGASSEN is a results-oriented leader with deep expertise in process and assay development for molecular diagnostics and cell therapy. He currently leads the Miltenyi Bioindustry Assay Development group, driving innovation in San Jose, CA, USA.

Windgassen boasts an impressive track record of commercializing cutting-edge diagnostics. Previously, he led teams at Exact Sciences and Thermo Fisher Scientific, ensuring successful market launch of novel molecular assays. His experience extends to cell therapies, having

played a key role in the BLA submission for Provenge, a pioneering cellular immunotherapy, at Dendreon.

Windgassen holds a PhD in bioprocess engineering from the University of Erlangen-Nuremberg and completed his doctoral thesis on *ex vivo* T cell expansion at Northwestern University.

AFFILIATIONS

Giorgio Zenere PhD MBA
CMC technical project lead,
Global QC Technology Innovation Team,
Kite Pharma

Dirk Windgassen PhD
Director of Analytical Development,
Miltenyi Biotec

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Enabling and optimizing perfusion for intensified production of CAR-T cells in stirred tank bioreactors

As the CAR-T cell therapy sector battles to reduce manufacturing cost of goods and processing timeframes, **Qasim Rafiq**, Professor of Cell and Gene Therapy Bioprocessing at UCL, and **Julia Hengst**, External Collaborations Manager, Cell Culture Technologies at Sartorius, tell BioInsights about recent studies demonstrating the value of stirred tank bioreactors in accelerating CAR-T process development, intensification, optimization, and streamlining scale-up.

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Q What critical challenges are faced by therapy developers and producers in engineered cell manufacturing, particularly for CAR-T cell therapies, and what new emerging solutions and technologies can they use to address these issues?

Although there are now multiple commercially approved CAR-T products, key challenges remain including high COGs, unsustainably high production failure rates, long vein-to-vein process times, and lengthy quality control and product release testing regimens. Furthermore, supply chain management and the logistics of individualized products are complex.

In a bid to overcome those challenges, new trends in the CAR-T cell market are being observed. Specifically, rapid manufacture of CAR-T and *in vivo* CAR-T cell therapy are emerging as new strategies, as is the generation of novel CAR constructs to improve the potency of allogeneic approaches. The intensification of manufacturing processes (e.g., using stirred tank bioreactors) allows the availability of 'off-the-shelf' allogeneic cell therapies at a greatly reduced cost. This requires bioreactor platforms with small-scale, automated models that can be applied for screening and process development to allow for a seamless transition to robust, large-scale manufacturing platforms.

Q What are the advantages and disadvantages of expanding CAR-T cells in stirred tank bioreactors (STRs)? Do you have an example of where CAR-T cells have been successfully expanded in STRs, and if so, what were the results?

STRs are the workhorse of the bioprocessing sector and are used extensively to produce monoclonal antibodies, vaccines and protein-based therapeutics, and there is a reason for this. STRs are scalable, well-characterized from an engineering standpoint and allow for extensive process monitoring and control capability. For CAR-T cell expansion, therefore, we can monitor and control key process parameters such as pH, dissolved oxygen, and agitation. Importantly, we can also adapt the mode of operation, for example moving from batch to fed-batch, to perfusion. In our studies, we found that moving from a fed-batch to perfusion process resulted in a 4.5-fold increase in final CAR-T cell yield. STRs offer proven scalability from a mL scale up to thousands of liters, with significantly reduced facility footprint and improved yield compared with more traditional flask or bag technologies. This also enables process development and optimization at a small scale, thus minimizing the COGs. However, there has generally been a broad misconception in the field that such systems are not suitable for T cell production due to potential sheer stress.

To determine whether CAR-T cells can be manufactured in STRs, our group at UCL has been pioneering the investigations using STRs for CAR-T production since 2017. Working

"In our studies, we found that moving from a fed-batch to perfusion process resulted in a 4.5-fold increase in final CAR-T cell yield."

closely with Sartorius, we have successfully applied the Ambr® 250 bioreactor system for CAR-T cell expansion.

In an initial study the fundamental capabilities of STRs for the expansion of CAR-T cells and the process parameters that impact CAR-T cell quality and functionality were analyzed. In a second study, a quality by design (QbD) approach based on MODDE was applied to investigate whether process intensification in the Ambr® 250 High Throughput Perfusion bioreactor is beneficial for CAR-T yield, and can lead to the reduction of both processing time and COGs.

In the first study, isolated primary T cells were activated with DynaBeads, transduced with lentivirus, and pre-expanded for 7 days in T-flasks. Then, the CAR-T cells were transferred into the unbaffled Ambr 250 vessels with a single 'elephant ear' impeller for improved cell suspension at lower agitations. The CAR-T cells were expanded for 7 days at five different stirring speeds, ranging from 100–500 rpm. As a comparison, CAR-T cells were cultured in static culture in flasks. The viability was observed to be between 90 and 100% for all conditions.

The CAR-T cells were shown to expand well in the Ambr 250 system, and the final cell yield achieved upon the 7-day culture was even higher than in the static condition. It was also shown that CAR-T cells cultured with 200–500 rpm stirring speed reached the highest expansion rates. This is important because it demonstrates the CAR-T cells are not negatively impacted by agitation rates which typically might be required at larger scale, especially for allogeneic approaches. The CAR expression was neither affected by the culture system (static culture or stirring) nor by the stirring speeds investigated [1].

Q How does shortening the pre-expansion period impact CAR-T cell growth kinetics and phenotype?

The question of whether CAR-T cells would grow well after only 3 days of pre-expansion and a single activation on day 1 was investigated. We compared a 3-day and a 7-day pre-expansion process prior to inoculation in T-flasks. It was found that the CAR-T cells that are pre-expanded for 3 days grow better during the 7-day culture in flasks and reach a higher fold expansion, whilst maintaining all desired quality attributes.

Analyses by flow cytometry revealed that the 3-day expanded cells are less differentiated, meaning they have increased frequencies of naïve and central memory CAR-T cells. They are also less activated and exhausted due to lower CD69 or PD-1 and Lag-3 expression levels, respectively. Using the Incucyte®, for quantitative live-cell imaging, we were able to qualitatively

"The culture in the Ambr 250 bioreactor confirmed the data obtained in T-flasks with better CAR-T cell growth..."

demonstrate that both conditions resulted in effective killing of the target cancer cells *in vitro*. In addition, the secretion of interferon γ and tumor necrosis factor α was measured upon the co-culture of CAR-T cells and target cells with iQue Qbeads®. In contrast to the untransduced T cells, CAR-T cells from all conditions secreted both cytokines.

In summary, the shorter seed train is beneficial for CAR-T cell expansion in T-flasks, especially regarding growth kinetics, phenotype and, to a lesser degree, functionality. We then analyzed the effect of a reduced pre-expansion prior to inoculation in the Ambr 250. The culture in the Ambr 250 bioreactor confirmed the data obtained in T-flasks with better CAR-T cell growth when applying a 3-day pre-expansion. This brought us to the question of how further process parameter optimization could improve the CAR-T cell expansion in STRs.

Q How was the CAR-T cell expansion process intensified?

We compared the fed-batch process to a perfusion process with the Ambr 250 High Throughput Perfusion. To do so, we applied a design of experiment (DOE) approach to study a range of process parameters in the Ambr 250 in order to understand their impact on CAR-T cell expansion, phenotype, and function, with a view to optimizing the process.

The DOE methodology provides a systematic approach to studying the impact of each process parameter and its concomitant effect on the other investigated parameters, thereby enabling a larger design testing space to be investigated. Consequently, a more representative optimum can be identified when comparing with one-factor-at-a-time studies.

In our case, an experimental space based on 32 CAR-T cell expansion runs in the Ambr® 250 High Throughput Perfusion was established with the aim of identifying critical process parameters (CPPs) for optimized CAR-T cell growth. In this setting, two cell culture media and the impact of two perfusion parameters were tested. As donor-to-donor variability is a challenge for CAR-T cell production, the robustness of the identified process parameters was tested by including T cells that originated from three different healthy donors.

The MODDE® software platform was used to establish the DOE studies and applied to analyze the data and identify the best operating window for each medium while taking the two tested perfusion parameters, as well as the CAR-T cell fold expansion, into account. The analysis revealed that there was some heterogeneity between the donors, and that the best operating window for the process differs between the two cell culture media of which one was the serum-free, xeno-free 4Cell® Nutri-T cell culture medium.

Q Can process intensification methods lead to a reduction in process time or an increase in final cell yield?

The benefits of perfusion compared to a fed-batch process and the importance of the perfusion parameter settings became obvious when looking at the potential for CAR-T cell growth. When using the 4Cell Nutri-T cell culture medium the CAR-T cell expansion could be increased by more than 4.5-fold with perfusion as compared to the fed-batch process while maintaining a viability above 90%.

Furthermore, the expanded CAR-T cells had a beneficial phenotype, being mainly naïve and central memory cells. Interestingly, the continuous 7-day culture in the Ambr 250 resulted in the gradual down-regulation of the expression of early activation and exhaustion markers, which were increased at the timepoint of inoculation due to prior activation and transduction.

Therefore, process intensification can lead to the reduction of process time or to increased final cell yields, as CAR-T cell expansion is increased by more than 4.5-fold with the optimized perfusion parameter settings.

Q What was the culture duration from thaw to harvest in the STR experiments?

The primary T cells were thawed, activated on day 1, transduced on day 2, and then pre-expanded for 3 days. Thereafter, the CAR-T cells were transferred to the Ambr 250 bioreactor and expanded for a further 7 days.

Q How can the process be tailored for cGMP production?

As the Ambr 250 is not cGMP, this benchtop system is designed and used for process optimization and development prior to scale-up (or for later process characterization). The optimized process parameters from the Ambr 250 can be seamlessly scaled up to the Univessel® STR allowing for application of the intensified process for allogeneic approaches at multi-liter scale. To demonstrate this, we are currently up-scaling from Ambr 250 to the Univessel stirred tank single use (SU) 2 L benchtop bioreactor, which in future will be cGMP-grade. The Univessel Glass vessels, ranging from 2 L to 10 L, are also suitable for cGMP.

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BIOGRAPHIES

QASIM RAFIQ is a Professor in Bioprocess Engineering at University College London, with a focus on novel therapeutic modalities including cell and gene therapies. He is a multi-disciplinary engineer working at the life science, engineering and commercial interfaces with a research focus on the bioprocessing, automation and biomanufacture of cell and gene-based therapies. He currently leads a research portfolio of >£7.5 million as Principal Investigator and leads a dynamic interdisciplinary research group that collaborate internationally with high-calibre academic institutions, industry partners, and leading clinicians. Qasim is both a chartered engineer (CEng) and chartered scientist (CSci) and sits on multiple scientific and engineering committees including the the BIA's Cell and Gene Therapy Advisory Committee.

JULIA HENGST is an External Collaborations Manager at Sartorius, where she builds and leads partnerships through scientific collaborations around immune and stem cell therapies. She holds a PhD in Immunology and has several years of experience in product management for biomedical research and cell and gene therapy clinical applications.

AFFILIATIONS

Qasim Rafiq
Professor of Cell and Gene Therapy Bioprocessing,
UCL

Julia Hengst
External Collaborations Manager,
Cell Culture Technologies,
Sartorius

AUTHORSHIP & CONFLICT OF INTEREST

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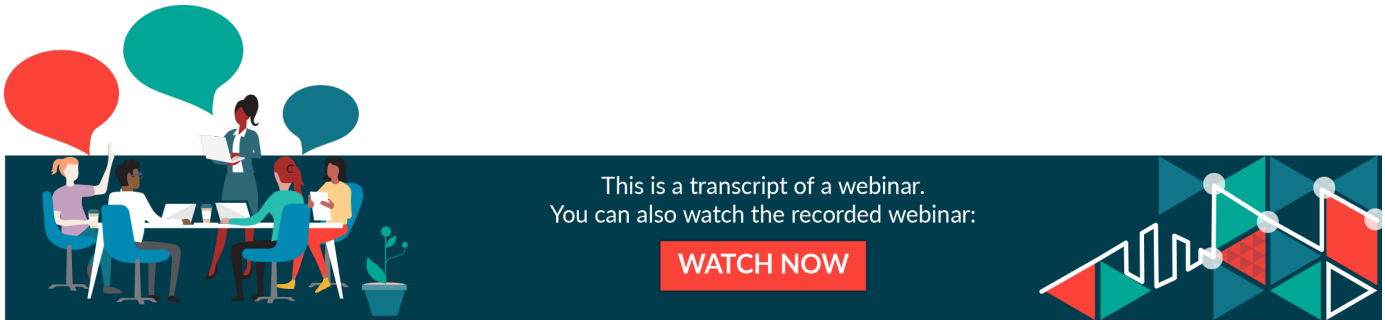

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Intensified production of recombinant vesicular stomatitis virus-based vectors by tangential flow depth filtration

Béla Brühlmann, Field Application Scientist, Upstream, Repligen and **Sven Göbel**, PhD Researcher, Max Planck Institute for Dynamics of Complex Technical Systems

Oncolytic viruses offer an elegant approach to cancer therapy. This poster explores how batch-based manufacturing strategies can be intensified to address the unprecedented demand for high-dose inputs of infectious virus. The content is based upon an open access paper published in *Applied Microbiology and Biotechnology* (https://link.springer.com/article/10.1007/s00253-024-13078-6).

INTRODUCTION

Oncolytic viruses offer a dual mode of action approach to cancer therapy, causing direct cell lysis while also stimulating immune responses to target the tumor. Fusogenic recombinant vesicular stomatitis virus-Newcastle disease virus (rVSV-NDV) is a novel and promising example of an oncolytic rVSV platform. This chimeric virus was created by replacing the native surface glycoproteins of VSV with the fusogenic surface glycoproteins of NDV, thus combining the benefits of both parental strains. Here, a proof-of-concept study to evaluate KrosFlo® tangential flow depth filtration (TFDF®) as a perfusion system and for continuous harvest and clarification of fusogenic oncolytic rVSV-NDV is described.

TFDF AS A PERFUSION SYSTEM FOR rVSV-NDV: A CASE STUDY

Case study set-up

The case study production run set-up is detailed in **Figure 1**. A 3 L stirred-tank bioreactor (STR) system was used for viral production. The perfusion rate was controlled either manually (I) or using a capacitance probe (II).

Figure 1. TFDF setup for perfusion cultivations rVSV-NDV.

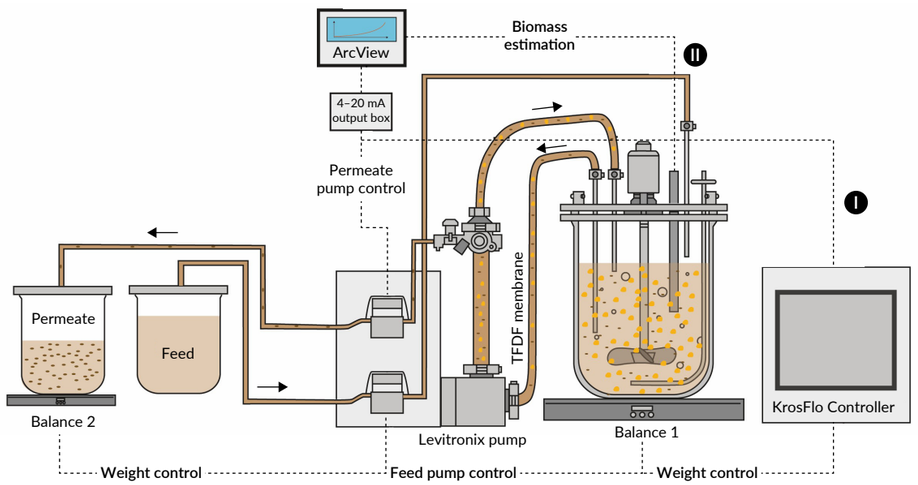
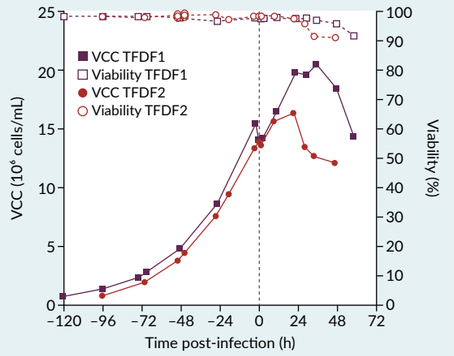


Figure 2. Cell growth of BHK in STR perfusion mode.



The production workflow began with an initial batch growth phase until a viable cell concentration of 4×10^6 cells/mL was achieved. Perfusion then began and cells were expanded until 4×10^6 cells/mL was reached. Complete media exchange was performed before infection with rVSV-NDV. After a 1–2 hour pause at 34 °C, perfusion was restarted and maintained at a fixed exchange rate before continuous harvest and clarification.

Case study results

Figure 2 shows the results of the cell growth of BHK cells in STR in perfusion mode. Two runs were performed: TFDF1 (manually controlled perfusion rate) and TFDF2 (capacitance-based perfusion rate). For both runs, viable cell concentration (VCC) was increased by 5-fold compared to batch processes, while maintaining high viabilities after infection until the final harvest.

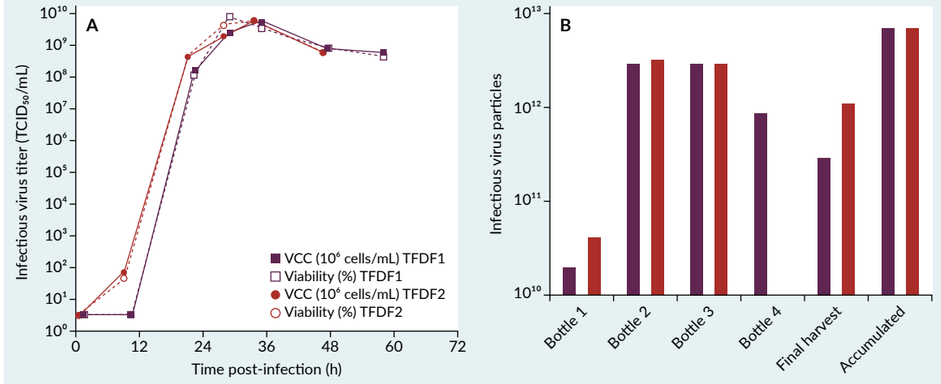
Low rVSV-NDV retention and reproducible productivity with TFDF perfusion were also achieved, as shown in **Figure 3A**. For both runs, maximum titers of ~5 to 7×10^9 $TCID_{50}$/mL were achieved, approximately 11-fold higher than optimized batch processes.

In addition, the permeate was separated into several bottles which were exchanged every 12–24 hours to prevent loss in viral infectivity. Through analysis of the total infectious viral particles per bottle, no benefit for continued production after 48 hours was found. The total media consumption during cell growth for TFDF2 was reduced by 15% and the production time was cut by 12 hours without any loss in accumulated infectious virus particles (**Figure 3B**).

SUMMARY

The intensified TFDF processes achieved the highest infectious virus titers reported so far. Compared to an optimized batch process, 11-fold higher infectious virus titers were obtained, resulting in a 460% increase in space-time yield. Overall, the proof-of-concept study for TFDF as a perfusion system enabeling continuous rVSV-NDV harvest and clarification in a single-step, was successful.

Figure 3. Infectious virus titer ($TCID_{50}$/mL) rVSV-NDV in BHK-21 in STR.



CELL & GENE THERAPY INSIGHTS

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In partnership with: REPLIGEN INSPIRING ADVANCES IN BIOPROCESSING; MAX-PLANCK-INSTITUT FÜR DYNAMIK KOMPLEXER TECHNISCHER SYSTEME MAGDEBURG

FASTFACTS

Leveraging rapid sterility testing to advance cell therapy production

Maya Dubey, Scientific Content Manager, BioProduction Group—Pharma Analytics, Thermo Fisher Scientific

Contamination can occur throughout the complex cell therapy manufacturing workflow. Rapid sterility testing provides a solution, as it can be leveraged for testing raw materials, in-process sterility monitoring, viral vector manufacturing, in-process control, and as part of lot-release testing of the product. This poster explores the Applied Biosystems™ SteriSEQ™ Rapid Sterility Testing Kit, which offers reliable results in less than a day while utilizing minimal sample volume, preserving more of the drug product for therapy.

Given the extensive role of living cells in the workflow, cell therapy products are susceptible to microbial contamination, thereby posing significant risks to product quality and safety. Traditional sterility testing methods rely on microbial growth-based detection, which requires either a 14-day culture period or a 7 day-rapid growth-based test. However, this timeline can be problematic for cell therapy products as they have limited shelf life, and most commonly require immediate administration to patients. To address this issue, the SteriSEQ Rapid Sterility Testing Kit utilizes rapid sterility testing methods—namely, qPCR to test for both bacterial and fungal contamination, providing actionable results within 5 hours.

THE STERISEQ RAPID STERILITY TESTING KIT

As shown in **Figure 1**, the SteriSEQ Rapid Sterility Assay workflow is streamlined, providing an easy-to-implement approach to contamination detection. This kit can be applied to a variety testing points within the production workflow, and is designed to detect bacteria and fungi within 5 hours. It is a multiplex assay with an internal positive control and a discriminatory positive control, as well as probes designed specifically for bacteria and fungi. These integrated controls allow for the reduction of false positives, helping to ensure consistent performance and accurate data.

Figure 1. The SteriSEQ Rapid Sterility Assay workflow.

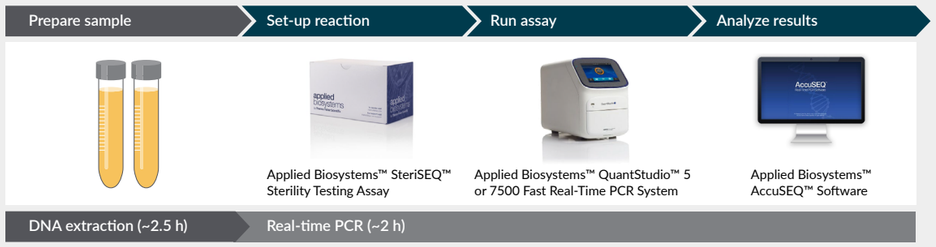
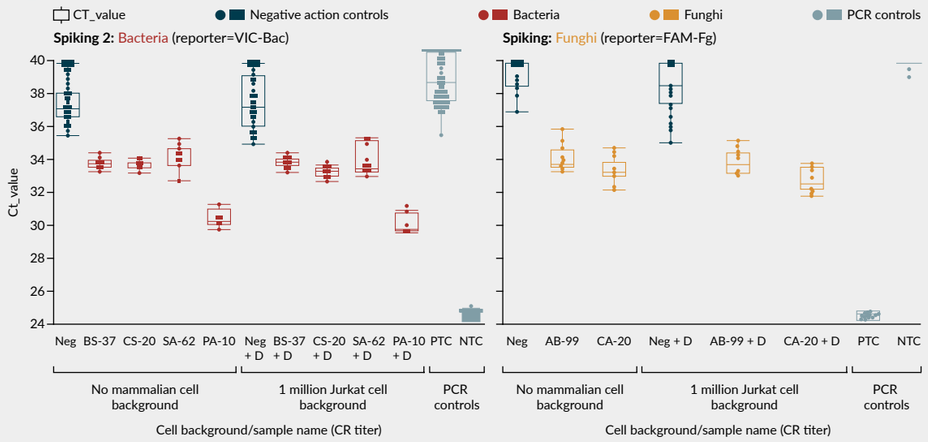


Figure 2. Limit of detection for SteriSEQ Rapid Sterility Testing Kit.



CASE STUDY: EVALUATING LIMIT OF DETECTION VALUES FOR USP <71> SPECIES

Using the experimental setup outlined below, the SteriSEQ Rapid Sterility Testing Kit was evaluated for limit of detection (LOD).

Experimental setup:

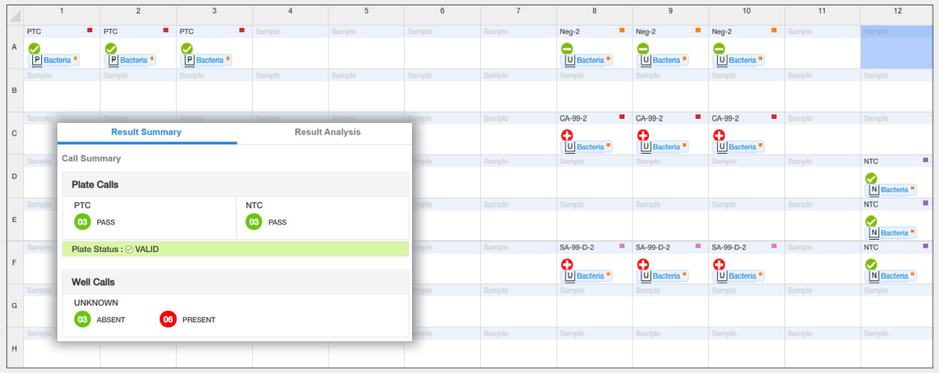
- Without matrix: 100 µL sample
- With matrix: 50 µL bacteria/fungi + 1 mL of matrix

Samples were prepared and eluted in 80 µL, and each PCR reaction was run with 13 µL of eluted sample. As shown in **Figure 2**, six species were detected within the LOD range, and the kit was shown to be compatible with cell culture matrices containing 10^6 mammalian cells. Species detected included: *Aspergillus brasiliensis*, *Candida albicans*, *Bacillus subtilis*, *Clostridium sporogenes*, *Pseudomonas aeruginosa*, and *Staphylococcous aureus*. Numbers following the species abbreviation reflect the titer (in colony forming units (CFU). LOD values were between 5–50 CFUs per reaction. The data consists of two independent experiments, two extraction replicates, and three PCR replicates. In addition, centrifugation followed by extraction was performed to reduce floating DNA by 99%, ensuring that the sample contained only live DNA.

EASY-TO-READ REPORTS

As shown in **Figure 3**, the reports produced by the SteriSEQ Rapid Sterility Testing kit are straightforward, simplifying the analysis process. On the left, the Applied Biosystems™ AccuSEQ™ Analytical Software summary illustrates positives and negatives; on the right, columns eight to ten show contamination.

Figure 3. Exemplar results summary produced by the Applied Biosystems AccuSEQ Analytical Software.



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In partnership with: ThermoFisher SCIENTIFIC

FASTFACTS

Efficient AAV purification with AAVX and AAV9 magnetic beads

Shu Uin Gan, Principal Investigator and **Kian Chuan Sia**, Senior Research Fellow, Phoenix Lab of Gene and Cell Therapy, National University of Singapore

The development and production of high quality recombinant AAV-based therapeutics relies heavily on an efficient downstream purification process. This poster explores an alternative to chromatographic affinity capture that eliminates the need for universal nuclease treatment by utilizing magnetic beads with CaptureSelect™ ligands specific to AAV. It outlines a quick and simple method for bead-based AAV purification, which could have significant implications for gene therapy research.

EARLY-STAGE PURIFICATION OF AAV

The recently launched Dynabeads™ CaptureSelect™ AAVX and AAV9 Magnetic Bead-based purification system provides a solution for the affinity capture of recombinant AAV. This method offers high purity and yield in a single purification step, high specificity, high capacity, and improved process efficiency. The AAVX/AAV9 Magnetic Beads use the same affinity ligand as a chromatography resin, and do not require columns for purification. General purification steps are demonstrated in **Figure 1**.

AAVX/AAV9 Magnetic Beads also do not require a universal nuclease treatment step. When a crude AAV9 supernatant with universal nuclease and a crude AAV9 supernatant without universal nuclease were subjected to AAV9 Magnetic Bead purification, the yield of AAV9 with or without the pre-treatment of nuclease was very similar. The Magnetic Beads were able to bind efficiently to the supernatant even in the absence of universal nuclease. Non universal nuclease treated AAV also proved to be just as pure after Magnetic Bead capture as the nuclease treated samples (**Figure 2**).

Furthermore, binding capacities for AAV9 using AAV9 Magnetic beads, were measured at scales from 0.5 mL to 20 mL crude supernatant harvest. This demonstrates a successful 40-fold scale-up.

CASE STUDY: AAVX MAGNETIC BEADS PURIFICATION OF AAV8 VIRUS

To further test the capabilities of this method, AAVX Magnetic Beads were used to purify AAV8 virus in varying parameters such as incubation time and sample volume.

As shown in **Figure 3**, to test for the maximum binding capacity of the AAVX magnetic beads, increasing total vector genome (vg) of AAV8 in a fixed volume of 0.5 mL was used. There was minimal breakthrough at 5×10^{11} total vg and breakthrough occurred when the titre is greater than 5×10^{11} vg. In summary, the maximum binding capacity using 0.5 mL of purified AAV is approximately 5×10^{11}/40 µL magnetic beads slurry.

Furthermore, the recommended sample volume to slurry ratio for capture is 0.5 mL to 40 µL (12.5-fold). When the AAV load was fixed at 1×10^{11} vg but the sample volume was increased from 0.5 mL to 2 mL (12.5-fold to 50-fold ratio), the AAV in the flow-through increased from 0.68% to 4.86%. With a maximum of 5×10^{11} vector genome copies, increasing the supernatant volume from 0.5 mL to 10 mL (12.5-fold to 250-fold ratio) significantly increased AAV in the flow-through, reducing recovery to 30%.

Lastly, after determining binding capacity using purified AAV, the capture of AAVX magnetic beads with filtered AAV8 supernatant from adherent packaged 293T cells (not treated with universal nuclease) was tested, achieving a good recovery of approximately 87%.

CaptureSelect ligands and resins: For Research Use or Further Manufacturing. Not for diagnostic use or direct administration in humans or animals.

Figure 1. Purification steps using AAVX Magnetic Beads.

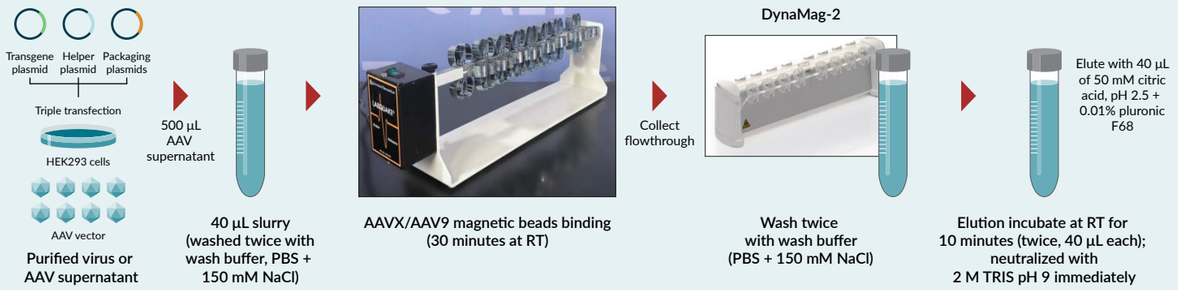


Figure 2. Purification steps using AAVX Magnetic Beads.

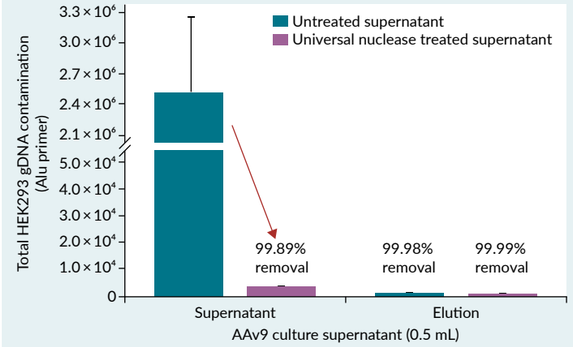
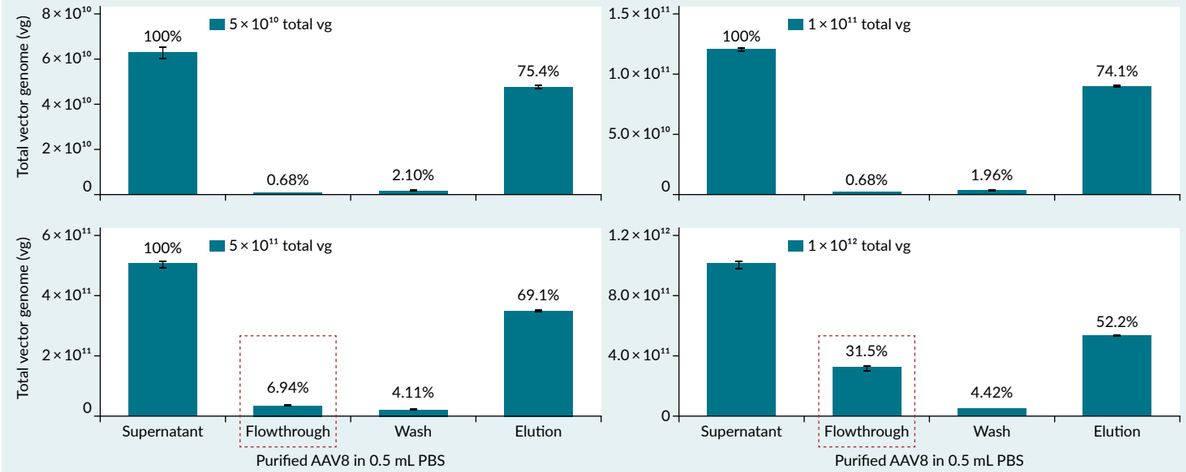


Figure 3. Binding capacity of purified AAV8 to AAVX magnetic beads.



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In partnership with: ThermoFisher SCIENTIFIC