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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Raw and starting materials

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CELL & GENE THERAPY INSIGHTS

Volume 7, Issue 2

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

FOREWORD

Raw and starting materials

ELIZABETH READ, MD is an independent consultant focusing on CMC development and CMC regulatory issues for cell- and tissue-based therapies. Dr. Read received her M.D. from the State University of New York (Buffalo, NY). After clinical training in Internal Medicine, Hematology, Oncology, and Blood Banking/Transfusion Medicine, Dr. Read worked at the National Cancer Institute and later in the Clinical Center's Department of Transfusion Medicine at the National Institutes of Health (NIH; Bethesda, MD), where she served as Section Chief and Medical Director of the Cell Therapy Core Facility from 1995-2006. Initially engaged with novel cellular therapies in the context of hematopoietic transplantation, she later worked on more complex cell, tissue, and gene therapies for a range of clinical indications. From 2007-2010, she headed the Cell Therapy Program at Blood Systems Research Institute (San Francisco, CA), collaborating with UCSF investigators on grant-funded stem cell projects. She previously served as Medical Director at the American Red Cross Blood & Tissue Services (Los Angeles, CA). Over the past 10 years, she held leadership positions at small biotech companies, including Fate Therapeutics (San Diego, CA), StemCyte (Baldwin Park, CA), Medeor Therapeutics (San Mateo, CA), and Adicet Bio (Menlo Park, CA). Dr. Read has authored over 100 scientific publications, and has served as a faculty lecturer at UCSF and for the American Course on Drug Development and Regulatory Science. She has served on advisory committees focused on quality, safety, and efficacy of blood products and cell, tissue, and gene therapies, including the American Association of Blood Banks, the US DHHS/HRSA Advisory Council for Blood Stem Cell Transplantation, and the US Pharmacopeia. She currently serves as an advisor on clinical-stage cell therapy projects funded by the California Institute of Regenerative Medicine.

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The global COVID-19 pandemic has had profound impacts on us as individuals and society. Many business sectors have suffered from loss of customers or under public health restrictions, but the biotech and pharmaceutical industries have proven resilient, despite difficulties in clinical trial execution and manufacturing. For cell and gene therapies (CGT), the pandemic provided an unprecedented stress test to the processes for ensuring the supply of high quality raw and starting materials. The current issue of *Cell and Gene Therapy Insights* addresses both old and new challenges for these materials, with perspectives from industry experts.

Cellular starting materials, arguably the most critical determinants of final product quality, are inherently unstable, and subject to a high degree of variability. Barbara Bonamassa and colleagues provide a regulatory perspective on the causes and impacts of starting material variability in both autologous and allogeneic settings. The editorial by Sanjin Zvonić highlights strategies for understanding and controlling cell collection variability during development of autologous patient-specific therapies. The expert insight of Ben Weil and Mark Lowdell analyzes pooling of cellular starting materials from multiple donors as a means not only to reduce variability, but also to overcome quantitative limitations in the commercialization of allogeneic, off-the-shelf products. An interview with Nate Manley describes evolving expectations for qualification of cellular starting materials, as well as new trends, such as increasing use of cryopreservation to manage cell stability over the supply chain.

Raw materials used in CGT are heterogeneous in characteristics, grade, and purpose. Use of risk-based approaches to qualification and control of raw and starting materials is the subject of a commentary by Sophie LeBrun and Carmen Brenner, who also provide practical examples. This theme is further expanded in an expert insight by Monica Nelson, who explains the USP risk-based approach and provides a quality perspective on measures for managing and documenting quality of raw materials. An interview with Richard Stout offers an enlightening view into management of supply chain risk, whether during a pandemic or not. Finally, Gary du Moulin addresses the critical role of quality audits, and details best practices for remote audits, in case a pandemic or another emergency impedes on-site audit activities.

Despite evolution of regulatory guidance and industry best practices for CGT raw and starting materials, the risk of transmissible disease from human and animal-derived materials remains a persistent issue, because cell-based therapies cannot be terminally sterilized. Regulatory requirements for screening and testing of human donors have been established, but require modification over time. The COVID-19 pandemic has demonstrated yet again that emerging infectious diseases will always be part of the landscape, and the CGT industry needs to respond to these threats. A recent example is the January 2021 guidance of the US Food and Drug Administration (FDA), “Manufacturing Considerations for Licensed and Investigational Cellular and Gene Therapy Products During COVID-19 Public Health Emergency.” [1] FDA acknowledged that respiratory viruses (which include SARS-CoV-2, the causative agent of COVID-19), are not known to be transmitted by administration of CGT products, but raised caution based on scientific reports of SARS-CoV-2 tissue tropism, and considering that much is still unknown. The guidance advises COVID-19 screening measures for CGT autologous donors similar to screening of allogeneic donors, but does not recommend testing. It also advises manufacturers to perform a SARS-CoV-2 risk assessment addressing donors, cell/tissue source, potential for viral propagation in manufacturing, and potential for transmission to facility personnel and contamination within the GMP environment. Description of the SARS-CoV-2 risk assessment and mitigation strategies are now expected to be included in regulatory submissions to FDA.

I’ve often reflected on how dynamic the CGT field is, and how much I’ve learned

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from the expertise of its diverse constituency. I hope you, too, will benefit from the experience of others by reading these articles, and even find nuggets of wisdom to use in your work now and into the future, when COVID-19 is behind us.

REFERENCE

1. US Food and Drug Administration. Manufacturing Considerations for Licensed and Investigational Cellular and Gene Therapy Products During COVID-19 Public Health Emergency. Guidance for Industry. January 2021.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

EDITORIAL

Considerations for controlling the variability of raw biological materials in the manufacturing of autologous patient-specific therapies

"...developers should consider seeking solutions to minimize the variability of the raw material driven by the collection practices as early as feasible in the product lifecycle."

SANJIN ZVONIC, VP, Product Development and Manufacturing

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Over the last two decades, the advances in the field have established cell and gene therapies (CGT) as viable therapeutic modalities with the potential to address previously unmet medical needs, as well as offer alternatives to existing therapeutics, either alone or in combination with small molecule and/or biologic drugs. In such context, perhaps the most exciting application of CGT may be in the area of personalized medicine. In fact, a large portion of currently commercially approved CGTs are autologous patient-specific therapies (APST), where the 'raw' biological material is sourced from the patient and manufactured into a drug product intended for the treatment of that same patient.

While the advent of APST has created unique opportunities for therapeutic development, it has also generated a unique set of challenges from the perspective of manufacturing and commercialization of these therapies. Notably, the inherent biological variability of the raw biological material (e.g., peripheral blood apheresis, bone marrow aspirates, primary tumor biopsies, etc.) collected from different patients creates a significant challenge in the development of the downstream manufacturing and analytical processes. These processes are expected to possess a high level of uniformity, reproducibility, and robustness, in order to unlock the operational and financial economies of scale to obtain regulatory approvals and position these therapies for commercial success.

Faced with this challenge, both drug and technology developers have made great advances in creating and implementing operational approaches and technologies throughout the downstream process, aimed at addressing the inherent variability of the upstream material input. However, given the fact that the collection of the actual raw biological material is performed at collection sites by local staff, there is a limitation to how much standardization (not to be confused with control, which is something essential to meet GMP and other regulatory requirements) a developer can implement, without the ability to make tremendous investment in the deployment of bespoke technological solutions for this specific purpose. As such, developers are often faced with a situation where they must carefully balance the existing capabilities and practices at the collection sites with the need to standardize material collection, without implementing solutions and procedures that will create undesirable operational, financial, and scalability challenges in the commercial setting.

Following are considerations for APST developers looking to standardize collection procedures with the goal of minimizing raw material variability:

UNDERSTAND YOUR PRODUCT

The first, and perhaps most important, step in the process is to understand the feasibility and value of focusing on raw material variability. Developers should perform detailed scientific evaluation to determine a) what factors/elements constitute biological variability in the context of their raw material, b) what are the impacts of these factors on downstream process uniformity, reproducibility, and robustness, and c) whether the customization and standardization of the upstream collection practices can effectively impact the variability of these factors/elements. However, to effectively answer these questions, one must develop a meaningful characterization dataset, which makes it less feasible to address this topic in situations where developers are creating a first-of-a-kind product or those starting an early phase trial. Therefore, before proceeding ensure there is a good understanding or whether and when one should undertake this work.

FOCUS ON VALUE CREATION

Aside from understanding whether and when to undertake the work, developers should focus the scope of work towards creating specific

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value (e.g., minimizing COGs by allowing standardized use of downstream materials at scale) while avoiding the creation of cost and scalability factors upstream (e.g., requiring bespoke/specialized equipment, or highly skilled labor). In reality, there will always be a tension between process optimization/standardization, and the overall impact on operational complexity and cost. The developer should understand the value that focusing on raw materials will generate for the overall program(s) and drive the scope and strategy of the work to be undertaken based on this understanding.

LEVERAGE EXISTING CAPABILITIES TO THE GREATEST EXTENT APPLICABLE

In the context of APST, raw material collection is typically performed at established and specialized collection sites that have been using relevant operational and technological capabilities in support of their own patient treatment procedures. Furthermore, their own procedures and capabilities are likely subject to a certain level of compliance and standardization by relevant bodies (e.g., FACT). The developers would be wise to leverage this existing framework of capability and expertise as the foundation of their own control and standardization strategy. To start, developers should focus on relationships with collection sites that have established the requisite level of capability and expertise. Then, a comprehensive review of the capabilities across multiple sites should be performed to understand the commonalities and differences among them. In order to minimize the complexity and the burden of oversight across multiple centers on the developer, one should consider leaving as many operational elements as practical to be performed 'per site procedure'. However, the developer needs to understand the impact of those considerations on the variability of raw materials collected at each site, as well as among sites. Therefore, in certain cases, a developer may need to introduce bespoke procedures/technologies to address the needs of their program(s) despite seemingly appropriate procedures already existing at the collection sites.

THOUGHTFULLY INTRODUCE ANY MODIFICATIONS

When planning to introduce bespoke procedures/technologies to the collection sites, the first thing to keep in mind is the fundamental difference in the focus among the manufacturing and clinical environments. Namely, while in the manufacturing environment the focus is on the product, in the clinical setting the focus is on the patient. Thus, the procedures/technologies being implemented should be designed in a way that does not deter the collection site staff from their focus on the patient. Furthermore, one should keep in mind that the collection site staff work on multiple programs and support multiple medical procedures. Many of these likely share a great deal of technical and operational commonalities. Therefore, if the developer keeps these attributes in mind while designing their bespoke solutions, they will be able to further leverage established operational expertise at the sites. Ultimately, the developer should regard the collection sites not as service providers but as end-users of their bespoke procedures/technologies. As such, the upfront work of understanding their users' needs, capabilities, and challenges should be performed prior to endeavoring to create solutions which balance the needs of the developer as well as those of the collection sites.

Managing the impact of the inherent biological variability of the starting raw material on APST manufacturing will continue to present the developers with significant challenges. As part of the overall strategy to address this challenge, developers should consider seeking solutions to minimize the variability of the raw material driven by the collection practices as early as feasible in the product lifecycle.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

Developing an understanding of the analytical landscape for testing complex biological raw materials in advanced therapy medicinal products: a CRO perspective

Alistair Michel & David Neville

Unlike antibody-based products that are generally single, highly purified proteins, ATMPs are complex products. Production involves the use of new technologies and older technologies that are adapted to ATMP production. Because of these additional complexities, the regulatory expectations for this type of product have also increased. An important aspect of the manufacture of cell and gene therapy products is the role of raw materials and the controls required to ensure consistent product quality and ultimately patient safety. Important aspects of analytical approaches to ensure raw material quality will be discussed in this White Paper.

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INTRODUCTION

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells and can generally be classified into three distinct categories: gene therapy, somatic-cell therapy and tissue-engineered medicines. All ATMPs function by manipulating the target biological system to treat the disease.

An important aspect of the manufacture of cell and gene therapy products is the role of raw materials and the controls required to ensure consistent product quality and, ultimately, patient safety. The fundamentals of Good Manufacturing Practices (GMP) still apply. Importantly, within the EU the terms 'starting materials' and 'raw materials' must also be distinguished. Starting materials (SM) cover the human cells and tissues used in the final product, whereas raw materials are defined within EU Directive 2001/83/EC [1].

Not all companies have the ability or resource to perform the required raw materials testing. In such instances, partnerships with CROs are considered, and the choice may be dictated by the regulatory status (EMA/FDA inspected and approved), experience and knowledge around the product area. The CRO must have an understanding of what may be required and can help to guide clients through the testing regime. This paper will not cover all of the aspects that are fundamental to ensure the quality of the product is as required but does consider the points that are important when you are forming partnerships with a CRO to perform aspects of the raw materials testing.

The characterization of materials from reliable suppliers, using developed and validated methods or pharmacopeial methods, are key elements to maintaining a dependable supply of products. Additionally, it is not just the raw materials used to manufacture the biological product, but the packaging used, possible interactions with medical devices, and impurities like sub-visible particles, leachables, extractables, which also need assessment. The identification and validation of appropriate methods, and the identification of appropriate partners to perform these analyses, are important aspects to consider.

As ATMPs are not single biomolecular entities, there may be differences in approach to what is required for antibody-based products. In certain cases, research grade chemicals may be used as part of the development process and may not be GMP-sourced. In such instances, the developer much provide evidence that the raw materials are of the highest grade/quality possible but must still provide evidence that the material is safe and does not provide a risk for the patient. For certain treatments, the donor cells are derived from the patient and it is not possible to test to defined specifications and, for tests that might be employed, greater variability might be observed, e.g. cell depletion or enrichment using monoclonal antibody-based approaches. Additionally, it is possible that starting materials may arise from a non-GMP facility. Therefore, there are additional factors that need to be considered through the product lifecycle for an ATMP, nit observed for more traditional antibody-based therapies.

Currently, the major products from the biopharmaceutical industry have been recombinant proteins and antibody-based therapies, both originator molecules and biosimilars. However, the number of cell and gene therapy medicinal products under development is increasing. Currently, there are 1109 clinical trials worldwide, with 97 in Phase 3 [2], and 154 trials ongoing in the UK as of Dec 2020 [3], which is approximately 14% of the world total. In addition, in 2019 there were 11 approved treatments within Europe [4] and 18 in the US [5].

This document does not cover all the required tests for all regulatory domains for cell and gene therapy products (e.g. FDA, EMA, MHRA) but, where referenced, will focus on the regulations for the European market in the main. The document gives a CRO perspective that may be involved in the testing

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process, and the awareness and expertise that the CRO must have to allow the client to produce a safe product of the desired quality. However, similar adherence to quality, testing, supply, etc. of raw materials will apply across the different agencies. For non-EU markets, the region-specific regulations must be understood and followed; further information that is specific to your proposed market can be obtained from the relevant regulatory authorities.

RAW MATERIALS

Within the EU and outlined in 'Guidelines of 22.11.2017 Good Manufacturing Practice for Advanced Therapy Medicinal Products' are the factors required to ensure product manufacture that complies with Good Manufacturing Practice (GMP). Sections 7.1 and 7.2 are applicable to the understanding of the thought processes that must be considered during the production of ATMPs. Importantly, Section 7.1 states "The quality of starting and raw materials is a key factor to consider in the production of ATMPs. Particular attention should be paid to avoiding contamination and to minimizing as much as possible the variability of the starting and raw materials".

Within the EU the terms 'starting materials' and 'raw materials' must also be distinguished. Starting materials (SM) cover the human cells and tissues used in the final product, whereas raw materials are defined within EU Directive 2001/83/EC [1]. These definitions are further clarified in a concept paper released by the industry association European Biopharmaceutical Enterprises (EBE). The paper is entitled "Management and Control of Raw Materials Used in the Manufacture of Biological Medicinal Products and ATMPs" [6] and states that for ATMPs, raw materials (RM) are process inputs that are not intended to be part of the final product. By the definitions found in the directive and concept paper, RM cover the chemically defined growth media, process buffers, cryopreservation solutions, chemical transfection agents, and also cytokines, growth factors, enzymes, etc.

An important point also mentioned within the concept paper is made regarding excipients, which although not a RM, can be managed and controlled by a similar approach. The paper defines excipients as "pharmaceutically inactive components of the final formulation that are required to maintain the activity and stability of the active pharmaceutical ingredient and bring suitable functionalities of the defined dosage form." For ATMPs, water for injections, simple buffer solutions and stabilizers such as sucrose would be defined as excipients. Excipients can also include higher risk excipients such as human serum albumin and dimethyl sulfoxide (DMSO).

RM are, therefore, subject to the full process and quality regimes of GMP regulations. For ATMPs, Annex 2 of the EMA GMP guidelines is also applicable and although not the subject of this white paper, some aspects will be more stringent than for conventional therapeutics [7].

One further point to note is that raw materials can be synthesized chemical entities or of biological origin. For raw materials of biological origin, General chapter 5.2.12 of the European Pharmacopeia – 'Raw materials of biological origin for the production of cell-based and gene therapy medicinal products' is relevant. This chapter's overall aims are the following:

1. Identify the critical quality attributes of raw materials of biological origin
2. Harmonize variable practices and make the regulatory expectations more predictable
3. Encourage raw materials manufacturers to provide consistent, predefined quality and to record and share information on the origin and quality of the raw material
4. Help users managing batch-to-batch variations and changes in raw materials.

RAW MATERIALS TESTING

As biological products, the testing of raw materials may involve the use of established pharmacopeial methods, as well as the development and validation of novel methods. RM should be produced following applicable GMP guidelines to provide documented evidence of purity, potency, consistency, stability and traceability. The quality assurance system must comprise major GMP procedures including change control, deviation, Out-of-Trend and Out-pf-Specification procedures. At all stages of manufacturing, processing, and QC, the use of Standard Operating Procedures (SOPs) by qualified and trained personnel following validated and consistent processes must be performed.

Though there are many standardized pharmacopeial tests for synthesized RM, the same does not apply for biological or complex materials. Testing for biological materials requires the use of many different techniques and many methods may be bespoke for the raw material used. Companies may not have all the facilities, or pre-requisite experience, to perform all the required testing. Therefore, the establishment of partnerships with contract research organizations (CROs) can be an important step in a new product life-cycle. In addition, the CRO must be licensed by the relevant regulatory authority (EMA, MHRA, FDA, etc.) to satisfy the requirements of both quality systems and GMP. An important consideration in a CRO partnership is data retention. A minimum of 30 years storage is required and the challenges that this entails (electronic data/computer systems/data formats/maintaining readability/ensuring the data can be removed from storage and read) are multiple. Data retention time is often based on standard small molecule drug shelf lives as based on best practice, as advised through regulatory agencies, or through specific client-contracted times. This time period may be as short as six years and can extend for longer periods. Therefore, a CRO must have the ability and processes in place to extend data retention times to meet the desired requirements (e.g. 30 years), and to ensure that the tests performed/results obtained/reports can be recovered for the client.

What are the reasons and why are the appropriate testing methods important for raw materials?

Within Europe, The European Medicines Agency develops scientific guidelines to help pharmaceutical companies and individuals to prepare marketing-authorization applications for human medicines [8]. This guidance covers testing regimes that must be applied. This testing is to ensure that processes are in control, raw materials are tested to the required standards and ensure the products are safe for human use. For raw materials, a risk-based approach must be undertaken to ensure quality (Section 2 of the GMP guidelines for ATMPs [9]). This approach is performed by the manufacturer to identify and define the criteria which must be inherent in the quality of raw materials used. To assist in identifying and prioritizing suitable criteria, a series of questions can be asked prior to any selection of raw material supplier – examples of questions that could form the beginning of a suitable framework can be found in Table 1. This list is not exhaustive, and other questions may be required. However, it forms the basis of understanding the types of issues that it is better to resolve early in the product life-cycle. This reduces the risk of non-compliance and also forms the basis of initial discussions with laboratories that may be used for raw materials testing.

Depending on the ATMP, more questions than listed in Table 1 may be required to implement the correct risk-based approach. The EBE concept paper previously described [6] expands on the questions asked in Table 1 and provides guidance, based on EBE member companies, on how to establish a suitable framework. As it has been written from a commercial understanding of the regulatory requirements, it is advised reading for anyone wanting to establish raw material management.

Where raw materials are of biological origin, the General Chapter 5.2.12 of the European Pharmacopeia is relevant. The general

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requirements refer to: Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Material batch), Storage, and Labelling.

These general quality requirements apply for tests, which include, but are not limited to the following:

- Appearance
- Solubility
- Osmolality
- pH
- Elemental impurities
- Total protein
- Related substances
- Microbiological control
- Viral contaminants
- Bacterial endotoxins
- Mycoplasma
- Stabilizer
- Water

Many biological-derived materials may be complex mixtures, where it is not always possible to characterize completely the components of the mix. It is important that the testing regime can define the consistency,

TABLE 1

Choosing a raw material of suitable quality.

Supplier quality	▸ Is the supplier GMP-qualified for the raw material? ▸ Can they consistently produce the material to the desired quality? ▸ Can they consistently produce the material in the desired quantities for production needs? ▸ Is there sufficient stability data on the RM?
Multiple suppliers and Qualification of suppliers	▸ Is there more than one supplier? ▸ Do the raw materials have the same effect in your process? ▸ How similar/different are the raw materials? ▸ Are differences a risk? ▸ Qualify all suppliers and ensure that the quality criteria that you define can be met by the suppliers ▸ Try to avoid single, unique suppliers if possible
GMP materials	▸ The earlier the raw materials, produced under GMP, are used in the development process, the easier the subsequent processes of lab, pilot to production scale to generate clinical material
Partner contracts	▸ Have the appropriate defined contracts in place with your supplier to ensure robust quality and supply
Understand and monitor the material source	▸ How consistent is the product quality? ▸ Is the company stable financially? ▸ Where does the company source its raw materials, and is this secure? ▸ What happens if the company is bought out by a larger company? ▸ You may want to consider/explore whether the supplier has a business continuity plan in place (to cover natural disasters, hacking of systems, etc.)
Understand the testing regime	▸ Are the tests robust? ▸ What happens as regulatory requirements change? ▸ What happens if tests are not available due to kit availability, end-of-life of equipment, etc. ▸ As testing/equipment advances, will the requirements for impurities, protein purity, etc. change?
How variable is the raw material?	▸ Understand the important aspects of your raw material – purity, bioactivity, source, etc.

TABLE 2

Examples of impurities that can be introduced during the production process.

Impurity	Process or product related	Method that could be used
Aggregates	Product	Sub-visible particles, SEC, DLS
Degradation products	Product	HPLC methods, cIEF, cSDS, WB
Host cell proteins	Process	ELISA, LC-MS/MS
Host cell DNA	Process	qPCR
Vector-derived DNA	Product	qPCR

performance and safety of the material being used.

This is undertaken by a combination of testing and, where required, bioassays. The risks that were identified in initial scoping of raw material quality (using the risk-based approach) act to guide as to the combination of tests required to ensure quality of the raw material.

Where cell growth, expansion and/or maintenance is an aspect of the manufacturing process, for example during the manufacture of chimeric antigen receptor T-cells (CAR-T), raw materials can include:

- Sera and serum replacement
- Proteins produced by recombinant DNA technology
- Proteins extracted from biological materials
- Cell growth factors
- Cytokines required for cell differentiation
- Vectors

For many raw materials used in the manufacture of cell-based therapies, it is important that they are sterile, and if not, of known biological contamination with full justification for the non-sterile status. Therefore, sterility testing is a prerequisite prior to use. Within Europe, ICH Q4B [10], and, in particular, ICH Q4B Annexes 4A, B and C are relevant and define the relevant considerations for sterility testing and refer to the desired pharmacopeial chapters for the EU, US and Japan.

In a similar fashion to antibody-based therapies, if manufacture of the ATMP generates process-related and product-related impurities (Table 2), it is up to the manufacturer to ensure that all potential impurities have been identified and limits defined.

There are many possible tests that can be performed in the biological world to define raw material characteristics that are important to ensure product consistency and to define the quality attributes of the raw materials. The tests must meet pre-defined quality requirements for identity, purity and biological activity. No one test can define the total quality attributes of a raw material, and the desired quality attributes must be defined to guide the testing regime. The tests are orthogonal to each other and ensure that a consistent product is used in the manufacturing process.

The method(s) for each test must give consistent performance and undergo a validation process, in accordance with ICH Q2 [11] guidelines before being used for routine testing. In addition, the supply of reference materials, where possible and the evaluation of the stability of representative batches of raw materials to ensure that the test is in control, must form part of any testing plan.

Testing of chemical raw materials for ATMPs will generally be defined within the pharmacopeial compendia. In terms of biological raw materials, examples of experimental approaches can be found in Table 3. As well as developing bespoke methods, commercial kits designed to detect common cytokines, cell proteins and other common biomolecules are available and can be used to create GMP validated raw material methods. However, these kits often require additional expertise in adapting them to ensure that either the kit can detect the raw material within the sample matrix, or if interference is observed, a suitable sample preparation procedure is developed to allow the raw material

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TABLE 3

Potential testing approaches for common categories of ATMP raw materials.

Sample type	Testing required	Suitable technique that can be implemented	Comments
Serum	ID	SDS-PAGE/Western Blot	SDS-PAGE provides a protein profile that can be used to confirm serum type. When combined with a species-specific western blot, both species and serum type can be suitably identified
Serum free cell culture media	ID	CE-SDS for protein profiling can be implemented if available sample volume is restricted	
Defined protein components	ID and/or Quantitative	ELISA	ELISA methods can form the linchpin to any raw material testing regime as they are versatile, and a well-developed method can be created to be cost effective, robust and quick to run A qualitative ELISA can be developed to confirm ID of component by a positive/negative result. Alternatively, a fully quantitative ELISA can be developed to confirm ID and concentration of component
	Functionality	Bioassays	Ability of RM to selectively activate cells can be demonstrated by a bioassay
		Flow cytometry	Ability of a RM to bind to specific cells can be demonstrated by flow cytometry
		Occasionally a bespoke ELISA	For certain components, a bespoke ELISA could demonstrate depletion of specific cells by a RM, creating a method that is more cost effective than a cell-based method
	ID and characterization	HPLC – mass spectrometry	Intact mass – ID test for purified or expressed proteins Protein sequencing following tryptic and/or chymotryptic digestion Small molecule analysis/screening Leachables and Extractables Identification of post-translational/in-process/storage modifications
		HPLC – protein	Purity – Reverse Phase or SEC Aggregates/Oligomers – SEC, DLS
		HPLC – N-linked Oligosaccharides, following release using PNGase F	Fingerprinting/sequencing to confirm correct glycosylation
	Microbiological assays	Sterility, bioburden, endotoxin, and microbiological testing	Not required for all reagents but need to be considered when planning raw material testing. Virological detection and Baceriophage testing may be necessary, and should be additionally considered
Buffers	Presence/absence of: sugars vitamins amino acids other chemically synthesized small molecules Ions	HPLC – small molecule May be coupled to UV, fluorescence, CAD, ELSD, PED/PAD, MS) or combinations of detectors	Usually defined pharmacopeial methods
	Ions or heavy metals	ICP-MS	Can be used to detect ions or heavy metals
	Microbiological assays	Sterility, Bioburden and microbiological testing	Not required for all reagents but need to be considered when planning raw material testing

to be tested using the commercial kit. In addition, these assays need to be validated with the appropriate matrix.

In addition, a CRO such as RSSL can also provide clients with the ability to extend beyond raw materials testing (both biological and chemical) and enable testing through other aspects of the product life cycle. This may include:

- Extractables and leachables from single-use processing components/fill vials/culture bags/etc
- Stability testing using GMP testing regimes of temperature and relative humidity, and photostability may be required for some light-sensitive RM (Media) according to ICH Q1B [12]
- Dissolution testing of raw materials into conditions similar to process conditions
- Emergency Testing of raw materials

Additionally, your CRO should have the expertise to develop and validate methods to ICH standards and additionally, stand up to scrutiny by the commissioning company and regulatory authorities.

CONCLUSIONS

The prime objective of any medicinal product is to provide the patient with a product that is meets the defined quality, safety and efficacy requirements. Therefore, the processes for manufacture, process control, testing, release and adverse-reaction reporting are heavily regulated. For all active ingredients, raw materials, excipients, cell lines, media, DNA, etc. the principles of GMP apply. This is to ensure that control of all materials used are within the limits defined by the manufacturer, are acceptable to the regulatory authorities and maintain absolute product quality. Raw materials for cell and gene therapies are more diverse and often bespoke tests are required to demonstrate quality. The approaches outlined in this White Paper will help in the decision process to define the questions that need to asked about raw material supply, quality, a testing regime, types of tests and start the process of choosing where testing may be performed. Not all companies will possess the required expertise, and partnerships with suppliers and CROs may be required. When choosing a CRO, such as RSSL, the regulatory status of the CRO must be ascertained, in addition to whether they have the experience and knowledge to help with the testing required. Often this will require consultancy, ability to perform pharmacopeial testing, develop and validate methods to GMP standards, and have the required quality management systems in place. No one test will define your total process. A combination of testing will be required to confirm identification, quantity and quality of raw materials.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

EXPERT INSIGHT

The case for the use of pooled donors in the manufacture of allogeneic cell therapies

Benjamin Weil & Mark Lowdell

Cell and gene therapy (CGT) continues to be an expanding field of biotech, however, current therapies are frequently associated with complex manufacture and distribution platforms which result in high costs of goods. To provide the benefits from economies of scale, quality control batch testing, and an 'off-the-shelf' supply model, allogeneic treatments are attractive where possible. This Expert Insight article will assess both the bioprocess and therapeutic dependency of donor material, and present pooled donor therapies as a necessary consideration for commercial CGT relevance.

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INTRODUCTION & DONOR DEPENDENCY

Medicinal products are fundamentally reliant upon starting material for quality. For allogeneic cellular therapies, the source of donor starting material will directly impact the clinical safety and efficacy of products. Critical analysis of donor dependency will be subdivided into two categories: proliferation and variability.

Proliferative capacity

In order to gain marketing authorization from the European Medicines Agency (EMA), or

approval from the Food and Drug Administration (FDA), the unmet medical treatment landscape must be considered. If a drug cannot be manufactured at a commercially relevant scale, approval will not be granted.

Mesenchymal stem/stromal cell (MSC) therapies will be used as platform for quantitative assessment of clinical manufacturing, with a median patient dose of 100 million cells for intravenous injections [1]. Assuming the indication is a rare disease, defined as fewer than 1 in 2,000 affected people within the population, and that only a single dose is required, to treat the UK alone would require 3.5 hundred trillion cells (10^{14}) to be manufactured.

To treat this rare patient population, assuming 1 million cells are isolated from procured donor tissue, the cells would have to undergo 500 million population doublings to achieve this figure, excluding cell loss through the bioprocess, retention and reference samples, and quality control (Figure 1A).

Indefinite expansion of somatic cells is inhibited by the process of senescence, which instils a finite limit of cell division. Senescence is congruent with biological aging, and associated with telomere shortening and epigenetic dysregulation. Although research into cellular rejuvenation shows some promise [2], increased cell population doublings has been shown to negatively impact immunological effects and efficacy [3–5].

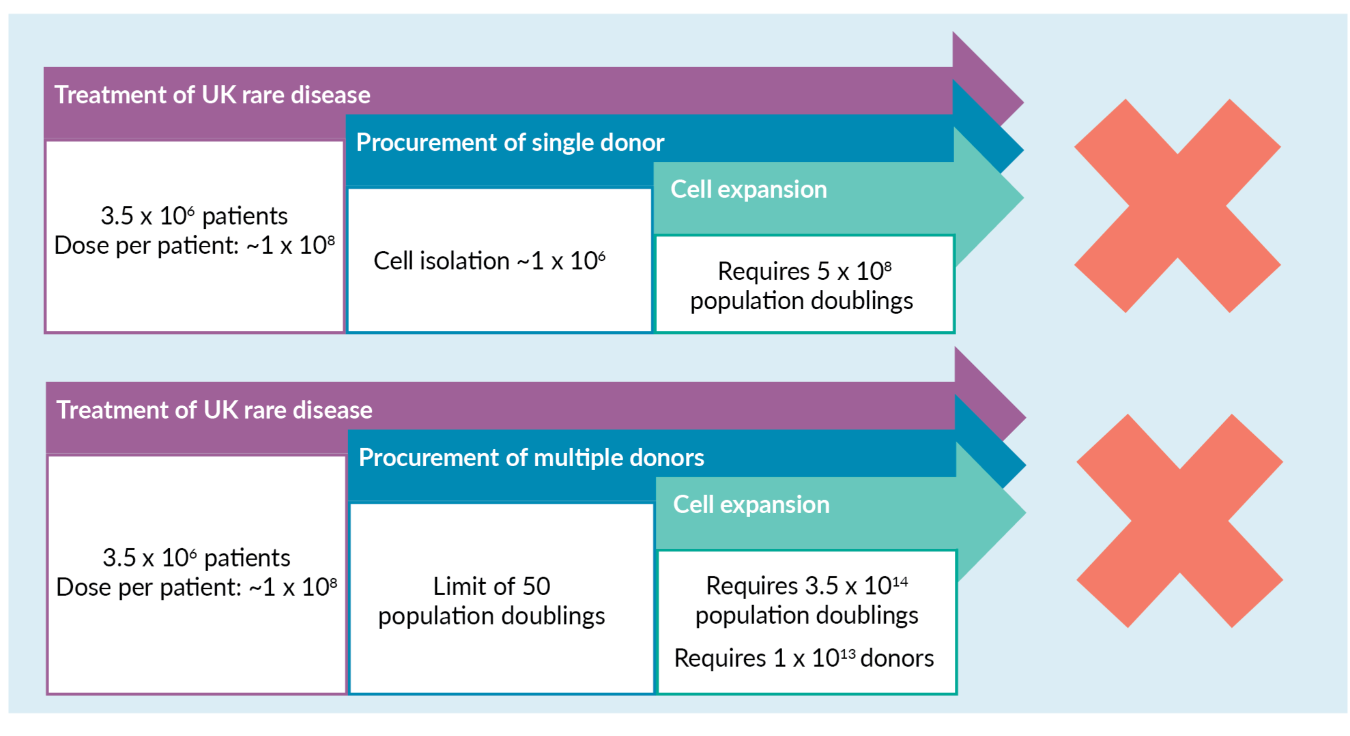
Incorporating a practical limitation of 50 population doublings, but retaining the single donor product model, 10 trillion donors would be required to meet UK clinical demand (Figure 1B). Again, this results in a manufacturing platform that is not economically or logistically feasible.

Innate donor variability

To produce commercially relevant single-donor cell stocks would require huge numbers of donors. However, beyond the logistical and economic challenges of procurement, innate biological variability of starting material presents a significant challenge. The dependency of donor and *in vitro* manipulation upon product characterization and efficacy has been widely observed [6–10].

FIGURE 1

Single donor models to treat a rare disease in the UK; both present infeasible manufacture platforms.



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Investigational new drug (IND) applications require preliminary safety (pharmacology and toxicology) data to be demonstrated, as well as manufacturing information detailing a consistent supply of drug product. Once safety and function has been proven, a consistent state of compliance is required. To replenish a depleted single donor stock requires rolling the dice of biological variability, in the hope that a comparable safety and functional profile will be achieved from a new starting material source (Figure 2).

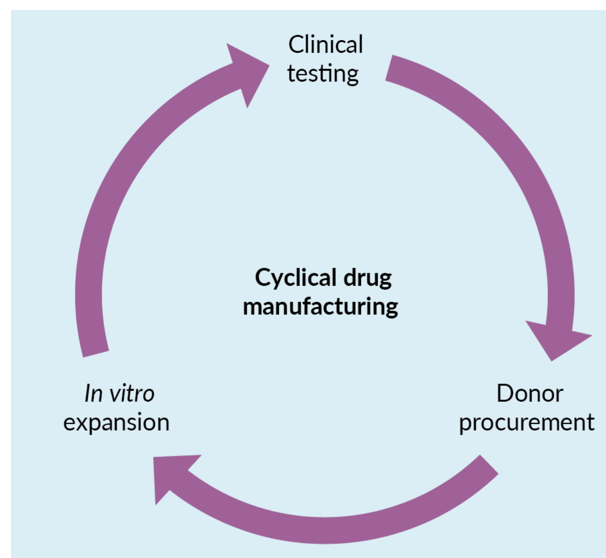
Cyclical product manufacture, depleting and replenishing single donor cell stocks, introduces batch variability. Ensuring that new donors will produce a consistent drug product is challenging. Characterization and quality control assays are based on established minimal criteria, such as the International Society for Cellular Therapy's (ISCT) position on MSCs [11], which limits product knowledge. Efficacy is also of specific note here, as highlighted by the recent FDA rejection of Mesoblast's allogeneic MSC therapy for steroid-refractory acute graft-vs-host-disease. A failure to demonstrate clinical relevance of potency assays to clinical performance was noted [12]. The successful repetition of potency and other assay for each new donor stock adds cost and risk.

HISTORY OF POOLED MEDICINAL PRODUCTS

To counter the challenges of manufacturing scale and batch variability, compendium licensed medicines derived from pooled donors present a well-established utility. Plasma-derived medicinal products date back to the development of plasma fractionation by Cohn and colleagues in the 1940s [13]. The technique was scaled up into industrial clinical manufacturing, exemplified by the production of anti-hemophillic factor (factor8) concentrate [14]. In the 1970s, however, the risk of viral transmission from blood plasma was little understood and had dire consequences. It was not until the 1980s when

FIGURE 2

Diagrammatic representation of cyclical drug manufacturing, requiring new donor procurement for each expansion cycle of drug product.



profound regulatory change occurred to improve the safety of blood-based therapies, such as through viral inactivation.

Human albumin solution from large donor pools is now widely used in the CGT industry, such as an excipient for cryopreservation. There is also growing use of pooled human platelet lysate (phPL) as an alternative to fetal bovine serum (FBS) for cell culture. Beyond global supply limitations, Transmissible Spongiform Encephalopathy (TSE) and xenogeneic risk of FBS, phPL reduces the batch-to-batch variation and ambiguity of other cell culture supplements [15]. However, there are still risks which must be critically assessed for pooled-donor products. For example, pharmacopeia-grade batches of phPL of more than 50 donors are widely commercially available, although the German Federal Regulatory Authority has issued a recommendation to limit pools to 16 donors [16].

DONOR POOLING FOR CELL & GENE THERAPY

The innate complexity of biological tissue presents warranted uncertainty and risk when

defining specifications and characterizing donor-derived medicines. In mathematics, the Monte Carlo method uses the repetition of random sampling to draw conclusions of probability distribution. Variance reduction increases the precision and clarity of a simulated result by increasing the number of inputs (in this instance, the number of pooled donors). Convergence upon a mean will apply with increased inputs, moving towards an asymptotic standard deviation of zero by the square root of n. Contextualizing this, by using pooled donors rather than a single donor for manufacturing, product heterogeneity (i.e. the probability space) is decreased. Increasing the number of donors will decrease variability, with limited gain once normal distribution is approached according to the central limit theorem.

Pooled donor products provide a scalable solution to the premature depletion of single donor cell banks. Assuming model in vitro expansion of eight donors pooled at passage 2, with uniform doubling times across each donor, by 11 cumulative population doublings 10^{14} cells could be manufactured (Table 1) – enough to meet the UK clinical demand to treat a rare disease condition.

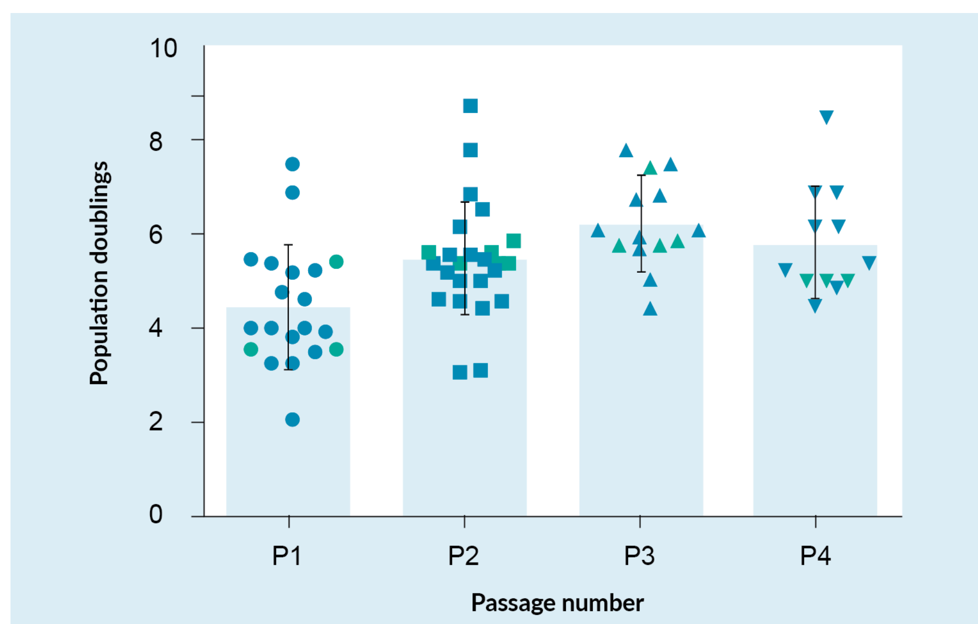
However, to critically assess how pooled donor products practically deliver a scalable solution, experimental data is required to evaluate the theoretical assumptions. The derivation of population doublings per passage, the onset of senescence, and the equivalence of donor expansions once pooled will be investigated.

Population doubling

A population doubling of five per passage was selected for pooled donor modelling. Single donor and pooled donor umbilical cord tissue-derived MSCs (UC-MSCs) were cultured in serum-free, xeno-free medium across four passages to assess population doubling. A mean doubling between 4.5 to 6.2 was noted, with no distinguishable variation between passage, or between pooled and individual donor material. A selection of five population

FIGURE 3

Population doubling of UC-MSCs, assessed across passage 1 to passage 4 for individual donors and pooled donors.



The scatter dot plot displays the mean with standard deviation, as well as each data point: red labels are pooled donor samples (from ≥3 donors) and black labels are individual donors.

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TABLE 1

Calculation of theoretical doses and patient treatment capacity for an allogeneic product consisting of 8 pooled donors.

Passage number	Cumulative population doublings	Cumulative cell output	Theoretical doses manufactured	Theoretical patient treatment capacity
Passage 2	5.6	8.19E+09	81	27
Passage 3	6.3	2.62E+11	2,621	873
Passage 4	6.9	8.39E+12	83,886	27,962
Passage 5	7.5	2.68E+14	2.68E+06	8.95E+05
Passage 6	8.1	8.59E+15	8.59E+07	2.86E+07
Passage 7	8.8	2.75E+17	2.75E+09	9.16E+08
Passage 8	9.4	8.80E+18	8.80E+10	2.93E+10
Passage 9	10.0	2.81E+20	2.81E+12	9.3825E+11
Passage 10	10.6	9.01E+21	9.01E+13	3.0024E+13
Passage 11	11.3	2.88E+23	2.88E+15	9.60768E+14
Passage 12	11.9	9.22E+24	9.22E+16	3.07446E+16
Passage 13	12.5	2.95E+26	2.95E+18	9.83826E+17
Passage 14	13.1	9.44E+27	9.44E+19	3.14824E+19
Passage 15	13.8	3.02E+29	3.02E+21	1.00744E+21
Passage 16	14.4	9.67E+30	9.67E+22	3.2238E+22
Passage 17	15.0	3.09E+32	3.09E+24	1.03162E+24
......	......	......	......	......
Passage 25	20.0	3.40E+44	3.40E+36	1.13427E+36
....	......	......	......	......
Passage 33	25.0	3.74E+56	3.74E+48	1.24715E+48
....	......	......	......	......
Passage 41	30.0	4.11E+68	4.11E+60	1.37125E+60
......	......	......	......	......
Passage 73	50.0	6.01E+116	6.01E+108	2.0041E+108

Passage 0 isolation of 1×10^6 cells, and 5 population doublings per passage are assumed; 8 donors are pooled together at Passage 2, and the specific growth rate of each donor is equal. A dose of 1×10^8 was selected, with 3 doses per patient.

doublings for the model seems appropriate within this range (Figure 3).

Cellular senescence

As mentioned previously, growth kinetic and population doubling are limited by cellular senescence. A cumulative pooled population doubling of 11.3, equating to passage 11, was posited to meet disease target demands. Cumulative population doubling has been assessed in literature for both bone marrow and umbilical cord tissue-derived MSCs, with a maximum of ~40 and 60 doublings noted respectively [17]. The value of 50 population doublings from Figure 1 lies within this range and therefore remains applicable.

Equal donor expansion

To produce a pooled cell stock that can be expanded to provide enough cellular

material for commercial value, it is important to demonstrate the continuation of each donor line across multiple passages following pooling. To examine individual donor kinetics within a pooled product, three independent UCT-MSC donors were pooled and expanded for 4 subsequent passages. Using polymerase chain reaction (PCR) amplification of short tandem repeats (STR), common alleles were identified that were expressed by each donor. By comparing the target allele across each pooled passage, the unique relative contribution of each donor within the pool could be assessed (Figure 4).

There are two key conclusions from the data presented:

1. The initial relative contribution from each individual donor is unequal, and asymmetrical donor expansion is observed;

2. MSC heterogeneity is maintained throughout the expansion process and therefore will be present within the final product.

Whilst increasing the number of donors in the pool will decrease the impact of inter-donor proliferation heterogeneity, variation between donors is expected and must be embraced. Figure 4 demonstrates how uniform donor expansion is idealized, with unequal donor contributions observed experimentally. A key finding, however, pertains to the maintenance of each individual donor throughout manufacturing. Although the percentage contributed by each donor varies over time, the presence of all N donors is retained; hence the ability to provide a heterogeneous product.

By maintaining population doublings per passage, delaying cellular senescence, and providing a mixed donor product, pooled cell stocks provide not only a theoretical solution, but a practical method to implement and de-risk clinical manufacture within the CGT field.

CONCLUSION & TRANSLATIONAL INSIGHT

Although autologous donor-derived therapies support a diverse range of clinical applications, the advantages of affordable, off-the-shelf allogeneic products are numerous. By embracing donor variation, not only can product variability be diminished, but the creation of low passage cryopreserved cell stocks will support continued manufacturing with a successive banking strategy [18] (Figure 5).

The generation of pooled cryopreserved cell banks enables product consistency, and

FIGURE 4

Relative donor contribution of each donor to the overall pooled of MSCs across 4 passages following pooling.

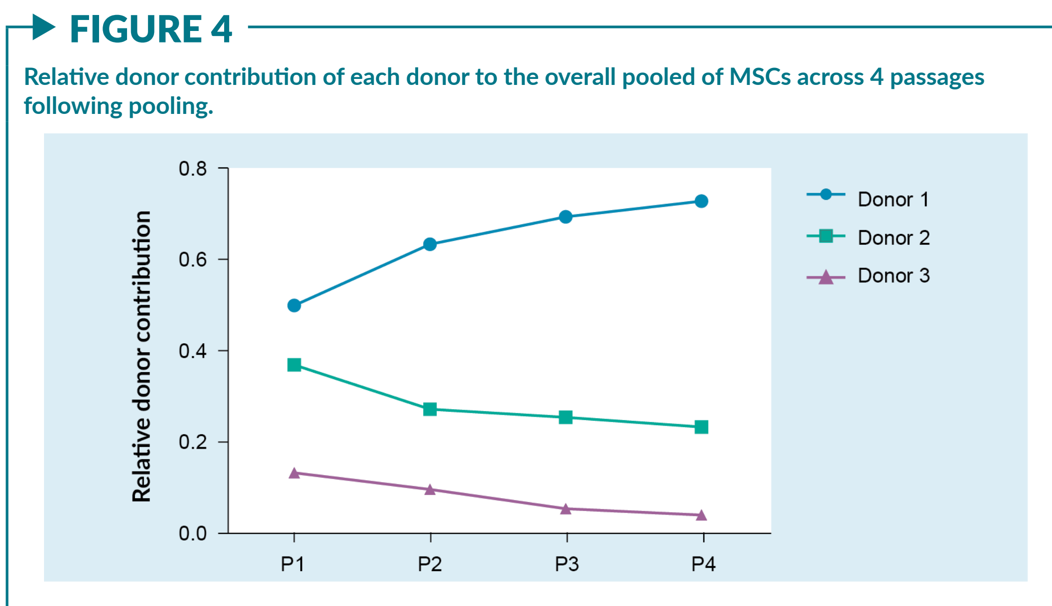

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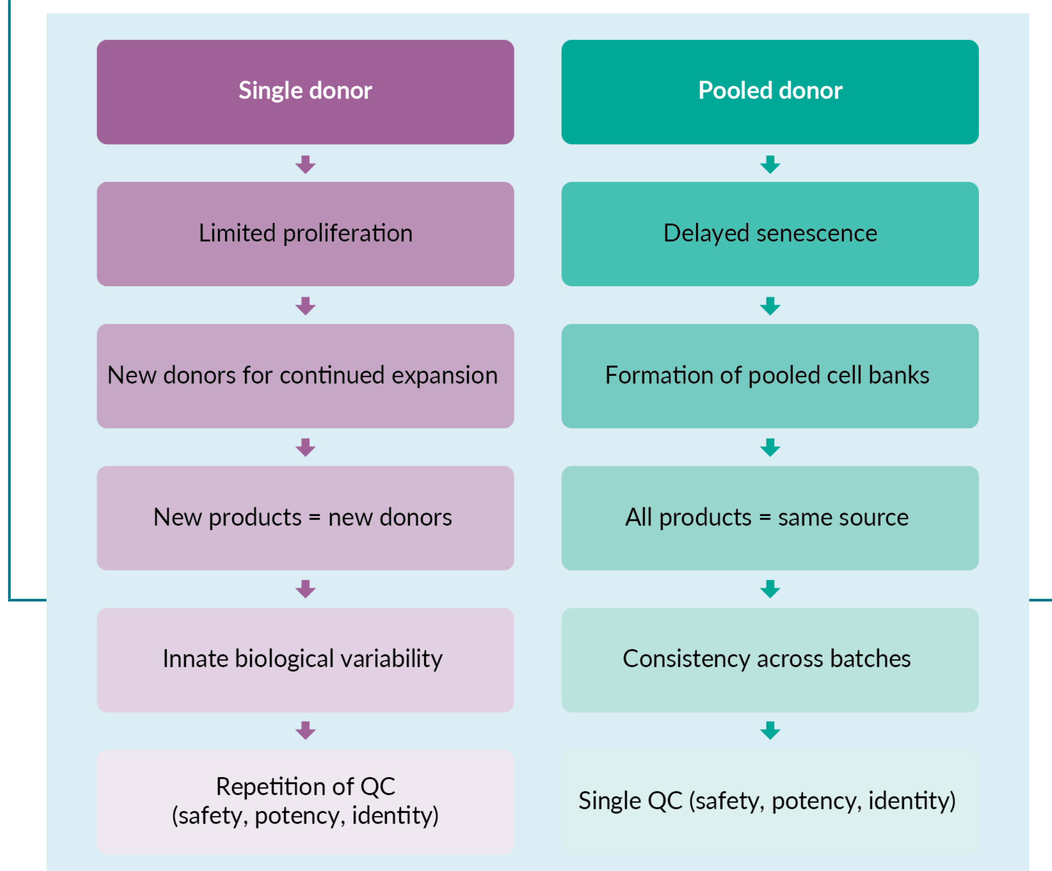
FIGURE 5

Comparison of single and pooled donor bioprocess manufacturing strategies.



ensures that a reproducible drug product can be manufactured through clinical development and commercialization.

Consideration of the risks pooled donors present must be duly noted, however, numerous clinical trials now utilized pooled donor products to successfully mitigate donor-to-donor heterogeneity and create commercially relevant cell stocks:

- Pediatric treatment of GvHD from a cell bank derived from 8 bone marrow donors [19];
- Treatment of non-small cell lung cancer using genetically modified, pooled MSCs without tissue matching or immunosuppression [20];
- Pooled MSCs for critical limb ischemia due to Buerger's disease [21];

Nevertheless, there are still many challenges for the commercialization of pooled CGT products; an emphasis upon quality control, with appropriate cellular assays, must be taken to ensure clinically-relevant identity, safety and potency testing. The application of a systematic Quality-by-Design (QbD) approach to manufacturing can further support continued process and product understanding.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INTERVIEW

Expect the unexpected: maintaining and optimizing a cell collection network during COVID-19

AMY HINES, RN, BSN, is the Director of Collection Services for the NMDP/Be The Match. She works with senior leadership to develop the strategy for the growth of the NMDP/Be The Match-owned network of apheresis facilities and is responsible for the implementation of that strategy. Hines was an instrumental member of the NMDP/Be The Match team that planned and launched the Be The Match Seattle Collection Center, which opened in January 2020.

She joined NMDP/Be The Match in 2013 managing the NMDP/Be The Match Apheresis and Collection Center Network of more than 90 apheresis center and 80 collection center partners. She then became the Director of Collection Network Management for Be The Match BioTherapies. In this role, Hines oversaw the performance of apheresis centers and cell therapy labs in the Collection Network, and ensured their ongoing compliance with FDA and international standards and criteria, industry best practices, and appropriate regulatory and accrediting entities.

Hines has spoken about the challenges apheresis centers are facing and then need for standardization during multiple conferences and webinars, including Cell and Gene Therapy World U.S. 2018, the Adoptive T-Cell Therapy: Development track of the Immuno-Oncology Summit 2018, Phacilitate: Leaders World 2019 and World Advanced Therapies & Regenerative Medicine Congress 2019.

She has nearly 20 years of experience in the cellular therapy field, starting her career as a stem cell transplant nurse. She received her Bachelor of Science in Nursing from Grand Valley State University in Allendale, Mich.

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Q We last spoke to you in 2018, before the COVID-19 pandemic began, about optimizing the cell collection network to support commercialization of cell and gene therapy. What has changed over the past few years, and what do you think still needs to be achieved?

AH: Since 2018, the industry has become increasingly aware of the challenges that come with managing consistency and standards across a wide variety of cell collection facilities, and also the value and benefits of working together to overcome these challenges.

A few years ago there was a lot of talk about the need for consistency and standards, but there was no real agreement on how to move forward, and no real cooperation as to who was going to take the lead and push these things through. One major change over the past year has been the continued growth of collaborative efforts towards standardization across the whole industry.

It has also been great to see the industry working together with apheresis centers, accrediting bodies, and other key stakeholders, towards identifying opportunities to develop common standards and expectations, with the realization that when we are all on the same page, we all benefit.

These efforts are incredibly important, and we have definitely come a long way since 2018. But there is so much yet to be done so that developers of new therapies can take these standards and incorporate them into their development process early on.

Q Do you feel Be The Match has cemented itself as one of the key voices in driving that initiative?

AH: I do, and in addition, I think Be The Match has played an instrumental role in kicking off that cooperation.

We are very specialized within this industry. We have been doing a lot of these processes that are similar to the cell and gene therapy industry needs for a long time, but we have done it in a unique way, through our cell collection network and through overseeing activities at multiple facilities.

One of the things we have brought to the table over the course of the past few years is in helping to pull together the right stakeholders to collaborate and talk about standards and consistency. I would like to take a little bit of credit for our organization in drumming up some of that excitement to participate amongst the apheresis centers. It is key to have those hands-on experts helping to drive what is feasible and what is not when it comes to developing standards.

Q The impact of COVID-19 on cell therapy supply chains is impossible to ignore. Could you go a little deeper into how Be The Match has been able to successfully navigate this incredibly challenging period?

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AH: There were three key components. One was an unwavering dedication to donors and patients. Two was the ability to leverage the longstanding relationships our organization has built over the past 30+ years. Third was some amazingly innovative thinking.

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Our operational management model allowed us the success that we have had during the pandemic. While the depth and duration of this long year of dealing with COVID may have been a surprise, at Be The Match that is part of our role. We are constantly at the ready for unexpected events.

Perhaps we hadn't expected the pandemic, but we were well suited to handle these events. Whether it is due to weather, power outages, political events, airline strikes, or anything else, our organization is 100% dedicated to mitigating whatever challenges arise in order to ensure that patients can receive the therapy that they need.

I will comment too on our large, geographically dispersed, and highly capable network of collection centers, and our diverse donor pool. This allowed us to focus collections close to where donors are located, helping to minimize donor travel, which was obviously extremely complicated and continues to be somewhat challenging. It also meant we were able to maximize our ability to be flexible to collect, whether because of COVID hotspots or weather events – we have just had Winter Storm Yuri in the southwest, and that caused a lot of issues. We are able to mitigate these challenges because of the model that we have developed. Our managed logistics model helps to provide flexibility in moving cell product shipments when flights get cancelled and things get challenging.

We have a great emergency preparedness team, and their relationships are really critical to help us work across the world with partners to ensure we can still have couriers moving across borders. If and when donors do travel, and travel is impacted, they are able to get really creative to get a donor from his or her home to one of our network collection centers.

Additionally, the capability and willingness of our network partners to cryopreserve products has been absolutely critical to ensure that before a patient starts prep, the cells are where they need to be, and they are ready with zero delay when the patient is ready to receive them.

Q Looking to the future, what would you say are the key lessons or benefits that the COVID-19 experience has brought to Be The Match's cell collection operations and network management?

AH: In the interests of full transparency, I will say that the pandemic has put our organization to the test. We have successfully navigated this past year, but we have learned quite a bit about our capabilities, and also our opportunities.

A key lesson was that it is critically important to be prepared for the unexpected in order to ensure business continuity, especially when the business at hand has such direct impact on patient lives. But no matter what innovative and creative pathways we can forge to continue donor collection activities despite these unexpected events, what cannot change is the focus on compliance and product quality.

For example, back in 2018 I spoke about the complicated process of coordinating donor availability with apheresis collection availability, and ultimately with manufacturing availability. What we realized through our experience over this past year is that something like cryopreservation of the collected product really helps permit some flexibility in that core patient, and that sometimes helps to mitigate a complex challenge.

As I mentioned, a few weeks ago we had Winter Storm Yuri, which was in and of itself challenging, but also had widespread effects – for example, in terms of moving Filgrastim from the pharmacy to the donor. These types of challenges are not going away.

While we as an organization have been, and always are, prepared for these types of events, going through the COVID experience has made us more proactive in identifying opportunities to successfully continue our business throughout whatever events might happen. Cryopreservation has played a huge part in that. Being able to collect early means that despite whatever challenges there may be in getting product from a collection center to a patient or manufacturing facility, the product is collected, and we face instead a logistical challenge of getting it from point A to point B, with no detrimental effects to the product.

Going into COVID we had been addressing these challenges for years successfully, but we have realized some new opportunities that we can keep in our toolkit for both anticipated and unanticipated events in the future.

Q What are the main opportunities to mitigate the variabilities between collection centers in particular?

AH: In my view, the best way is starting with a challenge to help uncover what opportunities there might be. The biggest challenges in mitigating the variabilities between collection centers lies in the fact that no two centers are fundamentally organizationally alike. This is something we have known for years, and in some ways it is just the nature of the game.

Certainly, there are commonalities between blood center-based collection centers and even somewhat amongst hospital-based collection programs. But the ability to standardize detailed policies and complicated procedures is not necessarily feasible. Things like the laboratory, the staffing, and all sorts of other components go into the collection process, and they likely impact not just the collection center team but also other departments within that center's organization. The opportunities to mitigate these variabilities can come from identifying the commonalities, coupled with what aspects of the process necessitate consistency in order to establish industry acceptable standards.

Good work towards developing proposed standards has been done, and continues to be done, for things like labelling, site training, and site qualification, and this will undoubtedly decrease variability and promote consistency across the collection center network.

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Q What is the current state of play in terms of the drive for standardization around cell collection? What are the chief areas of focus to drive both efficiencies at cell collection sites, and increased quality and consistency?

AH: As I mentioned before, it is very exciting to see so many experts coming together and driving efforts for standardization, as opposed to everyone trying to do it on their own.

There are representatives from all aspects of this work, from cell and gene therapy companies, to accrediting bodies, to collection centers administrators, to apheresis clinicians, and everyone is actively working together to develop standards around the cell collection process.

For example, standardization around collection center qualification has had lots of attention in the past couple of years. Most of industry is aware of the term "audit burden". When we last spoke in 2018, I shared that some collection centers report up to 30, or maybe even more, audits a year, and many of them are almost cookie cutter repeats of previous audits.

So while we still have a way to go, there has been some progress towards an acceptable standard for the onboarding and qualifying of new collection center sites, which increases collection center satisfaction by freeing up their resources and time to actually do the work we are asking them to do. It also brings a realization that we can leverage each other's qualifications of a center, if we come together and determine what those standards for onboarding and qualification should look like.

Probably the biggest success so far in standardization has been around labelling. The use of the ISBT 128 labeling platform has provided some much needed consistency, and is a great example to show there are areas in which standardization is possible, and can be successful.

Q How is the industry's increasing focus on allogeneic cell therapy products, particularly in the cellular immunotherapy space, impacting Be The Match, and how are you preparing for continued growth in this area?

AH: It has been an exciting few years as this industry has really blossomed. Because of our vast network of amazing and diverse donors, our organization is really well suited for this increased focus on allogeneic cell sourcing.

When available, most transplant centers will select younger donors for their transplant patients, which is completely understandable, but leaves those of us upwards of 35 wondering how we can help. We are on the registry, we are signed up to be able to help someone, but we probably are not going to be the first selection for a patient for transplant.

Because not all allogeneic donor source protocols in cell and gene therapy require a certain donor age, we are able to offer altruistic volunteer members of our registry to help to save a life, or to continue a mission, by participating in some of the cell and gene therapy protocols requiring cellular starting materials.

We are continuing to invest in the biotherapies side of the business, to ensure that we have scalability once emerging cell therapies enter the commercial scale environment. This is something that we are very familiar with, and we are readily prepared to be at the forefront of assisting and helping to source allogeneic cell products at that time. And of course, we continue to work with clients to bring our expertise and work to influence standardization, and to help clients get therapies to patients faster.

Q What other key future trends do you anticipate relating to cell collections for cell therapy R&D and commercial applications?

AH: There are a couple of areas I think we might anticipate seeing more attention. One is more research into what aspects of the actual collection process and product truly influence therapy efficacy. If we are going to look at standardization, we have to ask what aspects of the product itself are most appropriate for standardization. What areas of the collection process are product-specific, and will truly impact the end therapy efficacy?

Identifying those areas is going to greatly advance standardization efforts, and is going to improve overall protocol compliance, and decrease the pressures and challenges apheresis centers face with current collection protocol expectations.

Secondly, as I mentioned above, we realized throughout the pandemic that cryopreservation certainly permits flexibility in scheduling. It allows collections to occur when the donor is ready and available, and allows the receiving entity, whether it is a transplant center or a manufacturing facility, to accept the cellular therapy product when they are ready. Ultimately, it enables the patient to receive his or her lifesaving therapy when timing is optimal for a successful outcome.

An increase in research on how cryopreservation affects the end therapy, as well as other cryopreservation techniques and comparisons to fresh products, will help to provide flexibility in the supply chain. This seems like an area that definitely needs some further development, and there are some great opportunities there.

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RAW & STARTING MATERIALS

SPOTLIGHT

INTERVIEW

Managing raw and starting materials for viral vector manufacture: managing risk and staying flexible

ERIC HACHERL holds a PhD in Chemical Engineering from Rutgers University. As the Head of Manufacturing, he is responsible for Internal and External Manufacturing, Materials Management, Shipping & Receiving and Manufacturing Science & Technology. Eric joined Spark in September 2020 with over 20 years of experience in the pharmaceutical industry. His functional expertise lies in Operations, Supply Chain and Manufacturing Science & Technology. His focus has been primarily on vaccines and large molecule manufacturing. Prior to joining Spark, he was the Site General Manager and VP of Operations for Catalent Gene Therapy in Baltimore, MD.

CHRISTOPHER KLEM is an experienced professional specializing in technical and logistical oversight of external/contract manufacturing operations in the emerging gene therapy space. Effectively managed external manufacturing projects for over 15 years, specializing in relationship building and execution of program deliverables. Current experience in oversight of all aspects of outsourced supply chain partnerships: drug substance, drug product, secondary packaging and specialty distribution of commercial and clinical gene therapy products. Also have managed and directed internal Primary and Secondary operations in both pharmaceutical and biopharmaceutical manufacturing, directing large multi-tiered teams to meet internal manufacturing objectives.

MARK GALBRAITH has worked in the biopharmaceutical industry for 20 years, where he has gained diverse experience and knowledge in all phases of product development from preclinical to commercial. Mark began his career at Merck & Co, where he held positions of increasing responsibility in several functional areas related to vaccine clinical development and vaccine commercial manufacturing including analytical R&D, regulatory sciences, commercial analytical support, and quality control operations. Mark continued to develop his career in quality control management and analytical development by directing the quality control activities at Celldex Therapeutics, Bristol Myers-Squibb, and Gilead Sciences. Mark has gained a broad knowledge of vaccines, biotechnology products, sterile pharmaceuticals, and now gene therapy. Mark is currently serving as Head of Quality Control and Analytical Sciences for Spark Therapeutics Inc. in Philadelphia, PA. Spark is a leader in the field of gene therapy, seeking to transform the lives of patients by developing potential one-time, life-altering treatments for debilitating genetic diseases.

RYAN BARTOCK is Vice President and Head of Technical Strategy & Operations at Spark Therapeutics, a fully integrated gene therapy company and member of the Roche family of companies.

Ryan joined Spark Therapeutics as Head of Supply Chain and Network Strategy in 2017 where he established the Supply Chain function and launched the first ever FDA approved Gene therapy. His current responsibilities include leading the Supply Chain and CMC Program Management functions, as well as strategic initiatives across Research, Technical and Commercial organizations. He is also interim Head of Technical Development, overseeing Process and Analytical development, Automation and Pre-Clinical Operations.

Ryan holds BSc in Engineering and Business from Drexel University. His 18 years of industry experience spans pre-clinical and clinical development, manufacturing operations, supply chain, CMC, corporate strategy and new product launches at both GSK and Merck.

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Q What processes do you utilize in both your in-house and your outsourced viral vector manufacture, and can you give us some background to how you arrived at them?

At Spark, we rely on our cross-functional program teams to meet regularly to discuss strategies for the product development life cycle. We encourage standardization of

processes and methods so we can leverage information from one program to the next, and we are moving to platform types of processes for both upstream and downstream.

- On the upstream side we are standardizing on cell lines and cell culture methodology and equipment.
- On the downstream side we encourage the research team to utilize a core set of capsids (where possible) so we can utilize the same or similar purification processes. The standardization of capsids allows for easier formulation development and analytical testing as well.
- On the clinical trial and development side, it's important for us to obtain early reads on what type of products are advancing. This is because the clinical development window is so short.
  - We ensure we have a sufficient supply of clinical material and are ready for a rapid pivot to Phase 3.
  - At Phase 3, we like to lock in the process and have a process we understand well and that demonstrates consistent performance.
- On the analytical side, we have invested in advancing both traditional and new technologies to better measure and ensure the quality and safety of our products. By fostering a culture of innovation, leveraging our collective expertise and applying sound science, we have been successful in applying approaches product testing and other activities that accelerate product development work through barriers.
  - For example, given the complexity and long lead time on potency (activity) assays we like to start the development of those assays as early as the pre-clinical stage of development and focus the efforts in an in-house centralized team that possesses that critical expertise.

Q How do your raw materials sourcing and management considerations and approaches differ between in-house and outsourced manufacturing?

For the most part, our outsourcing partners manage the procurement of their own materials. They base their ordering on periodic forecasts, but they plan and manage their own materials requirements. This includes any planning necessary to manager order lead time and safety stock requirements.

We approach supply chain risk management by looking beyond our tier 1 and 2 suppliers and put in place mechanisms for both passive and active risk management. We also look to go beyond purely transactional supply agreements and create strategic partnerships with our most critical suppliers and external

> "...we manage our raw materials through our production planning process."

partners so we can more effectively assess and mitigate risk and collaborate on solutions to issues that arise.

In-house, we manage our raw materials through our production planning process. We rely on a monthly Sales and Operations Planning process to capture changes in Demand and Supply. Via this process, we adjust our production plan, inventory levels, safety stock, and order frequency accordingly.

Q In what specific areas of raw/starting materials and consumables are you feeling the greatest impact of single source supplier issues and associated IP constraints?

We plan for our single source items well in advance and we have seen lead times dramatically increase with little to no advance notice. This places a strain on planned production runs and maintaining flexibility to cover the cone of demand. While we maintain a healthy inventory position for our commercial program, this has in some cases caused us to revisit our strategies around pre-purchase of materials or boosting on-hand inventory to ensure we keep momentum on clinical development.

We are trying to resolve these supply issues by working with qualified vendors who are willing to work with us creatively to resolve their supply constraints.

Q To what extent – and in what specific areas – has the COVID-19 pandemic exacerbated the situation?

Our production has been stable through the COVID-19 pandemic, and our materials needs have been predictable. In spite of this, it has been difficult to manage our order lead times that continuously get extended.

Specific materials shortages have been in constant flux. Even before COVID, certain media and single use components such as bioreactor and other single use bags were in very short supply. The COVID crisis caused materials shortages due primarily to allocation of materials to manufacturers of the COVID vaccines. We have found that some suppliers are prioritizing government and hospital needs which is resulting in extended lead times for consumables that were once considered off the shelf items. There was significant impact to PPE availability. We've lived through shortages of masks, hairnets, sterile gloves, cleanroom booties.

> "The pandemic has presented its fair share of challenges, but it's also created a number of opportunities to think differently about what we want to translate back into 'normal' operations."

We are also starting to see delays with international shipments of materials by air

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freight. Courier services utilize cargo space on commercial flights, and the significant reduction in international commercial flights has contributed to challenges with delivery lead times.

The pandemic has presented its fair share of challenges, but it's also created a number of opportunities to think differently about what we want to translate back into 'normal' operations. At Spark, we have turned the conversation from "what went wrong" to "what went right, and what have we learned" about the resiliency of our supply base, agility of capacity and sourcing. It has also prompted us to look at social responsibility during a pandemic (e.g. by sharing PPE to assist local hospitals and front-line workers who were encountering shortages). This wasn't something that was part of our initial business continuity plans, but something we can translate into 'normal' circumstances.

Q How are you seeking to manage risk in this regard on an ongoing basis – for instance, in terms of devising alternative sourcing strategies?

We have always had a program for developing and qualifying secondary suppliers for critical raw and starting materials. As a result of the pandemic, we have extended our supply chain risk mapping beyond tier 1 and 2 suppliers and have increased our active and passive risk monitoring our of top 10 critical suppliers.

We are also exploring mechanisms to share or trade inventory with strategic partners and the broader Roche network. For example, can we leverage some of their on-hand inventory to bridge a potential shortage owing to a lead time delay, and then replenish their stock once our delivery arrives.

Finally, we have brought and increased focus to an existing program set up to standardize materials from product to product to reduce inventory SKUs

Q Can you talk us through your approach(es) to raw/starting material testing - and again, how does this differ between in-house and outsourced scenarios?

We have developed and adopted a risk-based model that best leverages the benefits from both in-house and outsourced approaches. For compendial raw materials, we partner with contract laboratories that specialize in this area. For starting materials and non-compendial materials we have developed or insourced in-house methods. Given our platform approach to manufacturing processes, we have been successful in minimizing the overall number of raw materials we have to test and manage.

Q As a trailblazer in bringing gene therapy to the market, what would you pick out as the keys to success for Spark Tx in this regard, from the raw and starting materials perspective?

Supply reliability has always been discussed in materials sourcing decisions, but often undervalued. The importance of high quality, reliable suppliers cannot be understated. In gene therapy, where we are developing treatments for rare diseases, our treatments are often intended to be life-saving or life-sustaining. If we have patients scheduled to visit a clinic for treatment, it is imperative that our product is there for them. One of our Core Values is to Champion the Patient. In this regard, we have spent a significant amount of time and effort prior to the COVID-19 pandemic on risk mitigation and business continuity planning. We see the fruits of that effort now, and also we clearly see areas for learning and future development.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

COMMENTARY/OPINION

Considerations for performing virtual quality audits on manufacturers of gene therapy viral vectors: an auditor's perspective during the COVID-19 public health emergency

Gary C du Moulin

The capabilities of a viral vector manufacturer should be commensurate with Chemistry, Manufacturing and Control (CMC) expectations for phase-appropriate current Good Manufacturing Practices (cGMP). As a critical part of the selection process, a comprehensive quality audit should be planned and conducted. The ongoing COVID-19 pandemic has introduced challenges for an industry increasingly dependent upon Contract Development and Manufacturing Organizations (CDMOs) that produce critical ancillary materials, products, and services. These audits would include an overview of the leadership team, company stability, values and quality culture, talent pool and subject matter expertise, Quality System maturity, and Quality by Design philosophy and processes. During the COVID-19 public health emergency, where the opportunity for onsite audits is restricted, the elements of a virtual audit need to be considered and incorporated into robust audit planning and execution. The virtual audit is becoming a critical event, as IND sponsors are ultimately responsible for the quality of raw and ancillary materials, final drug product, and the safety of patients participating in gene therapy clinical investigations.

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INTRODUCTION

While the US FDA has historically been primarily concerned about safety, efficacy, and durability of cell and gene therapy products, there is continued strong support within the agency for innovation and continued development. Peter Marks, Director of CBER, recently noted that, "As the regulators of these novel therapies, we know that the framework we construct for product development and review will set the stage for continued advancement of this cutting-edge field, and further enable innovators to safely develop effective therapies for many diseases with unmet medical needs. Scientific development in this areas is fast-paced, complex, and poses many unique questions during a product review; including how these products work, how to administer them safely, and whether they will continue to achieve a therapeutic effect in the body without causing adverse side effects over a long period of time." [1]

A number of vector-based gene and cell therapies are in development with several already in commercial phase. According to the US FDA, over 600 cell and gene therapies are in preclinical to Phase 3 development. Viral, microbial, and plasmid vectors are effective vehicles for delivering the next generation of gene therapies. Key Critical Quality Attributes for vectors employed for gene therapy applications include high packaging capacity, stable transgene expression, and low immunogenicity. Final product release testing is dictated by FDA established requirements and pre-determined specifications for safety, purity, concentration, identity, potency, and stability. [2] Under 21 CFR 312.23 (a) (7), (i) sponsors must show in their IND filing a section listing all components used in the manufacturing of the gene therapy product. This information would include a detailed description of the vector manufacturing process, all components including master/working cell bank (MCB) and master/working viral bank (MVB), and all reagents (raw and ancillary) used to produce the banks and vector products. Description, history, and details of the derivation of the vector construct, including the sequence analysis, must be included. Recent FDA Guidance for Industry provides specific CMC information for human gene therapy investigational new drug applications. [2–6]

Developers of gene therapy products are dependent upon manufacturers for the production of high yield and high quality vectors. In accordance with FDA guidance and USP General Chapter <1047>, Gene Therapy Products, the selection of a vector producer is critical for sponsors formulating drug substances or drug products that demonstrate safety and efficacy when used in clinical trials. [2,7] Due to the promise and progress made to innovate vector engineering, delivery, and safety, many vector manufacturers have entered the field, some now with a large client base. [8] Selecting a CDMO with the experience to produce these materials must be a thoughtful and well-coordinated effort. When a gene therapy developer lacks the resources and expertise to adequately perform this assessment, an experienced third party auditor is usually called upon to organize and perform the audit.

Recently, the FDA has issued guidances for sponsors of licensed and investigational cellular and gene therapy products during the COVID-19 public health emergency. [9–10] A disruption in routine in-person auditing for the purpose of vendor selection and qualification has been one result of the pandemic. Official inspection activities throughout the regulated life science industry have also been curtailed. [11] In March of 2020, the FDA announced it was scaling back on foreign and domestic surveillance facility inspections, and relying instead upon (among other measures) the past compliance status of the facility and a remote review of the company's records - an authority granted in Section 706 of the Food and Drug Administration Safety and Innovation Act (FDASIA) amendments of 2012 to the FD&C Act. [11–13]

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FDA PERSPECTIVES REGARDING VIRTUAL GMP INSPECTIONS

To host Quality Assurance audits led by qualified third parties, sponsors are increasingly turning to remote or "virtual" audits. In the European Union these events are termed "distant assessments". The challenges of remote auditing for both auditors and auditees require strategies and best practices for overcoming problems to adequately execute the assessment process. [14] Planning, document review, video communication resources for facility, process review, and competency of personnel are among issues that must be considered in successfully executing virtual audits.

The FDA's Center for Devices and Radiological Health (CDRH) has issued criteria for remote audits during the pandemic through the Medical Device Single Audit Program (MDSAP). [15] However, CBER and CDER have been slow in establishing criteria for virtual GMP inspections. Deputy Director of the FDA's Office of Policy for Pharmaceutical Quality, Brian Hasselbalch, announced recently that the agency was developing industry guidance in support of a move to virtual current good manufacturing practice (cGMP) inspections for drug manufacturers during the pandemic. [12] In his remarks he noted how remote interactions would be used to make decisions about pending applications. Bruce Ross, Director of the Office of Global Operations, noted that FDA global investigators have been conducting "remote regulatory assessments". [13] The European Union (EU), Australian Therapeutic Goods Administration (TGA), and Health Canada have already provided this guidance and have conducted remote inspections, one recently conducted by the EU at the Thermo Fisher Scientific CDMO facility in Alachua, Florida [16–18]. Hasselbalch reportedly advised manufacturers to have the necessary technological capabilities in place for interactive video conferencing, if they have submitted or plan to submit applications likely to necessitate an inspection such as new facilities, operational or control changes, or other situations that would require a pre-approval inspection. While there was reportedly no mention of specific tools or systems to facilitate remote inspections, regulators suggest that industry incorporate tools allowing regulators to connect remotely and facilitate facility and operations document reviews. Until such time as industry guidance is developed to support a move to virtual GMP inspections, the FDA is careful to refer to these reviews as "remote evaluations" rather than "remote inspections". [12]

MANUFACTURE OF GENE THERAPY VECTOR PRODUCTS

The FDA emphasizes four objectives required by sponsors to initiate Phase 1 clinical trials; (1) a reproducible manufacturing process, (2) appropriate testing at critical steps, (3) adequate control of the quality of the raw and source materials and (4) adequate records and record keeping systems. Because of obvious safety concerns for clinical trials involving gene therapies, FDA is particularly interested in viral vector manufacturing processes. In the regulatory action document for the first chimeric antigen receptor (CAR) T cell therapy approval (tisagenlecleucel), the FDA noted that "generation of replication competent lentivirus (RCL) during the manufacturing process for tisagenlecleucel is a theoretical safety concern. To date, no RCL has been detected in any clinical trials using a lentiviral vector transduced cell product, as tested on the vector product with a sensitive co-culture RCL assay or on the final transduced cell product with the same RCL assay or a PCR based RCL assay" [19]. Manufacturers are asked to submit a description of the history of the vector and details of the derivation of the vector construct, including a vector diagram outlining the essential elements of the vector. Currently, the FDA requires a full sequence analysis of gene therapy vectors that are less than 40 kilobases in size prior to initiating a clinical trial. The FDA will evaluate vector manufacturing

information, including batch release, stability, shipping conditions, and quality control functional testing to evaluate safety, identity, purity, and potency. Additional testing is performed for the expressed transgene, infectious titer, and assays for detection of replication-competent viruses. To alleviate any regulatory concern and underscore the importance of the viral vector manufacturing process, the FDA performed two pre-licensing inspections at the facilities involved in the manufacturing of the lentiviral vector with the CAR transgene.

Viral vector production requires a complex series of technically demanding manufacturing steps (Box 1). Depending upon the requirement of mammalian or insect cells in either adherent or non-adherent cell-based systems, vector production can engender a number of possible manufacturing approaches. Laboratory scale systems require manipulation of large numbers of flasks, roller bottles or cell factories, which tend to be difficult to scale-up. Limitations in incubator space and increased number of open manipulations and processing time can result in the potential for failures in aseptic processing. Management of systems for cell and virus banks requires extensive attention to detail in order to ensure line clearance and lot segregation, especially in facilities producing viral vector products for multiple clients [2,20]. However, improvements in scale-up technologies have led to innovations in large single-use disposable culture systems and the employment of bioreactors. These systems are designed to increase cell culture density through a consistent supply of nutrients, while maintaining control of dissolved oxygen and pH preventing the accumulation of cell culture byproducts such as ammonia and lactic acid. Moreover, manufacturers adept at employing a robust Quality by Design philosophy have inculcated principles leading to continual improvements in safety and efficacy of their products. Additionally, these organizations have also developed expertise in developing, qualifying, and validating the analytics required for in-process and release testing. [21–24]

Given the significant financial investment and potential regulatory and safety risks a gene therapy developer will undertake, the assessment of a vector manufacturer by a third party auditor is necessary to adequately assess these capabilities and provide insight for the sponsor into the processes, site metrics, and culture of quality of a manufacturer. Traditional on-site auditing provides the optimal environment for such evaluations. However, the COVID-19 pandemic has prompted a re-examination and re-evaluation of the ways in which audits are conducted. By applying technology, virtual audits can result in a comparable outcome and provide information allowing the gene therapy developer to make an informed decision regarding the choice of a vector producer.

BOX 1

Summary of vector manufacturing steps [21-24]

Upstream production steps

- Vector production platform (AAV, adenoviral, herpesviral, lentiviral, retroviral)
- Adherent or suspension cell lines (mammalian cells, insect cells)
- Thaw cell stock and perform passages (flasks and bioreactor)
- Transfection or infection of cells at final scale
- Lysis/harvest
- Filtration
- Clarified harvest

Downstream purification steps

- Depth and other filtration
- Chromatography
- Filtration (UF/DF) for concentration/diafiltration
- Adventitious viral inactivation and removal
- Formulation (compatibility with cell/organ target/route delivery device)
- Aseptic fill-finish
- Storage, packing, and distribution

TRANSLATION INSIGHT

Auditing a CDMO producing Viral Vector Products

Conducting an audit of a CDMO dedicated to the manufacture of viral vector products

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will be challenging. Much like traditional biomanufacturing processes, the production of viral vectors is complex. Organizations producing vector products for multiple clients require a robust culture of quality executed by a competent workforce. The understanding of the concepts and goals of phase-appropriate GMP for each client's development program depends upon a facility whose manufacturing processes are well controlled, and whose vector product can be manufactured to parenteral drug specifications, or specifications required for ancillary materials. [25]

In selecting a contract manufacturer of vectors, a sponsor (especially one with a limited experience in assessing the capabilities of a viral vector manufacturer) will rely upon a third party auditing consultant to provide insight into the selection. The third party auditor should be qualified by sufficient prior experience in quality positions of increasing responsibility, especially in the biopharmaceutical arena. The depth of knowledge of the auditor cannot be underestimated when selecting a CDMO for vector manufacturing.

Initial preparatory steps for the virtual audit

Depending upon the size and scope of the supplier's products and services, a manufacturing organization may have to host many audits. Some companies may have not planned ahead for the possibility of hosting virtual audits, or may not have the resources to conduct these effectively. Additionally, an auditor may not have the experience of executing these audits remotely, leaving both parties unprepared for this important task. If one or both parties are unprepared to perform or host this form of inspection, the chances that the objectives of the audit are accomplished could be in doubt. Scheduling the audit with a long lead time provides the best opportunity for preparation to be undertaken by both parties, increasing the chances of a successful audit. Box 2 summarizes suggested general timing of the events encompassing the planning and execution of a virtual audit. Audit planning should begin a minimum of 8 weeks prior to the proposed audit date. Depending upon the CDMO, this lead time could be longer.

The auditor should identify key points of contact, usually from the company's Quality Assurance organization. The manufacturing and Quality Control subject matter experts responsible for vector production and analytics should also be identified at this time. The auditor should promptly develop an online and telephone relationship with these individuals, and immediately begin the dialogue in confirming the timing, agenda and expectations. Pre-meetings are an opportunity to build trust and credibility between the two parties. Both parties need to agree to these elements earlier rather than later. The auditor needs to additionally develop a point of contact with the project manager who ultimately will be responsible for the client's specific vector manufacturing program. If a quality agreement exists between the company and the client, these important individuals should be listed within this document.

The depth of the audit should be commensurate with early development and phase-appropriate GMP expectations of the client. [25] The regulatory standards should be understood by both parties. If clinical trials are planned in offshore settings, the regulatory environment of the development program could include guidances from US and international regulatory bodies.

Elements of a virtual audit

United States or international cGMP regulations, standards, and guidances do not prohibit the use of remote or virtual auditing techniques. In response to the ongoing pandemic, recent directives from the EU and TGA have been issued [16–17] In fact, the Pharmaceutical Inspection Convention and Pharmaceutical inspection Co-operation Scheme (PIC/S) have had guidance published since 2018 describing an inspection process for assessing satisfactory levels of GMP compliance

BOX 2

Proposed Lead Times in Preparing and Executing a Virtual Audit

- Preplanning meeting 1: First contact with the organization to be audited. Points of contact identified. Audit date proposed and agreed upon: 8 weeks out
  - GMP Quality Audit Questionnaire is sent to auditee
  - Confidential Disclosure Agreement (CDA) addendum provided by company legal staff is signed
- Preplanning meeting 2: Audit agenda supplied by auditor: 6-7 weeks out
- Tour element and Information Technology (IT) resources committed: 4-6 weeks out
- Preplanning meeting 3: Auditee supplies SOP lists: 4-6 weeks out
- Auditor selects SOPs to review: 3-4 weeks out
- Auditee makes requested documents available on online portal: 2-3 weeks out. Auditee confirms video communications are available and operational
- Final or Preplanning meeting 4: Auditor makes SME interview request: 2-3 weeks out
- Initial list of questions and discussion topics to be provided by auditor, SME interviews scheduled: 3 weeks out
- Auditee hosts Audit Day 1 (Opening meeting)
- Auditee hosts remaining Audit Days including closing meeting

without an onsite inspection. [26]. However, the success of a virtual audit must be balanced against the relative risk posed by the organization. For example, organizations with a prior history of compliance issues such as recalls, frequently rejected products, and other negative quality trends, are probably not good candidates for a remote or virtual audit.

The same objectives associated with a conventional onsite audit must be engineered into the planning and execution of a virtual audit. However, some auditing activities may be difficult to execute on a virtual basis. Alternative methods should be thoughtfully employed using a risk-based approach to achieve the same objectives. In auditing manufacturers of viral vectors, the complexities of the manufacturing processes should require a review of the major key quality systems. These include (1) Quality system, (2) Facility and Equipment system, (3) Materials system, (4) Production system, (5) Packaging and labeling systems, and (6) Laboratory control systems. In each system examined, representative SOPs, associated training, and documentation should be selected and critically evaluated.

As in all audits, robust communication between auditor and auditee remains a critical attribute. This will require detailed pre-planning and scheduling of the audit with full agreement on goals, methods, and outcomes. If technologies are to be employed, these should be designed to be compatible with those in use by both auditor and auditee. Pre-audit mock audit exercises should be planned in advance to ensure that the technologies to be employed are workable, especially during plant tours to facilitate inspection of manufacturing processes, reading of labels and documents, etc. As part of pre-planning, all legal contracts and CDAs must be executed in advance, especially if language is inserted to allow the auditor access to online documentation portals or other proprietary sources of corporate data.

Eight weeks prior to the audit, a GMP Quality Audit Questionnaire is sent to the company's audit point of contact along with a draft audit agenda. The questionnaire, similar to a vendor qualification questionnaire, is important as it can provide early insight into the regulatory history and quality systems of the company. The questionnaire seeks general information about the company, regulatory/quality information, facility and equipment data, inventory and materials management, etc. The draft audit agenda identifies the objectives and goals of the audit and initiates the schedule and timing of the audit. This is a

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draft document that will be finalized with the company audit representatives as the date of the audit approaches. Robust communication and pre-planning that can be accomplished days and weeks before the actual date of the audit can result in a significant advantage in optimizing the success of the audit. Three to four pre-planning teleconferences may be necessary to accomplish this task.

Organizations that intend to host multiple virtual audits would be well served by creating a Standard Operating Procedure that directs the guidance and planning elements. A step-by-step guide with flow charts that articulate timing and audit choreography will be useful for future regulatory inspections or vendor qualification audits. This planning is also necessary in the advent of audits requiring multiple auditors, time zone variability, language differences, or where the auditors have limited experience of conducting virtual audits. The Quality Assurance organization should conduct mock audits, making adjustments to the procedure on the basis of these internal auditing experiences.

Documentation review

The "currency" of the company is revealed in the quality of its documentation. Following the issuance of the CDA allowing the auditor access to the documents, a number of documents and procedures should be requested (at least 3–4 weeks prior to the date of the audit). Box 3 identifies suggested policy documents that should be made available upon request. Specific SOPs representing Quality System elements should also be requested. An example of these documents is shown in Box 4. An online portal should be made available to the auditor so these documents can be easily accessed. Auditors must be given sufficient time in which to review these documents. Time-consuming document review during the virtual audit is usually not feasible.

It is critical for the auditor to spend the time carefully reviewing documents for content and compliance to GMP regulations. The documents should reflect all quality systems. Any questions or concerns that emerge from the review of the documents is noted. Issues prompted by this document review will be addressed during the audit with the appropriate subject matter experts. It is important to note that a virtual audit requires that this document review be conducted by the auditor prior to the audit. If not, a successful audit is unlikely, as the auditor will not have the opportunity to appropriately assess the quality architecture of the organization during the virtual audit format. While the company may have time restrictions for access to electronic documents by a 3rd party auditor, a three-to-four-week lead time to review documents prior to the audit is not unreasonable.

Information Technology (IT) & video communication & preparation

Successful virtual audits will largely depend upon information technology and video communication resources. During pre-planning meetings, auditor and auditee should know well in advance of the planned audit what teleconferencing or videoconferencing capabilities will be available. Information technology resources of the company become critical members of the auditee's digital support team, and should assess available bandwidth, firewalls, software limitations, and security risks that would impact the deployment of these resources. The auditor's expectations of visibility of manufacturing, packaging, or quality control locations and processes need to be clearly defined with the capability of live sharing of screens displaying computerized systems used at the site. The company being audited may not have planned for the eventuality of a virtual audit, and consequently may not have resources on hand and in place to assist an auditor conducting the audit. Audits have been performed in a virtual mode using modest equipment, e.g., videoconferencing and iPad technology. However, organizations should be proactive and develop standard operating procedures that incorporate online digital

BOX 3

Suggested Quality System Evidentiary Documentation to be requested and reviewed in advance of the audit

- Organization chart/current reporting responsibilities
- Last two years of regulatory inspection history (including FDA Establishment Inspection Reports and 483s)
- List of Standard Operating Procedures
- Diagram layout of the facility (including heating ventilation and air conditioning (HVAC) plan
- List of equipment (manufacturing and Laboratory)
- List of computer systems
- Quality Manual
- Site Master File – or diagram layout of facility, process, waste, and personnel flows
- Validation Master Plan (List of validated processes and methods)
- Qualification Master Plan (Qualified equipment and tests)
- Lists of change controls, deviations, nonconformances, and corrective and preventative actions (CAPAs) that have occurred in the previous six months
- List of all out-of-specification events, including stability results for the last twelve months
- Training records for personnel involved in audit-related activities
- Calibration and qualification/validation protocols and maintenance reports for equipment involved in relevant production/analytical testing
- Quality Control laboratory controls
- Classified area information with contamination controls, including environmental monitoring and trending reports
- Quality Management Review (QMR) agendas

conferencing platforms to host and facilitate these audits. Some organizations have planned ahead and are very well prepared. For example, an employee should be identified as the "eyes" of the auditor to act as narrator during live (or real-time) tours. A virtual audit conducted by the EU at the Thermo Fisher Scientific viral vector manufacturing site in Florida is a recent example [18]. An excellent summary of this experience by this well-prepared organization has been described. As a result of this audit, a GMP certificate was issued [27]. Most other organizations will not be so well prepared and can learn from this experience. In such cases, the basics outlined below (Table 5) can help ensure that a virtual audit can be successfully managed by the auditee.

Execution of the Audit: best practices

Remote auditing is an intense activity on both sides of the computer screen. The auditor is under pressure to ensure that sought-after information is obtained during each part of the audit. However, in the event that a pre-planned interview with an SME cannot be met, the auditor must, in consultation with the company audit representatives, make quick adjustments. One way in which this can be achieved is to conduct the audit for a four-to-five-hour block of time each day, which recesses for the remainder of the day. This hiatus allows the auditor to review the days past events, and review additional documents and data, whilst preparing specific questions for SME interviews during the next day's activities. This format also allows company personnel to tend to other commitments.

Efficiencies gained during virtual audits can result in a better use of time and management of resources. It can minimize environmental contamination risks within controlled areas. It also allows document reviews to be performed prior to the audit and better prepares the auditor. The primary limitation of

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BOX 4

Suggested Standard Operating Procedures to be requested and reviewed in advance of the audit

- Quality agreements
- Validation program, preventive maintenance, and calibration program
- Employee training, document and record control
- Raw material release, Material Review Board
- Manufacturing Batch record and QC test record review
- Label control lot release
- Deviations and CAPA
- Change management
- Internal Audit/inspection and vendor qualification procedures
- Complaint management
- Contamination Control/Environmental Monitoring trends
- Documentation and record archives
- Warehousing procedures
- Preapproval inspection readiness

these audits is the loss of face-to-face interaction and personal connections.

The experiences to date can lead to additional best practices that could improve the success of future audits. Some of these are as follows:

- Early preparation for these audits is crucial.
- The audit host must always be available and authorized to make schedule changes and modifications if and when the situation dictates
- Access to a secure cloud server to share documents
- Digital support teams are critical elements and must be easily accessed when needed
- SMEs to participate in discussion and answer questions should be identified ahead of time and available at predetermined times
- Virtual audits can work well when the organization being audited has a robust record of regulatory compliance. Companies with a history of regulatory issues and questionable follow-up will not be acceptable candidates for virtual audits. PIC/S has noted that "desk top assessment of GMP compliance of facilities in other countries is possible when an acceptable level of GMP compliance can be confirmed and assured from the activity of another compliance audit." [26]
- Video tours of manufacturing sites, warehouses, and laboratories are technically demanding, and must be choreographed and practiced.
- Multiple auditors, time zone, and language differences can exacerbate potential difficulties in successfully executing auditing activities and must be accommodated through robust planning. For example, using a live videoconferencing platform, breakout rooms may be reserved to facilitate separate channels of discussion between different inspectors.

Audit closure & conclusion

1. The last day of the audit will include a closeout meeting usually prepared by the auditor during the previous day's recess.
2. Both parties will review and discuss findings and action items in an audit summary
3. Follow up items will be agreed upon and a timetable will be established so that these items are addressed in a timely manner.
4. Audit results are categorized depending upon the severity of the observation.
    - Acceptable or minor deficiencies

- Conditionally accepted or major – problems that require auditee responses before an "Acceptable" status is issued
- Rejected or critical observations – reveals a facility to be of high risk.

5. Audit report should be expected within 30 days post-audit
6. Once the audit report is received, a point-by-point response with corrective actions and timeline should be received within 60 days, following issuance of the audit report.

SUMMARY

Selecting a CDMO that offers the experience necessary for high-quality vector production services is essential and requires more than an understanding of the science involved in cGMP vector production. While a virtual audit is not equivalent to an on-site audit conducted by an experienced inspector, it can serve as an interim solution, allowing the industry to maintain its development timeline. For the virtual audit to be successful, extensive pre-planning with open, honest, and transparent relationship-building. Planning and communication between the parties must start very early. CDMOs should develop Standard Operating Procedures specific to virtual audits, determine the technical resources that will be needed, and conduct mock audits that simulate regulatory inspections or vendor qualification audits. Choreography with built-in timeframes can maintain the discipline needed to ensure that all aspects of the audit have been adequately addressed. All parties involved, including SMEs and digital resource personnel, must be on hand to support the audit. Finally, all electronic resources must be in place and field tested prior to the start of the audit. In the end, the auditor is seeking maximal transparency and confidence that all participants can maintain the objectivity of the audit process and are fully engaged in providing the information, resulting in a clear and complete understanding of the organization's fitness for the client's needs.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

Fitting product to process: raw materials customization for cell therapy manufacturing success

Lili Belcastro, Mitchell Brabec, Lindsey Clarke, Raymond Luke & John Paul Tomtishen III

Cell therapy manufacturing involves highly complex processes, with large numbers of inputs, and therefore a high amount of associated risk. A robust supply of high quality raw and ancillary materials is crucial to successful manufacture of cell therapy products – but selecting the best supplier, and the right product, can pose a challenge. Raw material customization may require more up-front investment from manufacturers, but even relatively small modifications to packaging and fill sizing of off-the-shelf materials can provide cost-effective products that better fit process requirements, and help to de-risk manufacturing. Identifying risks up-front and customizing products where required can save time, money, and ultimately speed up commercialization of therapies.

In the following case studies and expert roundtable, the benefits and drawbacks of both off-the-shelf and customized raw materials for cell therapy manufacture are discussed

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SMALL CHANGES THAT CAN TRANSLATE TO BIG BENEFITS

Case study: A simple fill modification of an off-the-shelf reagent

When considering customized raw materials, even small changes can help to drive a more streamlined manufacturing process. Simple fill modifications of off-the-shelf products can help to manage risk, improve cost, and save time in the clean room.

In this case study, a manufacturer wishes to add an optimal quantity of cytokine to media used for culturing cells. Introducing this reagent into the process is often an open

step. Cas cytokines are commonly supplied lyophilized in glass vials, while the media required for closed or automated cell culture is provided in bags. The use of predefined 'off the shelf' sizing also means the quantity being added may need to be modified manually for each manufacturing run. As cytokines are a reagent of biological origin, further complexity is added, as there may be lot-to-lot variability. This can result in the need for lot-standardization efforts in-house.

As illustrated in **Figure 1**, there are several ways a manufacture could approach the need to introduce an optimal dose of cytokine into a bag of media:

Option #1: Take a bulk quantity of cytokine & aliquot the exact amount for the process

This provides the optimal predetermined quantity for the cells, and is optimal for process standardization as the same operator is measuring each aliquot. However, this also introduces risk – if the cytokine is contaminated, or resuspended in the wrong volume, multiple patient products could be affected before the error is discovered.

Option #2: Off-the-shelf 'process size' vials

This option carries less risk as the vials are all coming straight from a supplier, and will be sterility tested. With no pre-aliquoting required, this approach will also save time. However, it may not be optimal for standardization between every manufacturing run. It is also possible to use a process size vial to oversaturate the culture, to eliminate potential measuring errors and improve standardization. However, this may not be optimal for the cell culture.

Option #3: Customization of fill & packaging

If suppliers provide the cytokine aliquoted at the exact requirement of the manufacturing process then it is both optimal for cell culture, and more standardized. If that packaging could be further streamlined to being closed system compliant (e.g. Bio-Techne ProDots [1]) then the risk of having an open step is removed. If manufacturing is being performed on a significant scale then bulk savings, alongside reduced risk and cleanroom time, will balance additional costs.

BESPOKE PRODUCTS FOR SPECIFIC NEEDS

Modified off-the-shelf products may be suitable for some manufacturers – but in some cases, the product a manufacturer needs does not exist. The following case studies highlight the important factors to be considered before committing to a critical reagent that may not be appropriate for future clinical development.

Case 1: Licensing & freedom to operate

The project

A client has been using a conjugated and hybridoma-derived Research Use Only (RUO) antibody for all of their preclinical work. Now, after risk assessing they have determined they are unable to move this material into Phase 1.

The problem

The product is not available in GMP grade, is not manufactured under animal-free conditions, and use is restricted by licensing issues.

The foremost consideration is whether the client has Freedom to Operate using this clone, as the owner of the clone may have sublicensed it to a different vendor, or there may be restrictions on commercialization or modification of the product. Having an open dialogue with the vendor to ensure the material is suitable for the intended use, prior to committing to a specific product, is imperative. In addition, the future conversion

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FIGURE 1

Potential approaches to adding a defined activity/volume of cytokine to 1L of media in bags.

	Large cost effective vials	Off the shelf "process" size vials		Custom fill vials exact process size	Bio-Techne ProDots
	Aliquot EXACT amount for process	Measure out EXACT amount every time	Compromise - use the entire vial contents	Use the entire vial	Cytokines in bags: EXACT Quantity, Closed system compatible
CLEAN ROOM TIME	⏱⏱⏱⏱⏱	⏱⏱⏱	⏱⏱	⏱⏱	⏱
OPTIMAL for CELLS?	✓	✓	✗	✓	✓
OPTIMAL for Process Standardisation?	✓ 🧍	✗ 🧍	✓	✓	✓
RISK	🧍🧍🧍🧍🧍🧍	✓ 🧍	✓ 🧍	✓ 🧍	✓
COST	$	$$$	$$$$	$$	$$$

Balance risk versus cost.

of the product to a recombinant clone, or being able to manufacture it in a GMP facility that is also animal-free compliant, will be important factors from a regulatory perspective. If the product comes into contact with animal-containing components, this could put approval of the therapy at risk.

In this case the vendor was not only unable to manufacture under GMP conditions due to their capabilities, but their license also restricted their ability to do so. These issues may not be addressed during the usual purchasing process – but giving a vendor the ability to understand where a project is going, and providing them with information on a client's ultimate goals, will allow client and vendor to work collaboratively to ensure scalability and success.

The solution

A licensing conversation with the owner of the antibody clone led to them agreeing to a contract that allowed the material to be modified, converted to GMP grade, and supplied to the client at the scale required.

Case 2: Scaling up for future manufacturing

The project

A client is using a contract manufacturing organization (CMO) for RUO manufacturing of a proprietary protein sequence used in therapeutic discovery.

The problem

The current manufacturer is unable to scale the process to meet required yields, and cannot develop under GMP conditions.

In this instance, licensing is not an issue as the product is a property protein sequence being used in therapeutic discovery by a clinical development company. The company had outsourced manufacturing to a CMO that was able to provide a pure and active protein product which worked in all of their preclinical processes. However, once the company was ready to move into Phase 1, they approached the vendor to convert the protein sequence to a GMP product, and found the manufacturer was not able to meet their requirements.

These issues are frequently caused by the need for animal-free conversion of the manufacturing inputs while also scaling up to the multi-gram yields that are required, and the need to manufacture under GMP conditions.

From the client's perspective, asking whether or not it is possible to convert a given RUO product to GMP, or even being involved in the RUO development process, can help with the transition from preclinical to clinical work. However, many vendors will not allow input and transparency around their internal processes, or allow modifications to their processes. Finding a vendor that is able to meet manufacturing needs, and be flexible in how it fulfils those needs, will help with commercialization, future forecasting needs, and future supply runs.

In addition, regulatory and quality support are key when a company reaches the stage of submitting an IND and filing other required documents with regulatory bodies. If there are differences in how they test with validated assays, or if they are unwilling to share details about the manufacturing process, a client can find themselves in a situation where they have a product that works, but the documentation regulatory bodies require is not available.

The solution

The product was taken in-house (at Bio-Techne), and all processes were converted to animal-free and GMP grade. Appropriate regulatory documents were filed to support the client throughout development.

Case 3: Continuity through clinical development

The project

A client is using a RUO protein and RUO antibody from different suppliers during preclinical development.

The problem

Supply chain and manufacturing inconsistencies between critical reagents.

This case study highlights the importance of supply chain continuity to support and simplify clinical development. At the point that a product has been developed, and multiple products may be going in to a manufacturer's workflow, difficulty can arise in managing several different vendors. This particular client had been purchasing RUO antibody and protein from two separate suppliers during their preclinical development, and chose to condense down to one vendor that could supply both in a GMP fashion.

The main problem the client faced was supply chain and manufacturing inconsistences between critical reagents. The need to coordinate deliveries, and coordinate and align the quality systems that were used during manufacture of the different materials, was leading to concern about the quality of the final product.

Aligning the quality requirements between different vendors, and comparing them at an early stage, may be challenging. In contrast, selecting one vendor and ensuring they provide all the quality requirements a manufacturer needs for one product can make it easier to then meet requirements on additional products.

In addition, when developing a custom product, the stability of the raw materials used can potentially affect the final process. Off-the-shelf products typically have stability studies performed on them so that the manufacturer is aware of their shelf life. With a custom product, it may be possible to instead establish extended stability that is aligned with the shelf life of the intended final product. In addition, the client can specify the assays that are performed to better optimize the stability studies.

The solution

By condensing suppliers, it was possible to modify the certificates of analysis, and modify the post-vialing QC testing, cell line testing, and other analytical testing, either in-house

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or outsourced. This simplified both the client's workflow, and the documentation processes when they came to file for an IND with the FDA.

SUMMARY

Investing in the selection of raw and ancillary materials early on in the manufacturing process can save significant time in the later stages of development – not only does it allow for improved forward planning, it can also enable the development of tailor-made product that better meet a manufacturer's process requirements.

Customization options can offer more flexibility from off-the-shelf products that already exist by providing specialized bags or filling sizes, or specialized testing. Alternatively, developing a *de novo* product can provide a bespoke solution for the often complex requirements of cell therapy manufacturers.

Q & A

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Q What are the main issues that each of you currently face in sourcing and securing an adequate and continual supply of your critical raw and ancillary materials?

RL: One of the most important things is the pace of innovation in cell and gene therapy. The industry is maturing very rapidly, and has gone from an academic dream just

over a decade ago, to commercialization today. It is starting to outpace the ability of off-the-shelf materials to accommodate it.

There are two different reasons for that: the science, and the compliance. From a science perspective, what we are looking to do as we innovate is to bring something from the benchtop to the bedside as quickly as possible. Oftentimes in these kinds of atmospheres, we are finding something potentially from academic papers, from research, and we are trying to bring that into patients as quickly as possible. We also want to do it in a safe and complaint manner. This means we need to find a partner that can help bring these innovative materials from RUO to GMP. We will often have shifting specifications, or shifting needs, as we discover more about what we are going to do with this material.

On the compliance end, again as we mature as an industry and are approaching commercialization, it behooves us to increase our compliance and increase our efficiencies, by closing the process and making things more efficient in general. One of the issues with off-the-shelf materials is that often a lot of the materials that the industry had used previously were essentially holdovers from blood bank processing, and things like that. They don't fit the process very well. We are trying to find materials that fit the process, and often that will require customization. We have to find the right partner to do that with.

LB: In my experience, with the cell and gene therapy industry being in relatively early stages, often a lot of these materials are only available from one supplier. These single source materials present the greatest potential risk to your supply chain.

It is therefore incredibly important to develop a relationship with your supplier as early in the development process as possible. As the user, you need to communicate to your supplier what your needs are and provide as thorough an overview forecast as possible. This way, the supplier can either confirm that they can or cannot fulfill that need, or alternatively they can begin to develop their own internal manufacturing capabilities in order to meet your needs.

JT: One of the key considerations at the top of my mind, especially with the pandemic and how important business continuity is, is when you think about ensuring you have the appropriate level of safety stock, make sure you work closely with all your different suppliers. Even when you think about the significant demand last year with masks and gloves and things like that – it is about really looking at your overall business continuity strategy. That is a critical component we need to think about to ensure we have the critical raw and ancillary materials needed to meet the demands of the industry.

Q Looking at how to then mitigate those risks and challenges, what are the key tools at your disposal for managing risk in your raw material supply chain?

LB: You always start with qualifying your supplier and performing routine audits, ensuring that they are manufacturing at the appropriate GMP level, and that

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they have cross-contamination controls in place. You can ensure that their raw material specifications include some identity and safety testing.

You also want to try and identify an alternative supplier – you should at least try to have a dual source, at a minimum, for each of your materials. Creating a custom material with an alternative supplier is a great way to avoid risk to your supply chain.

You can use a risk assessment that includes supply chain risks to identify those risks, and implement mitigations at the supplier, or through your own internal testing. Then you will have your internal verification testing prior to manufacturing, and at a very minimum you need to include identity and safety testing.

As you move through the development process, you can add on more critical attributes as you become aware of them and as your process knowledge and material knowledge increases. In addition, keeping up to date with what others in the industry are doing, and what regulatory documents and guidances are available, will certainly help you reduce risk in keeping your supply chain.

Finally, it is all about your procurement. Having a really good relationship, having routine meetings with suppliers, and conveying any changes in your supply chain or demand are key. For example, can you give your forecast 9 months in advance, versus three? And of course if it is a larger manufacturer, ensure you have multiple manufacturing sites qualified and not just one location, for example.

JT: I would add that I really like the idea of secondary sourcing. That is a key component to mitigating risks to your overall supply chain. Something critical to that as you think about the risk to your supply chain is quantifying with each of the different suppliers the possibility of failure.

When you think of the overall materials that are needed, there are multiple different suppliers that companies are working with to produce cell and gene therapy products. It is about understanding where the highest risk is, and deciding what to focus on first. That will be a key component of building out an overall strategy as you think about supply chain risk mitigation.

RL: Something I think is really important, and a great tool, is the partnership between the supplier and the company. You want to be very transparent about what you need, how much you need, and when you are going to need it – especially in autologous cell therapies, where we are predicting the number of patients we might get that year. Let the supplier know there is some level of uncertainty in those patient level forecasts, and build those in to the supply agreement so there is transparency on both sides. This means that each side knows what kind of demand they are going to expect, and allows you to ensure that the supplier can commit to that. Transparency on the ability of both sides to supply for these autologous patients is crucial – each lot is a single patient, so we have to make sure we are able to do that.

Q Mitch, we have heard a great deal here about the importance of partnership as being central to managing risk – from the perspective of the supplier, would you agree with that sentiment?

MB: Whenever a supplier can plan ahead, and potentially sequester lots specific for that therapy, or manufacture additional material according to what is forecasted, that is always going to help streamline the process. As Raymond said, it is an estimation of how many patients you may get. Being able to operate with additional mass can be helpful if you know there are more or less patients, and have that flexibility in the supply agreement.

Q There is a great deal of discussion in the field at the moment over the question of whether off-the-shelf or customized raw materials are preferable. What does each option entail for you specifically?

JT: Everyone's goal is that off-the-shelf products will work. There are some challenges, and for certain processes maybe an off-the-shelf product won't work, and you will have to go to the customization route. But ideally, off-the-shelf is preferable, and it decreases the amount of time you will need from a development perspective, versus developing a customized solution with a supplier. You can bring it in immediately to your manufacturing processes or QC processes.

One of the key components to that when you explore options with suppliers from a customization perspective, is understanding what that customization really gets you versus what the off-the-shelf product is already capable of doing.

A good example would be if you are doing a harvest of cells during a cell and gene therapy manufacturing process, and your yield is, say, 40%. You need to understanding whether that is really significant. Do you need to look at a solution that increases that yield, or is 40% good enough for what you need to deliver to reach your final products and end goal?

The key is if off-the-shelf works, great – but if not, understand from a customization perspective what the data truly means, and understand whether that is a significant area you need to focus on, or if you should focus on another aspect within your process.

LC: I completely agree. This is going to depend on where you as an organization are with your process, what your goals are, and where you are along the pathway to commercialization.

In an ideal world, an off-the-shelf product is going to be a perfect fit for what you need it to do, will fit well with your strategy for manufacturing, and it is going to be scalable. But as you are moving forward towards when you need to secure that supply chain, there may be a point when you can stop and ask yourself if a customized option will confer an advantage. How do you decide if a custom product is going to be for you? Look at where your process is going, where you want to be, and what you would want of an off-the shelf product to make it fit better.

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RL: In an ideal world, off-the-shelf is exactly what you want. Less work goes into determining the specifications and what needs to go into it. But obviously, as I mentioned earlier, we are a growing and a quickly innovating industry. There are times where you have to take some perspective and understand that if the off-the-shelf piece does not fit what you need, you need to pursue a partner that can help you innovate a new, custom approach. And potentially in the future, that can become an off-the-shelf offering for that supplier as well.

If you are looking to improve a step in the process and what you are able to find from an off-the-shelf supplier does not get you to the goal you need, then you go out and speak to custom manufacturers and define exactly what is needed. So it really does depend – both of them have their benefits, but it is dependent on your goal.

LB: Again, it all depends on your process. Can you fit an off-the-shelf material to your process, or is your process in such a place and so unique that you need to have that custom raw material?

Off-the-shelf is nice, but some of these more complex materials are often protected by intellectual property (IP) at the supplier. Understandably, the supplier needs to protect their IP. But when you are trying to set up your own internal specifications for these raw materials, not knowing exactly what the raw material is made of makes it very difficult to set a specification for identity, for example. That is where a custom raw material can come in – then you own the material, you have full knowledge of it, and it is all right there for you going in.

Q Let's go a little bit deeper into some of the specific pitfalls we touched upon there, that are commonly encountered by the panel with each option. How can they be best avoided?

MB: One of the pitfalls we run into with off-the-shelf GMP products is the time lost, and the risk added in, from the direct handling by the operator. If you are able to close that process with, for example, a customized product in a bag, that can lower the potential of failure there.

Although off-the-shelf GMP can save time, if there is any issue with the processing or handling, or if that product doesn't exactly fit the workflow, then a custom option may actually be a more cost-effective and timely answer.

For example with products in a bag, if the mass isn't exactly what is needed to make your media prep, then theoretically, customizing that could save time. It may also be more cost-effective down the line if you don't need to purchase multiple versions of a product, and things like that.

The pitfalls of customization are upfront timeline and price, but ultimately that might be mitigated with a risk saving.

RL: One of the major pitfalls you have with the off-the-shelf materials is that you will tend to find that with the pace of innovation we are going at, you do not always have what you need to fit into the process. As an example, you find a cell

culture medium from an academic paper and it greatly enhances your products, but you are unable to find it as a GMP product off-the-shelf. What you will often do is go and partner with someone to provide you with that material. You cannot get that material off-the-shelf, but being able to convert it to a GMP process will enhance your product greatly.

LB: With custom material the biggest pitfall is obviously the cost and time the user needs to put into it. You sometimes have these biotechnology companies buying each other out, and then they may no longer be able to perform that manufacturing for you.

For off-the-shelf materials, I would say again running into IP issues for the more complex type of materials is the biggest pitfall. There is also a greater risk in terms of your supply chain. There may be other users of that exact same material, and if they suddenly go into a larger scale, from development into commercial, that can mess up your own supply chain. This is where the customization may be better, because you can direct how much you need manufactured each time.

JT: I would add one additional perspective from the business side of things, dovetailing off some of the earlier discussion about single or secondary sourcing.

When you think about going with a customized solution, you are almost creating that single source perspective. You are obviously going to partner with a supplier, so you understand the suppliers capabilities of continuing the supply of that customized solution. If you are investing a significant amount of time and money into a customized solution, you need to understand what their capabilities are.

The other component to that is as you think about a customized solution, how does this all fit into your overall framework of your product portfolio? Are you looking at something as a one-off for one asset you are producing, or a larger area? Look at how you can leverage these off-the-shelf or customized solutions across multiple different assets, as a platform your company can build upon. That has a much more broad impact, even on design of your facilities and things like that.

Q Can you give some further examples of what is involved in taking that off-the-shelf product and fitting it to the client's process, and converting that to a GMP model?

MB: I view this as two separate examples. One will be changing a small factor of that off-the-shelf product, whether it be the formulation, liquid versus lyophilized, a smaller pack size, or potentially putting it in a bag. Those types of projects tend to be slightly more straightforward, just due to the fact that the specifications may not change from what the off-the-shelf GMP product is. In addition, the process for making it won't need to change. It is a matter of reconfirming activity, stability, and all of those release criteria, but potentially not a lot of development work is needed.

The other example, which may be a bit more involved, is when you are changing a specification – whether that means starting from scratch with a new protein or a new antibody,

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or maybe just tightening the endotoxin specifications, and other things along those lines. If you are going to be changing a specification, that is sometimes where it becomes a bit more involved with the process, whether it needs to go to a different lab within the facility, or what the limit of detection is for the assays, and which assays are validated. There are a lot of factors that go into it, depending on whether or not it is effectively a size change, or we are actually changing the specifications of the product itself.

LC: When you are risk assessing your product, it may be that you don't need GMP yet, but what you have got is not good enough. There is a discussion you may want to have about what the intermediary step is, because when going to a full GMP manufactured product, that timeline may not fit with where you need to be.

We have had several projects, where an intermediate step is good enough for Phase 1, with the end goal in mind that if these programs are taken forwards, then products will in that timeframe be made to GMP, and be appropriate for that next step in the progress of the therapy.

Q When is the key time to partner with a raw material supplier, as far as each of you is concerned, and why?

LB: The simple answer is as early as possible. If you can do it during preclinical development, do it. It takes time to build that relationship with a supplier, and it takes time to get the CDAs in place, and audit and qualify them. It will help you avoid delays later in development.

You can also learn early on whether your supplier is going to be able to fulfil your needs. Let's say you do need a customized size or container – you need to know that at the beginning. If they cannot deliver that, then you might need to find another supplier. Sometimes it is about shopping around for the correct point of contact within a supplier site. Getting the right people, and developing a relationship there, is the key to being successful.

RL: I would agree with Lili and say the earlier you can do it, the better. As soon as you understand what you want, kick off a sourcing event. As soon as you have the ability to put at least the basic necessities of what you are going to need on paper, you can shoot that out. It is good to be able to start early so you can avoid pitfalls. Additionally, one of the things we find, especially with the pandemic, is that lead times can be much longer than you anticipated. If you wait too long to approach a supplier, it may be too long even for a customer project, because of lead times and especially during the pandemic.

JT: I also agree that when you think about the right time to partner, the sooner the better. One thing I would add to that is the importance of having that continuous and open dialogue with your suppliers as your projects progress and you go through clinical development, and through to commercialization stages. When you think about suppliers, you are probably going to leverage several of them across multiple different assets or programs as well. Understand what lead candidate you are focusing on, what is next within your pipeline,

what the timeline is with that, and be upfront with suppliers to ensure they can meet the demand for multiple assets within your overall product portfolio.

Q Mitch – from the supplier side, do you feel that people are engaging with you early enough in their development cycle?

MB: It is ideal when it is as early as possible. I would say it is probably about 50/50 whether someone is coming to us and needs a better product or a change to the product they are using, versus coming to us in the preclinical stage.

One other thing to follow on from what the other panelists were discussing is there is a lot of qualification that goes into picking a vendor. If you are able to collaborate and have a joint experience to optimize that process for whatever reagent it is, you will know those processes are in place to support your other pipeline initiatives as well. It can be a timesaving, once you have that supplier in place, to continuously use them for either off-the-shelf or customization. It can make the process for your other pipeline initiatives simplified as well.

Q Lindsey, what would your key advice be in terms of optimizing these partnerships, as you progress through development towards commercialization?

LC: Everyone has touched on it: talk to us early is definitely the recommendation.

Transparency and communication between manufacturers and suppliers is just so important. All of us on the supplier side want you to succeed; we want you to develop these therapies and have absolute success with them. Part of that is not being afraid to ask us those difficult questions early on. If we are not able to grow with you to supply those needs long term, then we need to be transparent about it. My recommendation is don't gloss over vague answers. As Mitch alluded to, people are coming to us late in the game and saying "this is where I am at, but this is where I need to be, can you help?" Perhaps its relating to licensing of a particular antibody clone, for example. They might have been better off getting the advice that this is a no-go for what they want to do earlier on. By having that transparency on the supplier side, we can then give that advice and say actually, maybe you would be better off generating an entirely new product to circumvent that.

We cannot emphasize enough that if you are asking those questions early on, and giving us some insight into where you are taking these reagents, we can advise you on what is going to be most appropriate. It might not be what is sitting off-the-shelf, as an RUO raw material – it might be something that you don't even know we have.

Q It would be great to hear some specifics regarding the issue of scale up. What are the needs of both supplier and end user that need to be met to ensure you can scale up efficiently?

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RL: As the industry matures this is going to happen more often than not, but again, it goes back to the ideas of transparency, understanding, and an open dialogue between both the supplier and the company. Saying to the supplier we are looking to approach commercialization, this is the quantity we expect to consume, and making sure they are able to supply that. Working that into your supplier agreement is important as well. Making sure there are contingencies upon that if they have a catastrophic event will potentially allow you to take that to a CMO to get it manufactured.

Open dialogue is probably the most important thing you can have, as well as letting them know you are approaching commercialization and there is going to be additional requirements on that, and potentially information beyond the drug master file that you will need to submit. It is about making sure you have constant communication, especially with your critical supplier, and letting them know what stage you are at and where you plan on taking things.

LB: I agree totally that being very transparent, and providing your forecast as much as you possibly can, will help make sure your supplier won't overcommit and leave you dangling there. Another thing to consider is ensuring they would not grant exclusivity of a certain raw material or certain target to one supplier over another, leaving you by the wayside.

JT: What it boils down to is having that open and honest transparent communication, and that applies to both parties. From the end user perspective, make sure that what you need is truly communicated to the supplier. From the supplier perspective, make sure you understand those requests or needs from the end user, and be open and honest on whether you think you can meet their expectations.

The other key component to this is as companies engage suppliers or vendors, we are not looking at a one-off solution, we are looking at building a relationship. The crux of that is whether you have that open and honest relationship on both sides.

MB: One of the factors from the supplier side is being able to plan for future need and get those clear forecasts. When you are working with dedicated equipment, and scaling up to potentially multi-gram levels, equipment ordering and procurement can create quite a lead time.

Just as we wouldn't want to oversell our abilities, we need to make sure we are appropriately managing the project, and have everything in place to pull the trigger when we move past those milestones. It all comes down to communication, along with proper forecasting from both sides.

LC: As a supplier it is ultimately our responsibility to get you what you need, when you need it and where you need it. For that to happen, we need to have transparency on your changing needs and a good forecast is critical to that. Open, clear communication and transparency sums it up.

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BIOGRAPHIES

Lili Belcastro

Scientist, Biotherapeutics Material Sciences, Janssen

Lili Belcastro is a Scientist at Janssen and is responsible for the qualification of raw materials used in Janssen's cell and gene therapy products. Dr Belcastro has over 10 years of experience in preclinical and clinical cancer biology, cell and gene therapy product development, and analytical method development. Prior to joining Janssen in 2017, she was finishing her PhD in cancer biology in a joint program with the University of the Sciences and The Wistar Institute in Philadelphia. Prior to joining industry, Dr. Belcastro led pediatric, preclinical, *in vivo* testing programs at the Children's Hospital of Philadelphia for small molecule inhibitors, oncolytic viruses, antibody-drug conjugates, and radiopharmaceuticals. Dr. Belcastro holds two bachelor's degrees from Temple University and the University of the Sciences.

Mitchell Brabec

Commercial Development Specialist, Bio-Techne

Since 2015, Mitch has filled multiple roles within Bio-Techne prior to joining the Commercial Development group. He began in Technical Support to support Research Use Only (RUO) and Good Manufacturing Practices (GMP) products for academic and industrial clients, followed by Product Marketing in the Protein Business Unit for RUO and GMP proteins. As a Commercial Development Specialist with the Custom Projects team, he helps clients find solutions for their workflows by managing GMP conversion, custom protein modifications and GMP fill-finish projects. Mitch works closely with Bio-Techne's development scientists and expert quality and regulatory groups.

Lindsey Clarke

Global Product Marketing & Commercial Strategy, Cell & Gene Therapy, Bio-Techne

Lindsey has spent the last decade working in Cell and Gene Therapy on the raw materials supplier side prior to running Bio-Techne's Global Cell and Gene Therapy Product Marketing team. She joined Bio-Techne in 2018 to establish their European Cell and Gene Therapy specialist team and has recently transitioned into a global role. She is focused on building the team to support customers applications, planning the roll out of new innovations, and developing strategic partnerships within the industry. Prior to this she was in Miltenyi Biotec's UK field team, working closely with numerous process development and manufacturing teams to assist in translating their varied cell therapies to the clinic. Lindsey holds a first-class degree in Pharmacology from the University of Bath, a PhD in Immunology from UCL and worked as a postdoctoral scientist at Imperial College before moving into more commercial roles.

Raymond Luke

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Associate Director of Process Sciences, Adaptimmune Raymond Luke has over 10 years of industry experience in life sciences. He leads a team that is responsible for process characterization, product characterization and process engineering. He plays a key role in selecting material for both development and cGMP uses.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

REGULATORY PERSPECTIVE

The importance of starting materials: quality and regulatory considerations for cell-based therapies

Barbara Bonamassa, Paolo Gasparini, Giulio Pompilio, Alessandro Aiuti & Paolo Foggi

With an increasing number of cell-based therapies obtaining marketing authorization in the EU, the availability of an adequate quantity and quality of target cells for use as starting material has emerged as an important issue. Cellular starting materials exhibit variability, which has an impact on downstream processing and final product quality. On the one hand, accommodation of the variability of cell starting materials is usually essential to allow widespread patient access to innovative and life-saving therapies. On the other hand, variability in the starting material can result in inconsistent quality of the final product and failure to meet desired specifications. In this commentary, we critically review factors contributing to cell starting material variability and provide a regulatory perspective on its management.

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INTRODUCTION

In recent years, the cell-based therapy field has demonstrated potential to achieve unprecedented success. The unique mechanisms of action of living cells serving as active pharmaceutical ingredients allow cell-based therapies to address important unmet medical needs, making striking breakthroughs in helping patients and transforming healthcare [1–6]. However, the 'game- changer' potential

of cell-based therapies comes with several regulatory challenges. Among these, consistent manufacture is a critical issue from the standpoint of quality. Manufacturing processes are often complex and may be challenging to standardize, especially when transitioning to the robust industrial processes required is in the commercial setting. The initial collection of an adequate quantity and quality of target cells as starting material is often overlooked, but is one of the most fundamental issues in the manufacture of cellular therapies. The cellular starting material has the potential to affect every subsequent step downstream, including the entire manufacturing process, and ultimately can impact the final goal of consistently delivering maximum therapeutic efficacy and safety to the patient. This holds true regardless the incoming cells' donor source (autologous or allogeneic), patient disease state [7], source material (bone marrow, umbilical cord blood or peripheral blood, adipose or cardiac tissue, etc.), differentiation stage (mature or stem/progenitor cells) and manipulation (genetically modified or not).

CONTRIBUTING FACTORS TO THE CELL STARTING MATERIAL VARIABILITY

Common to all cell-based therapies is the inherent variability exhibited by the cells from the outset, due to their biological and living nature. As the field of cell-based therapy has matured, it has become evident that additional donor-related factors may contribute to the overall biological variability. These factors may include the donor's medical history and disease, as well as current or previous medications [8–10] that are known to further influence the cell subset composition, phenotype, functional capabilities, and downstream biological activity. Additional factors include gender, ethnicity, age, and body mass index (BMI) [11,12].

In recent years, an increasing number of Advanced Therapy Medicinal Products (ATMPs) based on autologous or allogeneic cells have received EU approval. These include genetically modified cells such as CAR-T cell immunotherapies for treatment of cancer, as well as hematopoietic stem/progenitor cells for monogenic disorders or ex vivo cultured cells indicated for acquired disorders. Some therapies, especially CAR-T cell immunotherapies, have paved the way for use of cell-based therapies for treatment of diseases with a higher prevalence compared to ATMPs developed and commercialized previously.

To ensure a steady clinical supply, relevant starting material procurement capabilities must be addressed, and usually require expansion to meet the manufacturing demands of the commercial setting. As multiple collection centres are typically required, possible differences in donor management, staff training or collection methods must be considered. Pharmacologic-, patient- and technological-related variables include target yields for collections [13], mobilization regimens and the individual response to mobilization, apheresis equipment employed (e.g., COBE Spectra, Spectra Optia or Amicus cell separators), volumes of blood processed, and duration of the apheresis procedure, as well as post-collection handling, preservation, storage and transport [15–16]. These differences may affect the uniformity of cell collection efficiency as reflected by variations in the target and contaminating cell number, cell viability and recovery, and collection yield and purity, further contributing to the starting material variability and potentially impacting the overall downstream processing and final product quality [11,12,17,18].

REGULATORY PERSPECTIVES ON THE CELL STARTING MATERIAL VARIABILITY

It is acknowledged that several unavoidable factors may contribute to the qualitative and quantitative variability of cell starting materials and therefore of the final products. It is also recognized that additional factors may be identified as the knowledge in the field

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increases and more starting materials are scrutinized and/or used for manufacture of new therapies, e.g., medicinal products based on induced pluripotent stem cells (iPSCs). Consequently, the regulatory approach to address starting material variability is also evolving in response to this fast-moving field, with recent product approvals and a rich clinical pipeline of promising ATMPs. It is expected that the level of flexibility employed will evolve as more evaluation experience is achieved with cell-based medicinal products, and greater awareness is gained on the level of acceptable material variability for successful product manufacture and clinical outcomes. Overall, exploring and embracing the variability of starting materials will enhance the ultimate ethical goal of providing patients with wider access to life-saving treatments.

Nevertheless, identification of variability-linked factors, the understanding of the variability range and of its design space, and the implementation of measures to minimize such variability are of outmost importance from the regulatory perspective. In all cases, the primary goal of a high-quality starting material and consistent product manufacture to limit negative consequences downstream remains to be accomplished. Examples of such consequences may include failure to achieve the target therapeutic dose [19–21] and to comply with product specifications, for which regulatory tolerance might occur only in exceptional circumstances [22].

To ensure cellular starting material quality and consistency, it is critical to establish a robust quality management system and quality oversight, integrating a number of different strategies and taking into account several aspects to help product developers better account for variability. The degree of flexibility allowed is typically regarded as progressively reduced from the early development stages to the pivotal clinical use/commercial setting. Alleviating the starting material variability with an accurate center qualification strategy is often a first and essential step to limit downstream processing risks [23]. The collection sites' variability cannot realistically be avoided. However, it can be managed through qualification of collection sites, verifying their compliance with the ATMP manufacturer's requirements (or, as appropriate, the sponsor's or marketing authorization holder's requirements). In addition, compliance with regulatory requirements (as set out in directive 2004/23/EC or 2002/98/EC and the respective daughter directives, as appropriate, as implemented nationally by the National Competent Authorities) must be verified. To ensure continuous monitoring of the donation, procurement and testing, additional reviews may need to be performed on a regular basis to confirm that the sites maintain a state of compliance.

The qualification process should be based on criteria for center selection and evaluation, as well as readiness activities including establishment of procedures to meet the ATMP manufacturer's/Sponsor's/Applicant's requirements, and training on specific procedures. Experience and capabilities in donor management and apheresis collection are areas that often impact cell quality and lead to variability. Therefore, the procedures and training activities should address donor management, collection methods, instrumentation, data collection and donation handling, preservation, storage, and transport. The availability of clear standard operating procedures for cell collection are ultimately essential for success of the complex manufacturing process. However, it is acknowledged that there is an increasing burden for the clinical centers and the national health system due to the need for performing new qualification procedures and complying with specific collection requirements for each new product, as imposed by an increasingly large number of manufacturers.

A flexible yet standardized starting material collection is the foundation of any cell-based therapy to ensure that the established quality requirements (specifications) for the cell starting materials are met, and that cells of sufficient quality and quantity are available for manufacturing. For $CD3^+$ or $CD34^+$ cell-based therapies, pre-apheresis or mid-procedure cell counts are often used to assist in

tailoring the apheresis procedure to the patient and to predict the collection of a starting material with the intended cellular composition, as well as yield and purity of target cells [18,24].

Adequate systems should be implemented to keep bidirectional tracking of cells/tissues collected for ATMP manufacture, from the point of donation up to the delivery of the finished product to the recipient. For all cell-based products, these systems are designed with chain of identity and chain of custody. For autologous and other patient-specific products, these procedures must also ensure administration of the specifically intended product to the intended patient. Traceability information should also cover raw materials and all substances coming into contact with the cells or tissues. Importantly, no flexibility is foreseen on this aspect, as traceability requirements should be implemented in full, regardless the stage of development.

Once the apheresis material is collected, additional factors related to the complex supply chain may increase its variability and therefore require careful evaluation. Several ATMPs have made their way to the European clinical and commercial setting using manufacturing plants based overseas. As a result of global manufacturing, long transport distances between the clinical sites collecting the starting material and the product manufacturing sites, and from the manufacturing sites back to clinical sites for product administration, must be considered. Potential delays along the length of these supply chains should not be excluded a priori and must be considered to preserve the quality of the starting material and final product, to ensure that patients may obtain the intended therapy.

Product manufacture from fresh material may appear to be optimal. However, cell viability and function are known to decline soon after collection, and should be accounted for in case of lengthy transports. Measures to achieve longer stability and shelf-life extension of the starting material and final product should thus be explored. Optimization of the formulation and cryopreservation medium composition, and implementation of cryopreservation at the cells' peak quality (i.e., soon after collection) may be pursued. These measures may improve starting material stability, limiting inconsistencies with downstream manufacturing, and also allowing more flexibility in manufacture planning and scheduling, ultimately maximizing the manufacturing capacity.

A comprehensive set of data is expected in regulatory submissions addressing the quality of cells to be used as starting material for the manufacture of cell-based medicinal products [25–27]. To minimize cell starting material and product variability, deep understanding of the properties of the incoming material should be demonstrated. Therefore, a thorough characterization of the cell starting material collected throughout product development should be undertaken. Analytical evaluation of the cell starting material characteristics should include cell number, composition (e.g., presence and percentage of different cell types and subsets), and functionality to detect impaired, immunosenescent, or exhausted cells. Procedures that minimize microbiological and other contaminations of procured tissues and cells should be in place. Directive 2006/17/EC also requires donor testing for certain infectious agents (i.e., HIV-1 and -2, HCV, HBV and syphilis, as a minimum) by properly certified testing laboratories using EC-marked testing kits. The use of cell starting material in case of positive test results can be foreseen for autologous donors. Following a proper risk assessment, their use may be allowed if appropriately isolated starting material storage facilities and measures to prevent cross-contamination during ATMP manufacture are in place.

Investing relevant resources early on in an extensive starting material characterization pays off in many ways. It can drive the fine-tuning of the collection procedure for the procurement of an increasingly consistent staring material during product development and up to commercialization. Incoming material characterization assays represent a critical foundation for the establishment

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of a minimum set of relevant release specifications, which in turn determine the cell starting material acceptability for the product manufacture, therefore predicting manufacturing success. Lastly, depending on the cellular composition of the incoming material, optimized processing pathways may need to be designed to sequentially reduce the variability throughout the manufacturing process, i.e., reduction of non-target cells and enrichment of the intended cells using steps involving their specific selection, activation, and expansion. Such processing steps have been well documented for CAR T cell-based products, which typically have starting materials that are highly variable. For example, PBMCs from heavily pre-treated patients have been reported to contain abnormal levels of inhibitory factors, inhibitory cells or populations of T cells that respond poorly to stimulation [28–30]. Separating T cells intended for genetic modification from these inhibitory elements has been reported to improve the outcome of the T cell culture [31–33], and aid tailoring robust and reproducible manufacturing processes. Indeed, isolation, and simultaneous stimulation, of T cells from PBMCs using anti-CD3 and anti-CD28-coated magnetic beads in combination with large magnets has been successfully implemented in clinical manufacturing [34]. Furthermore, failure to expand in cell culture has been associated with PBMC concentrates containing greater quantities of myeloid cells [35]. As a result, initial manufacturing protocols including T cell selection with anti-CD3/CD28 beads, have been further modified to include a step depleting myeloid cells by plastic adherence [18]. Importantly, post-collection T cell enrichment has made its way into the commercial setting [5–7], thus stressing the importance of starting material characterization and understanding of input cell key properties for manufacturing process adaptation and commercial success.

The availability of analytical assays shown to be suitable for their intended use and able to discern appropriate properties of the cells is critical for standardization of input cellular material for cell-based therapy manufacture. The development and availability of analytical methods is particularly challenging for ATMPs. Analytical hurdles include lengthy development and execution timelines for tests, sample handling challenges, limited availability of sufficient sample material, logistical challenges with test timing, variability inherent to biological assays, difficulty obtaining appropriate assay controls and reference materials, and validation protocols for complex methods that are typically non-compendial. Additionally, while a few tests may suffice for chemical medicinal products and more standard biologicals, cell-based therapies may require many tests to address their characterization, release, and stability, to control the process and input materials, as well as assure comparability and consistency, further complicating the task. Ultimately, the availability of suitable methods is crucial to clinical and commercial success because material, as well as product and process, variability can be truly understood and managed only when these assays are properly established. In this perspective, the EMA guidance as reported in Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**) can be taken into account. Additionally, Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products can be consulted as well.

Finally, improvement in product consistency from variable starting materials may benefit from the development of automation, which may alleviate the variability associated with subjective, manual and operator-specific operations, as well as acquisition and analysis of data. Current processes for manufacturing cell-based therapies often involve several devices, requiring significant operator interaction and support. As the cell therapy field matures, automated technologies and processes may further evolve to further aid in the management of variability and achieve better consistency by limiting manual manipulations occurring at the clinical and manufacturing sites.

CONCLUSIONS

The ATMP field has experienced an unprecedented number of regulatory submissions, many of which cover cell-based therapies, and many have reached the commercial/post-approval setting. This represents a significant change from past years, when investigational products were predominant in the regulatory landscape.

Necessary for the granting of a marketing authorization is the submission of manufacturing process validation data, demonstrating that the process can effectively and reproducibly output products meeting their predetermined specifications and quality attributes. Manufacturing consistency is thus a regulatory requirement for commercial products, which is particularly challenging for cell-based therapies due to the biological nature of input materials. In particular, the living nature of cells used as starting material is a significant and unavoidable contributor to variability, and the starting material quality is a major determinant of final product attributes. Therefore, knowledge and management of the biological variability of the cell starting materials used for an ATMP manufacture is critical to ensure that the product can be manufactured successfully and ultimately result in improved patient care. Management of starting material variability should be pursued via rigorous and integrated quality oversight and quality management systems.

Attention and resource allocation to several areas, including collection site qualification, supply chain logistics, and analytical method development, are critical to suitably address the starting material, product, and process variability. In the near future, limitation of manual operations in favour of automation may further aid in control of the materials, product, and process.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

EXPERT INSIGHT

Upholding raw material suppliers to higher quality standards to better support cell and gene therapy manufacturers

Monica C Nelson, PhD

Health authority guidance recommends that all raw materials intended for use in the production and distribution of a cell and gene therapy (CGT) product be carefully selected and appropriately qualified. As part of this process, CGT manufacturers thoroughly evaluate the quality of materials to identify any potential risks to patient safety, process execution, product quality, and material management. This evaluation relies heavily on the review of supplier documentation, such as product descriptions, certificates of analyses (COA), certificates of origin (COO), and quality questionnaires. Currently, there is no standardization for the manufacture and characterization of raw materials, and the availability and details on each of these documents are at the discretion of the supplier. When limited information is available, it becomes the responsibility of the CGT manufacturer to work in cooperation with each supplier to seek, review, and audit their processing procedures and documentation to appropriately qualify the suitability of a material. Raw material suppliers can better support the CGT industry by committing to the highest possible quality standards in the manufacture, testing, and associated documentation for materials intended for CGT manufacture. This would provide a great service to the industry as a whole, because as more suppliers offer raw materials aligned with CMC guidelines and quality standards, the more CGT manufacturers can develop and deliver compliant products quickly, reliably, and safely to more patients.

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Health authorities expect cell and gene therapy (CGT) manufacturers to carefully scrutinize raw materials to ensure patients receive the highest quality and safest product [1,2]. For the purposes of this article, raw materials are defined as all materials intended for use in the production and distribution of a pharmaceutical product. Of most concern are materials that are directly incorporated or in direct contact with the therapeutic substance and can exert an effect in the final product, such as starting materials, excipients and ancillary materials. Starting materials are substances that are a precursor to the final therapeutic product or are a significant structural component that will be integrated into the final therapeutic product. Examples of starting materials for CGT include donor cells, bacterial cell or viral banks, plasmids, and viral vectors. Excipients are defined as substances that are intentionally contained in the final product dosage form along with the cell or gene therapy to aid in patient administration, long term stability and/or cryopreservation. Ancillary materials are components that are used during the manufacture of cell therapy products and are not intended to be part of the final dosage form, but may be present as residuals in the drug product. This category of materials includes media, media supplements, growth factors, cytokines, and other components used during cell culture, harvest, and other manufacturing steps. Ancillary materials will be the focus of this article; the term "ancillary materials" will be used interchangeably with "raw materials" throughout the discussion.

There are a growing number of guidance documents and concept papers that provide regulatory views on the classification of ancillary materials and the minimal expectations for appropriateness in the manufacture and formulation of clinical materials [3–11]. Focus is placed on the importance of using materials of the highest standards for safety, potency and purity, whenever possible. Ideally, these raw materials should be FDA-approved/cleared, pharmacopeial (USP, EP, JP), or clinical grade. However, when those options are not available, health authorities advise the selection of materials that are manufactured under Good Manufacturing Practice (GMP) or equivalent International Organization for Standardization (ISO) quality conditions. With the appropriate level of qualification, the use of in vitro diagnostic use only and research use only materials can be considered. Preferably, all raw materials should be available from the vendor with significant characterization and be free from adventitious agents (human, animal, microbial) and any other undesired impurities. In addition, guidance advises on sourcing materials from reputable vendors to limit process and assay variability, ensure a sufficient supply chain, and meet regulatory compliance.

Although guidance sets clear recommendations, it should be noted that raw material suppliers are not certified by regulatory agencies or bound by legal requirements to manufacture or characterize raw materials under any specific quality standards. It is at the supplier's own discretion to define their practices and principles for the production and documentation of raw materials. Instead, health authorities place the ultimate responsibility on the CGT manufacturer to evaluate and qualify raw materials as suitable for product manufacture. Consequently, the selection of raw materials can be complex and highly burdensome for CGT manufacturers.

To aid CGT manufacturers in material selection, USP <1043> [2] offers an excellent resource that provides a tiered risk category approach to help define appropriate qualification activities for the various types of available ancillary materials (AMs). A similar risk-based approach is provided in European Pharmacopeia General Chapter 5.2.12 [6]. Table 1 summarizes the typical vendor-supplied documentation that is available per material quality grade, and the qualification activities that are required to meet USP <1043> expectations. CGT manufacturers work in cooperation with each individual supplier to seek out this documentation and must establish confidentiality agreements to obtain and review their processing and testing

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procedures and supporting documentation to verify compliance with CMC guidance. For materials with incomplete supplier controls or documentation (Tiers 3 and 4 in Table 1), CGT manufacturer themselves will then have to dedicate additional resources to perform characterization/safety testing and vendor quality audits to further mitigate risks, all prior to qualifying the suitability of the material.

The entire process of sourcing and selecting raw materials for CGT manufacture can be streamlined and more efficient if raw material suppliers implement the highest possible quality standards in the manufacture, testing, and associated documentation for materials intended for CGT manufacture. The following paragraphs provide a proposal to raw material vendors on the level of detail and alignment with CMC guidelines and quality standards that would significantly benefit end users in the selection and qualification of raw materials.

MATERIAL QUALITY GRADE & INTENDED USE

Selection of raw materials begins with a review of the vendor label claim and/or product description sheet for a material to determine its potential for use in the manufacture of cell and gene therapeutics. Ideal, low risk choices for raw materials are licensed biologics and approved drugs for human use (Tier 1 materials in Table 1). These materials are manufactured with controlled, documented processes, are well characterized, and have a known safety profile that is regulated by health authorities. The next best option is to use Tier 2 materials, which are well-characterized materials

TABLE 1

Tiered, risk-based approach to raw material quality requirements.

	Material risk category			
Vendor-supplied documentation vs. USP <1043> expectations	**Tier 1: Materials intended for use as approved biologics, drugs, or medical devices**	**Tier 2: Well-characterized materials with intended use as AMs**	**Tier 3: Moderate-risk materials not intended for use as AMs**	**Tier 4: High-risk materials**
Material Quality Grade and Intended Use	Licensed biologic, drug or medical device (FDA-approved or cleared)	Pharmacopeial (USP, EP, JP), clinical grade, intended for CGT use, or manufactured under GMP or equivalent ISO quality conditions	Intended for *in vitro* diagnostic or research use, or locally produced under laboratory conditions	Intended for industrial or research use, may contain harmful impurities and/or animal- or human-derived components
Vendor Quality documentation (quality statement or questionnaire) review, if available	Available, information meets expectations	Available, but may need to be supplemented by vendor audit	May not be available, requires vendor audit	Not available, requires vendor audit
Certificate of Analysis review	Available, information meets expectations	Available, typically meets expectations	Available, but requires additional testing by CGT manufacturer to meet suitability	Available, but requires additional testing by CGT manufacturer to meet suitability
Certificate of Origin review	Available, information meets expectations	Available, typically meets expectations	Available, but may require additional safety testing by CGT manufacturer to meet suitability	May not be available, requires safety testing by CGT manufacturer to meet suitability
Regulatory Support/ Drug Master File cross reference (when possible or practical)	Available	Available	May not be available	Not available

that are intended for use as ancillary materials, are manufactured and tested under GMP and equivalent ISO quality standards, and if available, meet pharmacopeia and compendial requirements. However, very few suppliers offer materials used in the manufacture of CGT products, such as culture media, recombinant cytokines/growth factors, and serum, that fall under these two categories. Typically, these ancillary materials are labelled by vendors for the intended use in in-vitro diagnostics or research applications (Tier 3 and 4 categories in Table 1). A welcomed shift in the ancillary material industry would be the availability of more materials for use in CGT manufacture that fall in the first two tiers.

VENDOR QUALITY DOCUMENTATION

Many suppliers have recently committed to providing higher quality materials ("clinical grade" or "GMP") that are labelled as suitable for use in cell or gene therapy manufacturing. However, there remains a lack of harmonization on the definition of these terms across the raw material industry, and the manufacturing and testing approaches may vary significantly among the various suppliers. It would be beneficial to end users if raw material vendors provide an easily-accessible Quality Statement for each material, highlighting what aspects of GMP compliance is followed in the production of the material. For example, this statement can be certified by the supplier's quality department and offer a high-level summary of the supplier's manufacturing process controls and documentation, facilities and equipment, materials management program, product test methods and specifications, and other key areas of quality control (Box 1). The CGT manufacturer can then use this source document as part of their initial raw material selection process to screen and identify suitable materials for process development efforts. Only when the material is deemed suitable for its intended GMP use would CGT manufacturers proceed with a vendor quality questionnaire or audit the vendor's quality management system.

CERTIFICATE OF ANALYSIS

CMC guidance also places great emphasis on the importance of raw material characterization. It is expected that process raw materials be assessed by CGT manufacturers for several quality attributes, which include identity, purity, functionality, and freedom from microbial and adventitious agent contamination. Therefore, it would be beneficial if suppliers include lot release testing and specifications on raw material Certificate of Analyses (COA) that meet these CMC expectations. At a minimum, suppliers should have measurements of identity, quantity, homogeneity/purity, and biological activity specific to the raw material. It is understood that the intended use of the material may vary across CGT manufacturers, which makes it difficult to standardize the necessary specifications. However, having supplier-defined assays and acceptance criteria for critical raw material attributes would at a minimum ensure batch-to-batch consistency. Having this level of assurance from the supplier will then alleviate some risk of variability in raw material quality that may directly impact a CGT process

BOX 1

Quality Statements should offer high-level summaries in the following areas of raw material quality control.

- Personnel Training
- Materials Management
- Qualification and Validation Policies
- Manufacturing Process Controls and Documentation
- End-to-end Traceability, Chain of Custody
- Cross-Contamination Prevention
- Laboratory Controls and Documentation
- Product Testing and Release Documentation
- Risk Management Procedures
- Deviation/Change Management
- Facility and Equipment

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parameter or final product critical quality attribute.

In addition to material attributes, suppliers should include microbiological and process-related impurity assays on the COAs. Due to the aseptic processing of cell and gene therapy products, the risk of a raw material being a source of microbial contamination in the manufacturing process or final cell product is of great concern. Therefore, it is essential that any material labelled for use in CGT manufacture include the appropriate microbial assessments to ensure they are free from bioburden, endotoxin, and if applicable, mycoplasma. Other impurities of concern are recombinant host cell DNA/protein, metals, or toxins, which can pose direct toxicity or elicit an undesired immunogenic response in humans. For materials that are produced recombinantly, COAs should include assays and specifications for host cell substrates (e.g., host cell proteins and DNA) and culture components (e.g., media components and antibiotics) that may be residuals present with the material. Synthetic molecules should contain residual metal testing on the COA, if a possible contaminant. In cases where this information is not specified on the COA, a Certificate of Conformance from the supplier that states the material is safe for its intended use should be made available to the CGT end user.

CERTIFICATE OF ORIGIN

When sourcing raw materials, CGT manufacturers are obligated to identify and understand the origin and manufacturing process of raw materials to prevent the introduction of human and animal adventitious agents, which can transfer communicable disease to patients. FDA guidance recommends the use of non-animal- or human-derived reagents for the manufacturing of cell and gene therapies [2,3,6]. However, this might not be possible since there may be certain, necessary materials for critical process steps that either contain components directly derived from human/animal origin or utilize human/animal-derived components in their manufacturing process. To aid CGT manufacturers with this requirement, suppliers of these biologically-sourced materials should provide Certificates of Origin (COO) that meet CMC requirements to confirm the absence of adventitious agents. In particular, COOs should document the screening and sourcing (i.e. from countries of negligible BSE/TSE risk), processing (including viral inactivation or removal steps), and testing of human- or animal-derived components used in the manufacture or included in the final raw material. Having this level of appropriate controls and supporting COO documentation helps CGT manufacturers qualify the human- or animal-origin raw material for use in their product manufacture and reduces the burden on them to include additional mitigation measures as part of their own material qualification program (i.e. testing and treatment of raw materials prior to use).

REGULATORY SUPPORT FILE

Finally, it would be most beneficial for both CGT manufacturers and suppliers if raw materials labelled as suitable for use in biologic, cell, or gene therapy manufacturing have regulatory support files to aid in the regulatory approval process for a CGT, where applicable. The US FDA has a well-defined system for these regulatory support files through the use of Drug Master Files. Here, a supplier can submit a confidential regulatory document that contains complete, detailed information on the quality and safety aspects of the manufacturing and testing of a specific raw material. The CGT manufacturer could then cross-reference this document in their own filing to support the use of this ancillary material in their process. It would be at the raw material supplier's discretion on what non-proprietary information from this Master File document would be disclosed to the CGT manufacturer under a confidentiality agreement. Having this type of document in

hand, and committing to support the regulatory filing of an end user, would provide significant assurance to CGT manufacturers that the supplier is dedicated to providing high quality raw materials that meet CMC Regulatory expectations, while allowing the supplier to maintain confidentiality of proprietary information.

TRANSLATION INSIGHT

As noted earlier, health authorities place the responsibility primarily on CGT manufacturers to evaluate and qualify raw materials as suitable for product manufacture. Consequently, cell and gene therapy manufacturers devote significant effort on internal, quality-driven risk assessments in the selection of appropriate raw materials. In this process, a phased- and risk-based approach is used to identify and mitigate materials of highest potential risks to patient safety, process execution, product quality, and material management. Vendor product descriptions, certificates of analyses (COA), certificates of origin (COO), and quality questionnaires are key reference documents for the risk assessment. However, when limited information is provided in these documents, it is essential that CGT manufacturers establish supplier relationships early on in development with open, honest, and transparent communication to fully understand the quality of each raw material.

The number of materials intended for use in the production and distribution of a CGT product can be considerable, which, in turn, requires a substantial number of resources dedicated to material qualifications and supplier oversight. Therefore, it would be a great service to the CGT industry as a whole if raw material suppliers commit to upholding the highest possible quality standards in the supply of materials intended for CGT manufacture. Specifically, suppliers should focus on (1) manufacturing raw materials with appropriate quality controls, (2) testing these materials with characterization and safety measures aligned with CMC guidelines, and (3) providing easily-accessible and comprehensive documents summarizing these details. If suppliers took on this responsibility, more CGT manufacturers would choose their high-quality products to avoid using materials that require risk mitigation efforts associated with incomplete supplier controls or documentation. As a result, CGT manufacturers would be able to expedite raw material risk assessments, establish comprehensive material qualifications, and reprioritize focus on understanding a raw material's impact on CGT performance and residual risk. Most importantly, if suppliers strive to supply raw materials with highest possible quality standards, CGT manufacturers can continuously drive higher manufacturing performance for their products and deliver products quickly, reliably, and safely to more patients.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INTERVIEW

Emerging trends in control and regulation of immunotherapy cellular starting materials

NATHAN MANLEY, PHD is a principal consultant at Dark Horse Consulting with over 17 years of experience in stem cell biology, neuroscience, and preclinical modeling of human disease. Prior to joining Dark Horse, he led research and preclinical development efforts at Asterias Biotherapeutics, a pluripotent stem cell-based biotech company, and trained as an academic researcher in the departments of Biology and Neurosurgery at Stanford University. Over the course of his career, Manley has contributed to the advancement of a wide range of experimental and clinical-stage cell therapy products, authored scientific articles, and co-authored cell therapy patent applications. He also is a part-time instructor in the Biology Department at San Jose State University.

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Q What are you working on right now?

NM: As a member of the Dark Horse Consulting team, I have the opportunity to support a wide range of projects in the cell and gene therapy (C>) space. This ranges from the earliest of technologies emerging from academia and seeking a path to first-in-human clinical testing, all the way to commercial filings.

Right now, I am focusing on a number of different projects, including pluripotent platform technologies, cell replacement strategies for neurological indications, and a broad spectrum of targeted therapies for cancer. The cancer space is especially exciting because of the growing number of approved C> products and our resulting visibility into the development and regulatory strategies needed to reach approval. We are now seeing so many emerging new approaches, particularly in cellular immunotherapies for cancer and autoimmune disease, which are really hot areas in C> right now.

My core background is in preclinical and analytical development of C> products, which I use to guide programs on their nonclinical development plan and how to stage product development in a manner that is aligned with regulatory strategy. On the analytical side, I help clients build an appropriate set of characterization assays, both in-process and for their final product. This can be challenging for the new wave of C> products that often come with increased complexity. For a cellular immunotherapy, for example, what methods should be used to define the product and show that it can be produced consistently from batch to batch? Analytics plays such a core role in the development of these types of therapies, and it is really fun to work on such a broad range of programs through a common lens.

Q What is your take on the current state of play in terms of assays for cellular starting materials? What do you view as the state of the art, and equally where is further innovation required?

NM: **Across programs and C> product types, there is quite a range with respect to the type and breadth of analytics used to qualify cellular starting material in C> manufacturing.** In the case of cellular immunotherapies, leukapheresis products obtained directly from patients or from healthy donors is one of the most commonly used cellular starting materials and provides a good example of how analytics used to qualify cellular starting material are evolving.

The current state of the art for allogeneic leukapheresis product testing starts with donor eligibility screening and testing, followed by very basic assessments of the collected material performed at the collection facility or by the supplier. In most cases, this initial material testing is limited to total nucleated cell count and viability but may also include a basic assessment of cell types and their relative proportions either by hematology counter or flow cytometry. This is the extent of material testing information that CG&T developers can expect when they receive their leukapheresis starting material.

> "A big question that most developers have with their cellular starting material is how well it will perform in their process with respect to cell yield..."

Once in the hands of the CG&T developer, further leukapheresis product testing is highly process- and product-specific. A big question that most developers have with their

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cellular starting material is how well it will perform in their process with respect to cell yield, and if their process involves genetic engineering, the efficiency of gene modification. This typically is done by performing a small pilot run to evaluate cell proliferation and/or gene modification efficiency within the developer's own process and as a result, is quite difficult to standardize across processes.

Regarding where further innovation is required, testing of cellular starting material needs to become much more comprehensive, more like the level of analysis C> developers are performing on their final drug product to understand cell type composition, phenotype, and function. For example, the T cell field is swiftly moving towards a deeper analysis of phenotypic profiles – naïve, memory, and effector profiles that are present within the CD4+ and CD8+ subtypes of T cells – and realizing that these profiles have differing degrees of success in their manufacturing process, and have the potential to impact efficacy, and even safety, in patients. A number of C> developers are starting to look at these T cell profiles in leukapheresis products, but are not yet at the point of using such criteria to qualify their starting material. Instead, they are tracking these data and trying to understand the potential impact on their manufacturing process, their final drug product, and ultimately, whether there is any relation to patient outcomes.

Another exciting area of innovation is the realm of single cell-based assays. There are some interesting technologies coming out now for single cell omics, including looking at proteomic profiling and gene expression profiling on a single cell level. That kind of technology is really exciting and has a lot of potential to increase our understanding of cellular starting material, manufacturing process intermediates, and final drug product. However, it also comes with challenges, because it produces a lot of data. Understanding what it means, and what is really relevant to your process and therapeutic product is difficult. A lot more effort is needed to standardize how single cell assays are analyzed and interpreted, which will require more groups to generate the associated data and identify potential connections to process and product outcomes.

Q Are there any common pitfalls that you see cell and gene therapy developers and manufacturers falling into in terms of their cellular starting material testing?

NM: While some groups are looking very deeply at what is in their cellular starting material and trying to relate that to their process success and the intended characteristics of their drug product, there are also just as many or more C> developers who just aren't doing enough to assess suitability of their starting material. Some are just doing the basics; making sure they have enough viable cells and that a reasonable proportion of a given target cell type is present. From there, they are going straight into manufacturing and not probing deeper to understand how differences in their cellular starting material may be causing variability in their manufacturing process or the performance of their drug product.

Given the complexity and diversity of C> programs, it is difficult for regulatory authorities to clearly define what extent of characterization is appropriate for cellular starting material beyond known safety concerns (i.e., adventitious agent testing). Instead, the onus is on the

C> developers to decide what aspects of their cellular starting material should be explored, and in how much detail.

Another common pitfall for leukapheresis-based programs is that some C> developers are not engaging enough with the collection facility or supplier that provides their cellular starting material. As a result, the C> developer lacks sufficient understanding of the leukapheresis collection process including the specific apheresis instrument/settings used and subsequent handling procedures. This also can contribute to variability in downstream manufacturing operations, if for example, C> developers are not confirming that their cell starting material is being obtained in a consistent manner.

Ideally, communication between C> developer and starting material supplier should go both ways – the C> developer should provide information to the collection facility or supplier about their starting material needs and then receive information back to confirm that appropriate collection, handling and testing methods were performed. Again, this comes back to the need for increased understanding of what makes cellular starting material fit for use in C> manufacturing. It starts with better and more comprehensive characterization and ultimately, that should lead to much more open communication and an improved partnership between the collection facilities, the suppliers of cellular starting material, and the C> developers.

Q Dark Horse Consulting recently undertook a survey on cellular starting materials – can you give us some background on why you did this, and what you set out to discover?

NM: At Dark Horse, we aim to be aware of trends and upcoming changes that drive the evolution of the C> field. An example of this is the general shift in the C> development pipeline towards more allogeneic products. Relative to autologous products, allogeneic products offer the potential for increased scalability (depending on the type of cellular starting material), off-the-shelf availability to patients, and much more control over the attributes of the cellular starting material (providing you understand which attributes are important, of course).

Looking at this in the context of immunotherapies that utilize leukapheresis products as their starting material, we believe that the current number of suppliers of healthy donor leukapheresis material is unlikely to support the increasing number of allogeneic immunotherapy programs. We are not there yet, but in the next 5 –10 years we expect a major uptick in the number allogeneic immunotherapy products reaching late-stage clinical development and approval. In that eventuality, the availability of healthy donor leukapheresis products will need to grow to support the field.

To understand this better, we conducted a research exercise in collaboration with our strategic partner Arcline Investment Management to gain perspective from two different stakeholders. First, we interviewed CG&T developers to gain insight into their perspectives on starting material critical quality attributes and anticipated future demand. Next, we engaged former members of the US FDA and asked them about how the regulatory landscape is evolving to address the increased number of emerging and advanced allogeneic C> programs.

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> "Eventually, allogeneic products that are derived from a single donor source may be able to supply enough drug product to treat hundreds or even thousands of patients. In this scenario, the need to confirm that the cellular starting material is safe and fit for use in allogeneic C> manufacturing is a much higher bar than for autologous products."

Q What are the differences in testing requirements between allogeneic and autologous cellular starting material, and can you discuss the regulatory rationale for those differences?

NM: This is partly what got us interested in conducting this research and learning about what is needed from a regulatory perspective for allogeneic material. Through our work with a wide range of allogeneic C> developers, we have become aware of regulatory requests to perform additional testing on their cellular starting material beyond what is typically required for autologous cellular starting material.

Speaking with many regulators on this topic, what we learned – and this certainly makes sense in our minds – is that this is because allogeneic C> products have the potential to treat a very large number of patients. Eventually, allogeneic products that are derived from a single donor source may be able to supply enough drug product to treat hundreds or even thousands of patients. In this scenario, the need to confirm that the cellular starting material is safe and fit for use in allogeneic C> manufacturing is a much higher bar than for autologous products.

The other answer we received in speaking with regulators was about the 'zero tolerance' approach that the FDA and other agencies take when it comes to disease agent transmission. The zero tolerance approach means that even a single observed incident of disease agent transmission by a C> product will require all related products to undergo that relevant disease agent testing. While the observed incident may not be publicly disclosed, emergence of a new testing requirement may mean that the regulatory agency is reacting to something that happened in the clinic.

Another thing we can point to are the additional documents that the FDA is using to guide their recommendations to allogeneic C> developers. The FDA currently classifies gene-modified cell therapy products as gene therapies, and as such, donor and material testing requirements described in the FDA guidance document, *Guidance for Industry - Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy*

Investigational New Drug Applications (January 2020 release), are applicable. The FDA also draws from a second guidance document, *Guidance for Industry - Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications (February 2010 release)*. This second guidance document describes testing that should be performed on cell banks that are used to generate vaccines. The relevance of this to allogeneic cellular starting material is clear given the potential for allogenic C> products to treat patients at a similar scale to what we are currently doing with vaccines.

It is likely that in the near future we will see an updated guidance for industry that will harmonize the principles and testing recommendations currently found in these two guidance documents along with what is described in 21 CFR Part 1271, so that we can refer to a single document to understand the regulatory expectations around allogeneic C> products and their cellular starting materials.

Q Do you expect that additional donor or material testing requirements will come up in the near future? For example, will donors need to be screened for SARS CoV-2?

NM: We asked about this, and it is a near certainty that additional testing requirements will come up in the future as the list of known communicable disease agents continues to grow. Agencies such as the FDA are going to continue to operate with a zero tolerance policy and base their testing recommendations on all available data.

By that same token, we don't expect SARS CoV-2 to become a donor or material testing requirement for leukapheresis or related starting materials in the near future given that there currently is no evidence of blood transmission for SARS CoV-2. If this changes, i.e., evidence of blood transmission emerges, the likelihood of that becoming a near-future testing requirement will dramatically increase.

Another example is Zika virus, which in the not-too-distant past became a very relevant communicable disease. However, again, for Zika virus there is no current evidence for blood transmission, and we are now seeing a general decline in the number of cases. Given this, Zika virus is unlikely to become a donor or material testing requirement, and instead is likely to remain as part of the medical history questionnaire that is used to provide additional confirmation of donor eligibility.

What we really need to keep an eye on in order to be aware of upcoming or emerging new testing requirements, either for donors or the material itself, is the landscape of relevant communicable diseases, how they are transmitted, and whether or not we can generate reliable tests to detect the presence of that communicable disease agent in donors and/or cellular products.

> "Another area of genetic testing that is likely to increase in importance is human leukocyte antigen testing."

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One real challenge in this arena is our lack of effective test methods for the transmissible spongiform encephalopathies (TSEs and BSEs). They don't come into play as much with starting material, more in manufacturing processes. Currently for starting material, this is mainly handled through screening – looking at where the donor or cellular material came from geographically and the associated risk of TSE/BSE exposure. However, some innovative tests are continuing to be evaluated, such as the real-time quaking-induced test, and may one day provide a reliable means to confirm absence of TSE/BSE in cellular starting materials.

Q Do you anticipate that genetic testing such as screening for disease-linked polymorphisms will become required testing to establish donor eligibility in the near future?

NM: Based on our own understanding, and from speaking to regulators, it is not likely that disease-linked polymorphisms or broad genetic testing is going to become a requirement for donor eligibility or cellular starting material testing in the near future. Broadly speaking, we don't have enough evidence to definitely claim causality for most polymorphisms, even those that show statistical correlations to disease. There are specific instances, such as tumor suppressor genes, that if mutated pose a safety concern in a cellular starting material and the corresponding C> product. Consequently, we are starting to see more targeted genetic assays being developed and marketed by the supporting tools and technology industry, and this may be one area to keep an eye on for future cellular starting material testing requirements.

Another area of genetic testing that is likely to increase in importance is human leukocyte antigen (HLA) testing. This is a very useful tool for tracking the identity of cellular starting material, given that one's HLA genotype can be highly unique when high resolution genotyping methods are employed. In addition, some immunotherapy products require full or partial HLA matching to achieve their therapeutic effect and thus already include HLA testing to identify suitable starting material donors. Going forward, HLA testing of starting material donors is likely to become much more common and may even become a preferred method to confirm chain of identity of starting materials.

Q Switching focus to the logistics of cellular starting material collection and handling, what are the regulatory expectations with respect to quality systems and compliance? For example, to what extent should collection and handling be performed in accordance with GMP guidelines?

NM: Currently in the US, collection of cellular starting material including leukapheresis products for C> manufacturing must follow the FDA guidance document, *Current Good Tissue Practice (CGTP) and Additional Requirements for*

Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) (December 2011 release).

When we pressed regulators on the point of whether the leukapheresis collection process itself should fall under GMP guidelines, the majority of respondents stated that the CGTP guidelines already in place cover all of the important elements of that collection process with respect to chain of custody, chain of identification, ensuring proper training of personnel, and handling of the leukapheresis material.

For every step after the collection process, whether that includes cryopreservation or any other manipulations prior to manufacturing of a C> product, all of our regulatory respondents agreed that these activities must follow GMP guidelines.

Q Are there any other guidance documents on collection and handling best practice that are recognized by regulatory authorities at the moment?

NM: Specifically, in the context of the FDA, there are two additional guidance documents that are recognized. The first is a guidance document from the International Organization for Standardization, *ISO 21973:2020 - Biotechnology — General requirements for transportation of cells for therapeutic use (June 2020 release)*. This guidance document is currently recognized by the FDA as containing appropriate guidelines for transportation of cells for therapeutic use.

The FDA also recognizes recommendations put forth by the International Society of Blood Transfusion, as described in their guidance document, *ISBT 128 – Standard Technical Specification (January 2019 release)*. ISBT 128 provides additional guidance for the collection and handling of cellular starting materials such as leukapheresis products.

Q When working with cellular starting material that is cryopreserved prior to its use in downstream cell therapy manufacture, what additional testing requirements and logistics need to be considered?

NM: For cellular starting material that is cryopreserved, there needs to be qualified analytical methods to assess the stability of that frozen product over time and confirm that it is still acceptable for use in downstream manufacturing. What that testing should look like is a somewhat challenging question. As discussed earlier, the needs of C> developers can be very process and product-specific, as far as which cell types in that starting material are most important to them, what growth conditions will be used, and what performance attributes ultimately matter in the final drug product.

Engaging with both C> developers and regulators, we sought to identify a common set of testing requirements that could potentially be applied to cryopreserved cellular starting material such as a leukapheresis product. In the end, identifying a common set of tests methods that could be useful to almost everybody, we were left with something fairly similar to the

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current state of the art – total nucleated cell count, viability, and a basic characterization panel of cell types. Beyond that, it becomes very difficult to establish a set of testing criteria that will be broadly relevant to C> developers. Nonetheless, it is a decent starting point for understanding the impact of cryopreservation of cellular starting material, particularly for leukapheresis products that typically are used fresh, immediately after collection. As we get more information on additional critical quality attributes that can be applied across C> programs, that testing can be expanded.

"What groups should be doing as soon as possible is understanding if, and how, their process can work with cryopreserved cellular starting material."

One test that would be quite valuable to add is a performance measure of post-thaw cells. But again, we run into the challenge that everyone's culture conditions are unique. Could we identify a culture condition that is at least relevant, even if it is not identical to what each developer is using? Possibly, and that is certainly something that should be explored.

Q The increase in interest in allogeneic, off-the-shelf therapies is tremendous. What for you are the most pressing challenges and gaps for companies collecting and qualifying cellular starting materials when developing products for global use?

NM: One of the key things to consider here is the potential move towards cryopreserved products. Currently, most C> developers in the allogeneic space are using fresh cellular starting material, for most immunotherapies this means that they are obtaining a leukapheresis product that has been collected directly from a donor and shipped to their manufacturing site without any cryopreservation or processing in between. That presents some major challenges with respect to timing of manufacturing, i.e., making sure everything is set up and personnel are available and ready when the starting material shipment comes. Sometimes there are complications in the collection process that require downstream manufacturing to be adaptive.

What groups should be doing as soon as possible is understanding if, and how, their process can work with cryopreserved cellular starting material. Cellular starting materials that drive production of allogeneic C> products really need to follow the same suit that the allogeneic therapies themselves are aiming to deliver – an off-the-shelf concept that is well characterized and fit for purpose.

Q The raw and starting material supply chain has been one of the most challenging areas for cell therapy manufacture during the pandemic. Do you have any advice or best practices based on the experiences of Dark Horse clients over this difficult period?

NM: The pandemic is a perfect example to underscore the importance of being able to cryopreserve cellular starting material, in order to decouple the timing of collection from the timing of manufacturing.

The groups we have spoken with that have already begun that process of comparing fresh versus frozen cellular starting material in their manufacturing process, and are trying to optimize cryopreserved product in their process, generally are faring much better than groups that rely solely on fresh donor material.

Another thing that groups are doing is finding an intermediate point within their manufacturing process where they can cryopreserve. That allows them to have more control over when they are generating drug product, to make sure that it is available when they need it.

Right now, there are a lot of challenges with clinical work in general, including getting patients in for ongoing clinical trials, so we see a lot of groups using this as a good opportunity to focus more efforts on process development than they might otherwise have planned. This enables them to use research grade materials – both raw materials as well as the actual cellular starting material itself. You have a little more freedom in the type of material used when you are focusing on process development upstream of the actual GMP manufacturing.

So on the one hand I would say that this pandemic underscores the need for the allogeneic field and C> in general to move towards the use of cryopreserved cellular starting material. But on the other hand, times like this force all of us to ask what activities can be done and to shift our focus accordingly.

Q Could you sum up your chief goals and priorities over the next two years?

NM: At Dark Horse, our core goal is to help all C> developers reach their goals, and this is exactly why I signed up with the team. We want to see more exciting, innovative technologies reach the clinic, achieve commercialization, and reach patients. In order to do that, we need to continue to learn about the expanding range of C> products that are out there. There are new techniques coming out all the time, improving how we culture, differentiate and expand distinct cell populations, and broadening the analytical toolbox that can be used to assess product safety and efficacy. This is especially true for the rapidly evolving field of genetic engineering. The increasing number of gene editing modalities now available and the trend towards increased gene modification events per cell therapy product is triggering a need for a much greater understanding of the impact of those modifications on the safety and function of the resulting drug product. Our goal, and my goal personally, is to try and keep up with all of these fast-paced elements within the C> field, and really understand what these exciting innovative products need to succeed and get to patients.

Part of that is helping chart the development path of emerging C> products. Part of it is trying to foresee and react to the challenges that they face - both with respect to an increasing safety bar for C> products with increased complexity, and also as we try to treat a broader range of indications. I want to stay abreast of this, and really contribute to the field

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in any way I can, as a team member of Dark Horse. I want to help more of these treatments succeed and see cell and gene therapy become the next revolution in medicine.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

COMMENTARY/OPINION

Master ATMP processes by controlling raw and starting material

Anne-Sophie Lebrun & Carmen Brenner

Advanced Therapy Medicinal Products (ATMPs) must be safe, effective and of a high quality when administered to the patient. To achieve this, the activities carried out at each stage of the ATMP manufacturing process including supply chain must be controlled and standardized wherever possible to ensure safety of the finished product. In this context, the quality of starting and raw material is a key factor to consider especially in the ATMP field where the raw materials and starting material usually come from very different sources and include some of human origin. Regulatory frameworks are already in place for supporting raw material and starting material process control. The criticality of each raw material and starting material should be assessed using a risk-based approach. Following this evaluation, a control strategy should be defined. Ensuring reliable raw and starting material will allow consistency and performance of every batch and so a sustainable quality of the drug product.

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RAW MATERIALS

Raw materials is a general term used to denote reagents and solvents intended for use in the production of intermediates or APIs (EU GMP Guide). All raw materials must be checked at reception and released before entering the manufacturing process.

Regulatory requirements

Current requirements are highlighted in the following guidelines. These guidelines offer a good support in managing raw material. One target is to avoid any safety risk for the final product using an inappropriate material and

the other target is to ensure the consistency of the raw material.

- Eudralex volume 4 Part IV, GMP requirements for ATMPs
- EP General Chapter 5.2.12. Raw materials of biological origin for the production of cell-based and gene therapy medicinal products
- USP <1046> Cell and gene therapy products
- USP <1047> Gene Therapy Products
- USP <1043> Ancillary materials for cell, gene and tissue-engineered products
- Blood Directive 2002/98/EC

During early developmental stage, R&D material is for example tolerated however very quickly the requirements to control and to justify each raw material become increasingly strict. As it is very difficult to introduce changes in the process at later stages, it is important to carefully evaluate each raw material from the early beginning on. Having trusted and high-quality raw materials are mandatory to ensure a reliable and consistent manufacturing process.

The source of the raw materials

In the ATMP field, the raw materials usually come from very different sources and frequently include some of biological origin such as serum. Moreover, as ATMP products request highly specific growth and culture conditions, some raw materials are only available in an R&D grade and not in a GMP grade. Of course, it is highly recommended by all the guidelines cited above to use preferentially non-human and non-animal derived raw materials and to use GMP-grade material. The holy grail is to use only chemically defined raw materials that are produced in a GMP compliant manner allowing to decrease the risk related to safety and the variability of the material.

However, in most of the cases this is unfortunately not possible. As the ATMP's characteristics depends also on the way the cells are cultured, it is not obvious to implement major changes to the process. When the use of biological and/or non-GMP grade raw materials is necessary, it is mandatory to establish a risk-based approach and to set up a control strategy; the aim is to secure the quality and efficacy of the final ATMP.

Evaluation through a risk-based approach

The risk is evaluated on the basis of the criticality of the raw material that should be assessed on the basis of impact to product quality and ultimately the patient. The more critical the material is for the ATMP, the more the control strategy should be reinforced. Listed below are questions to be addressed in a survey that should be completed before ordering the raw material in order to establish the criticality related to the quality and to the vendor/manufacturer of the raw material. From this questionnaire a risk map can be established and applied to all new raw material. The list of questions is not exhaustive and should adapted by the user.

The criticality of the raw material should be evaluated through different parameters.

Knowledge of the raw material

What is the knowledge about the material and its impact on the quality of the ATMP? A deep knowledge on how the raw material acts on the ATMPS allows to detect batch to batch variability of the material but also allows to consider easier a vendor change or the raw material source.

Source of the raw material

Is the material from biological origin or animal? Is the raw material produced using

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substances of human or animal origin? Is the raw material or chemically defined and free of from substance of human or animal origin? Are there any equivalent sources available on the market to increase the quality of the raw material?

Quality attribute of the raw material

Is the material suitable for the intended purpose by its composition, its purity? Are product related impurities evaluated by the manufacturer? Is the raw material produced within a recognized quality management system? Are suitable specifications established for identity, potency, purity, safety? Has the raw material a monography?

Reliability of the vendor/manufacturer

Is the material GMP-Grade or R&D grade? Is the vendor a known and well-established vendor? Is a QTA (Quality and Technical Agreement) in place with the vendor? Has the vendor been audited? Are there alternative vendors proposing the same raw material with the same quality? Is it a customized product – if yes who owns the recipe? In here it is to make sure that the supply of the raw material is guaranteed, and that the vendor is reliable and informs in advance if there are any changes in the manufacturing of the product.

Risk related to the clinical benefit/risk

Is it in prolonged contact with the ATMP? Have the impurities been evaluated in the final product – is the raw material still present as impurity in the final product? Does the quantity of raw material as found in the final product present any risk to the patient?

The risk should be decreased as much as possible through actions on each parameter. Some are very easy to do – like increasing the reliability of the vendor through an onsite audit (or a remote audit because of the COVID-19 pandemic) and the set-up of a QTA.

Let us illustrate the risk-based approach by three examples: Serum, Trypsin and PBS.

Serum either from animal or human origin is a highly complex raw material with multiple risks. There is an inherent risk of transmitting infectious agents such as viruses and prions. The knowledge of the composition of this raw material is general quite low and the impact of the different compounds on the cell therapy product is often not well understood. Even if serum is collected from a pool of donors/animals, there are potential differences in quality between batches. Best practice is to try to evolve to a serum-free medium where all compounds are chemically defined, if this is not possible a tight control strategy should be established covering not only safety aspects such as viral safety but also potency aspects in general and especially with regards to the characteristics of the final drug product.

Trypsin is a very good example to highlight how a raw material has evolved over time from a high risk to a very low risk product. A few years ago, the gold standard was to use trypsin from bovine or porcine origin, implicating all the risks linked to the use of an animal derived product especially from a safety point of view. Even if the product is well controlled the safety of this kind of product remains critical and issues such as viruses, prions, and protozoa require extra tight safety tests. However, the market evolved and proposes now either a recombinant trypsin produced in plants, which reduces the viral risk for human applications, or even safer, from microbial fermentation. This recombinant trypsin is as efficient as the original porcine trypsin and can be inactivated through simple dilution instead of serum. This product is proposed in GMP-grade and some companies have a registered Drug Master File (DMF) at the FDA for this material.

PBS is a very simple, chemically defined solution that is sold in a GMP grade by many vendors. The identity and safety aspects can be checked easily and the solution has no major impact on the cell characteristics, as it is mainly used to rinse the cells and the contact with the cell product is very short. Impurities

are low. As it is a salt solution, even traces of PBS in the final product do not represent any risk for the patient. PBS is a very low risk material and with a reliable vendor, tests and sampling can be reduced over time.

Control strategy

The control and sampling strategy should be based on the risk-based approach. In general safety and identity tests are required for each raw material before being released for the manufacturing process. However, potency tests might be also very important especially for complex raw materials. Specifications should be set internally for each raw material to ensure that they fit for the intended purpose. The control tests mainly are articulated around three main parameters.

- Safety tests: sterility, endotoxins, mycoplasma, viral tests
- Identity tests: for example, identification of a protein, of ions, etc.
- Potency tests: to control batch-to-batch variability and to ensure the suitability of the raw material for the specific manufacturing process

Depending on where the greatest risk is identified, the control strategy should be reinforced for this parameter. Especially for complex biological materials such as serum and growth medium, specific tests should be set up to the intended purpose and adjusted to assess the quality and to check batch to batch variability but also the stability of the material over time. The identity tests are not sufficient for this purpose and tests described in a material's monograph might be not relevant for use of the material specified for a given ATMP. For complex biological material, no WHO Standards are available and so there is no general reference to rely on. Customized potency tests in an accurate system should be developed especially for those complex raw materials. For example, for cell culture medium, the growth promotion properties should be controlled in a suitable system that reflects the manufacturing process conditions and on the cells of interest. Moreover, for cell culture medium it is highly recommended to leave out any antimicrobials to prevent any interference in the sterility tests.

As a final consideration, I would like to add that the stability of the raw material and of the aliquots made from the raw materials is also a very important aspect. The expiration date provided by the manufacturer might be not applicable for the specific use of the raw material. Here again, this is mainly applicable to complex raw materials such as serum or growth medium. Sometimes the manufacturer performs a stability study by checking only a few major compounds of the complex product to establish a shelf-life, but minor compounds that might be very important for the manufacturing process and the quality attributes of an ATMP are not taken into consideration. As those minor compounds are not identified clearly as being critical compounds for the process and the quality of the final product, specific potency tests are required for those critical complex raw materials.

STARTING MATERIALS

Starting materials for ATMP's manufacturing processes are generally characterized by complex biological features with inherent variability. Starting material usually consists in human tissues or cells or blood-derived cells collected from either healthy donor or patient for allogeneic or autologous cell therapies respectively.

Regulatory requirements

The donation, procurement and testing of human body material (human tissues and cells or blood-derived cells) used as starting materials is strictly regulated with the application of the following regulations:

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- Eudralex volume 4 Part IV, GMP requirements for ATMPs
- Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells
- Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components
- CFR 21 Part 1271 Subparts A-D

The ATMP manufacturer must verify that the starting material supplier owns the appropriate accreditation, designation, authorization, or licensing according to the regulation mentioned here above, and in accordance with the terms of the marketing authorization/clinical trial authorization.

The source of the starting materials

For allogeneic products, ATMP manufacturers can usually rely on one or two supplier(s) for the supply of starting material. To limit the risk on the supply chain, it is important to work with more than one supplier. However, depending on the countries, the number of suppliers with the appropriate authorization is limited.

In autologous manufacturing processes, many different collection sites might be involved and therefore need to be qualified in the specific apheresis and/or tissue-collection methods along with the shipping preparation process. In addition to the variability of the starting material itself, some slight differences in collection methods and/or shipping time will increase the variability of starting material.

For each supplier (including blood and tissue establishments), the ATMP manufacturer must enter into a quality and supply agreement. This agreement should contain clear provisions about the transfer of information regarding the starting materials - in particular, on tests results performed by the supplier, traceability data, and transmission of health donor information that may become available after the supply of the starting material, and which may have an impact on the quality or safety of the product.

Donor selection

The quality of ATMPs is dependent on the quality of the starting materials which is dependent on the donor. There is inherent variability between donors however it is important to try to minimize this variability by carefully selecting donors. The identification of key parameters that are assessable prior to tissue or blood collection (for example blood markers) will help to ensure consistency of the manufacturing. In this regard, in addition to the donor eligibility criteria defined in the different regulations and focused essentially on safety testing, the ATMP manufacturer might add to the donor selection specific criteria such as gender, age, BMI or any other blood markers, etc. The ATMP manufacturer should define in the quality and supply agreement donor selection criteria that should be agreed with the supplier(s).

This approach is however not always possible - with autologous ATMP, for example.

Control strategy

The ATMP manufacturer should establish quality requirements (specifications) for the starting materials. These specifications should be in compliance with the regulations and with terms of the marketing authorization or clinical trial authorization.

The starting material specifications are divided into two main categories: safety aspect and cell quality (concentration, viability).

Safety testing is the most critical aspect to cover and relies mainly on the donor testing

that is performed by the supplier. The level of supervision and further testing by the ATMP manufacturer should be evaluated based on a risk-based approach. For example, for blood establishments and tissue establishments that are authorized and/or licensed under the regulations mentioned above, the ATMP manufacturer can rely on the data provided by the supplier.

In addition to serology testing, the ATMP manufacturer usually performs a sterility test on the starting material before or at the beginning of the manufacturing process. The sterility test takes more than 14 days, and it is usually permitted to start the manufacturing process without the results. In this case, the risk management plan should include the assessment of using a potentially failed material and its potential impact on other batches.

Regarding the cell quality aspect (cell concentration, viability), the specifications (at least minimal acceptance criteria) should be established during the process development to ensure batch consistency. Usually, these parameters are variables that should be considered in the manufacturing process. Specifications of these parameters can evolve at the product development stage.

Finally, the stability (shelf-life and storage condition) of starting material is important to consider. It is crucial to evaluate properly the stability of the starting material, as it can have a major impact on the finished product quality.

CONCLUSION

Raw and Starting Materials are key elements for the manufacturing process, contributing indirectly to the quality attributes of the final drug product. Guidelines highlight the most important parameters to be considered in a risk-based approach that will lead to define the criticality of material and subsequently define the control strategy. Each material should have specifications adapted to the intended use. Potency tests for raw and starting material are as relevant and important as the potency tests for the final drug product. Reliable raw and starting material will allow consistency and performance of every batch, and therefore, a sustainable quality of the drug product.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INTERVIEW

Sourcing and procurement best practices in the face of uncertainty

RICHARD STOUT has 20 years of Sourcing, Procurement, and External Manufacturing experience. Having spent his early career in the electronics and elevator manufacturing fields with Schindler Elevator and Tyco Electronics, he gained big pharma experience working in the vaccines and biologics divisions of both GSK and Merck, before bringing his expertise to the cell and gene therapy space with Adaptimmune. He has an MBA from Mt. St. Mary's University, Emmitsburg, MD.

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Q What are you working on right now?

RS: Obviously, much of the supply chain is being impacted by COVID-19 here in the US, in the UK, and around the globe. Consequently, there is something of a tactical element to almost every day: we take a look at how the pandemic has impacted our supply, identify where we may have issues, and try to put out any fires before they begin. I think the risk mitigations we did early on, prior to the pandemic, have helped us greatly - the number of issues we encounter has reduced - but they do still pop up from time to time.

I'm working on a few projects related to commercial readiness as part of the planning for our first product approval in 2022.

Managing the external manufacturing network is a further key priority of mine. I am in a unique position where I strategically source on behalf of our external manufacturing organizations. My team manages the procurement at the tactical level, but also the external manufacturing network from an execution perspective. It's all about executing to the contract and getting the work done.

So it's really a mix of the tactical and the strategic each day: the daily business that needs to be done, mixed in with pandemic-related problem solving, plus a constant focus on risk mitigation and running the sourcing process to ensure we pick the best supplier for Adaptimmune every single time.

Q What were your first impressions when coming to the cell and gene therapy space from big pharma?

RS: Coming from big pharma - huge campuses, global organizations, very tough to navigate - I found Adaptimmune (with 3 sites in the US and UK) to be incredibly well connected and a very intimate setting. We literally all sat beside each other on two floors of the same building, which made for a very cross-functionally collaborative environment that was quite different for me. You had manufacturing sitting alongside with quality, sourcing, regulatory affairs, and clinical. And it was really just a matter of going up and down the stairs and meeting with people to learn, to understand, make decisions, and keep the business moving forward. I think it was a great opportunity in that setting for face-to-face meetings here in the US, and through video conferencing with my UK-based colleagues, to really feel connected as an agile organization.

The really stunning thing for me was to suddenly be able to see my sourcing decisions have an immediate impact on the business. Whether it was sourcing a vector, a reagent, or even a disposable; being able to run the sourcing process, select the supplier, negotiate the deal, procure the material, and then see how quickly it got into manufacturing and had an impact on the patient was very rewarding for me.

Q How have you sought to adapt and leverage your learnings and experience from big pharma, and implement them with Adaptimmune's pipeline of cell therapy product candidates?

RS: My sourcing and procurement experience goes back to the electronics industry almost 20 years ago. I was in elevator manufacturing, and I learned early on that risk mitigation in my line of work is absolutely critical: if you are in elevator manufacturing and you don't have a nut, or a bolt, or some piece of hardware, the entire operation shuts down. That's also applicable to electronics, big pharma, and cell and gene therapy. You have to be so certain that you've got that assurance of supply. So I've taken all of those core learnings from

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> "...what do you choose to source first? In some cases, there is an immediate need to source something new; in others, there's a need to source an alternative to an existing item – you need to be able to weigh up which one to work on as the priority... should a part be incorrect, what is the impact on the patient?"

the past 20 years of mitigating risk and then running the sourcing process, and brought them into Adaptimmune.

And I really haven't made any alterations to my process. Risk assessment is the first step of risk mitigation and when you risk assess, you are seeking to understand what the most critical things are for running the company or manufacturing the product. You have to have the criteria down, because once you understand what the highest risk is, you assign a tier to that - tier one being the most critical and immediate; tier two being that mid-level risk that you don't have to work on right now, but you should focus on a year from now; and tier three being lower risk items where there may be lots of alternatives out there. You come to understand what the various components and their associated criticality and risk are in a very methodical way, which allows you and others to easily understand why you chose the parts you chose.

The second part of the process is the sourcing. So what do you choose to source first? In some cases, there is an immediate need to source something new; in others, there's a need to source an alternative to an existing item – you need to be able to weigh up which one to work on as the priority.

For example, coming into Adaptimmune, we did a really good job of risk assessing our bill of materials (BOM). We took a look at criticality to the business as a key criteria. We looked at the impact of non-conformance - should a part be incorrect, what is the impact on the patient? We asked if we have alternative solutions? Is a material sole-sourced, or was it single-sourced initially and capable of being supplied by other sources? How much does it cost, and what is the annual spend on it? We applied a percentage weighting to each of these criteria. Then, when we ran a full analysis, we were able to identify in which tier each item belonged.

For me, it is so important to start the sourcing function and process within a cell and gene therapy company in this way - or quite frankly, any sort of company. This ties in with the pandemic as well: we started risk assessing back in 2017 - we didn't wait for the pandemic or any other crisis to hit before we figured out what was important to us. Hopping on that train early was of great benefit to us when the pandemic arrived.

The next step is to take a look at the sourcing process and again, for me, that hasn't changed for years. The standard steps begin with defining the obstacle you want to overcome and really understanding it well, before you proceed to the next step of analyzing the marketplace

> "the step that is sometimes missed is ... where you do the continuous improvement with the supplier you've chosen, and you do good supplier management. "

- understanding what's really out there. That process is conducted using Requests for Information (RFIs) - nothing financial, but certainly understanding what a certain supply base has to offer. You also begin to set the AQSCI business requirements (Assurance of supply, Quality requirements, Service requirements, Cost, and Innovation) and really begin building out what's important to the business. If you build in your AQSCI requirements early, there tends to be much less debate on the back end as to which supplier to choose.

The third step is to create your strategy and generate your options. You understand from the RFI data the potential strategies available to move forward. That could be technical, such as deciding that now is the time you want a primary, a secondary, and a tertiary supplier. It's taking all those things into account.

The fourth step, the RFP (Requests for Proposals), is where the dollars and the cents come into play. You negotiate the deal, the back-up deal, maybe the tertiary deal, too; and you get that all set. Then you execute the contracts.

I think the step that is sometimes missed is that fifth step where you do the continuous improvement with the supplier you've chosen, and you do good supplier management. That is where, for critical strategic suppliers, you have the governance, the score cards, and the continuous improvement in the contract with what they are going to deliver, and you really manage them well. You get to the point where you have a smooth-running supply base and when something happens, like a pandemic or a natural disaster, you've got a secondary supplier and sometimes even a tertiary supplier built into that process.

That's a long way to say that I really haven't changed my methodology since coming to cell and gene therapy. It fits right into the manufacturing of cell and gene therapies: defining what's important, knowing what's less important (not that it's ignored, but it's placed elsewhere until we need to get to it), and then running a really good sourcing process. That is what we have been doing over the past three years since I joined the company.

Q So much of the process has remained the same – but is there anything you have had to tweak or adapt to fit the cell and gene therapy space?

RS: If you take a look at the supply base within cell and gene therapy right now, it is flooded. There is very little capacity within CMOs, CROs, and the suppliers of critical equipment and disposables. Cell and gene therapy is exploding and I think we are relying on a very defined supplier base.

The takeaway from that - and where I have needed to make some modifications - is going through the risk assessment and the need to focus on alternatives.

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I think we put a much greater focus on the alternatives and the 'what ifs' in cell and gene therapy. This means we will take a look at something and simply say that if we can't get a like-for-like, can we begin working on something that's sort of similar - and we spend a lot of time with the quality teams working on that. It's being aware that we are relying on suppliers that are incredibly stretched and also that we don't have the leverage of a big pharma company. I think it's also about networking with the supplier and making them understand we are working on potentially life-saving therapies for patients who are critically ill.

It all ties back to running those risk mitigations, a really good sourcing process, finding the alternatives, and building relationships with the secondary and tertiary suppliers out there.

Q You mentioned that you were well prepared when the COVID-19 pandemic struck – are there any examples you can share where the benefits of your preparatory work really shone through?

RS: I believe we took the right decisions on carrying inventory and not trying to run too lean.

I think there is a realization in the cell and gene therapy space that it is critically important to have inventory. It costs a little bit more, obviously, but while there is a cost to carrying more inventory, it is nothing compared to the cost of not being able to treat a person in urgent need for treatment. I think the leadership at Adaptimmune is on board with carrying the inventory and not taking a risk with it - putting dollars and cents in place to provide the level of assurance that we have and want to provide to the patients we treat.

That approach paid off very well for us when COVID-19 hit. I don't think anyone was fully aware of the scope and magnitude of this. I don't believe that supply chains in general were ready for it. There have been multiple times where we looked at the inventory we were carrying, looked at the sourcing we did (particularly for some reagents and for our vector supply), and appreciated the fact that we had a very solid inventory position. So, when the pandemic hit, there wasn't a panic across the board with the bill of material because we had some great inventory practices, great sourcing, and we had a lot of great people managing that.

On the flip side, if I were to be critical of myself, I was personally surprised on the PPE side to see the impact on the supply chain in terms of availability of masks, gloves, lab coats, etc. I know a lot of people were. Any type of PPE item, even down to the hand sanitizers, became incredibly difficult to obtain. We put a tremendous amount of time into expediting the procurement of essential items and finding alternatives. We had good inventory of those things to start with, by the way, but it still did not meet in every case the catastrophic nature of the deficits we saw in the supply chain. If I was to go back in time, I think I would put more emphasis on the PPE because our business has a regular need for these items, even before the pandemic - that's a lesson learned.

Q What future repercussions from the pandemic do you expect to encounter, and how will you continue to ensure a state of readiness for them?

RS: When the pandemic hit, we created a team tasked with looking at our UK facilities as well as our US facility on a daily basis. This team was completely focused on bill of material items and PPE - doing daily counts and understanding, on a daily basis, what suppliers were promising (or not promising, which could be more worrisome!). We created a good routine of being able to look at the supply chain that particular day and understanding if we had any issues. More importantly, we looked ahead 30, 60, 90, 120 days, to understand if we were going to have problems coming down the road. I mentioned finding alternatives earlier. If I need to find an alternative, I'd rather know four months rather than four days in advance, obviously.

We have kept that team and process alive - not on a daily basis, we do it weekly now. We continue to monitor the situation as it progresses in our facilities. We not only take into account the ramp-up of our manufacturing, but obviously how the pandemic could potentially impact it. We remain focused on the fact we are still in a pandemic, and we are going to be for some time.

I think there are likely to be repercussions in the supply chain for at least two more years. That's not to say there are going to be problems in every single area of the supply chain, but I think there is going to be a seismic change in how things like PPE items are consumed and distributed. I expect there to be some other fallout from the pandemic that we have to become aware of.

I think the best way to be aware is to force yourself on a weekly basis to take a look at the situation, to understand what's coming down the pike, and to build relationships with suppliers so you get transparency from them and understand why they may be facing problems. It is key to manage your contracts well, to understand what the supplier owes you and when those items are coming in, and to have a great team of people to manage that. It is not going to be over in a day. I think it's going to take some time. But I believe we are into a groove now that is sustainable and will make us successful.

Q Speaking of previously unforeseen but highly disruptive scenarios - Brexit: what challenges is it presenting for you in your role, and how are you seeking to address them?

RS: It is first and foremost about having an awareness of it. At this stage, I don't know if anyone has a definitive view on every single impact Brexit is going to have.

In my view, I think it is going to create longer lead times for some items. Again, I am not entirely sure what that exactly looks like right now, but certainly we have incorporated those longer lead times into our planning. I also think there is going to be more compliance coming down the road, and certainly, we will learn more about that as time goes by. Understanding how shipping lanes and shipping paperwork will need to be changed, for example. It could be changes in customs and duties that lead to those longer lead times. And it could result in higher costs, obviously, for all of the reasons I've just stated.

I think how it feeds into the sourcing process is maybe driving more of a focus on seeking out more "local" suppliers. For example, asking ourselves if we should we have two primary suppliers now, one for the US and one for the UK? We will need to consider those types of decisions and to build the topic of Brexit and its impacts into our sourcing decisions on an

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ongoing basis. Going back to the AQSCI, I think Brexit plays into that. It will be key to have business requirements that are built around our awareness of Brexit and its potential repercussions so we are not surprised by any outcomes.

I also think it will be important to stay flexible, as we learn how to deal with an issue like Brexit. I would compare it to the pandemic: you live through these things, you learn from them, and then you apply those lessons to the next thing that comes up, whatever it might be.

"it will be important to stay flexible, as we learn how to deal with an issue like Brexit... you live through these things, you learn from them, and then you apply those lessons to the next thing that comes up..."

Q Finally, can you sum up your chief goals and priorities in your role over the coming 12–24 months?

RS: Obviously, keeping an eye on the pandemic will remain a critical focus for some time to come. Beyond that, it's about keeping an eye on planning for our first product approval in 2022 and focusing on making the business successful in order to get to the subsequent launch. There are the various projects I mentioned earlier that are required in support of that key business objective - to deliver a product that we believe will address an unmet need for people with synovial sarcoma.

There will be ongoing risk mitigation of the supply chain, which ties in Brexit, to meet what we hope is a growing patient population and its demands on our manufacturing pipeline.

And strategically, operationally, it is about building out the sourcing, procurement, and external manufacturing team as the company grows, to allow us to provide services to our various stakeholders. I very much look at sourcing and procurement as a customer-facing organization. We are customer service, at the end of the day. And I want to be in a place with the team and bandwidth I need to go out to the marketplace with Adaptimmune's requirements and find the very best suppliers every time - suppliers that meet our AQSCI business requirements, regardless of whether it's related to CMC/manufacturing, research, clinical, or corporate needs.

Ultimately, to me, success is successfully treating our patients. No patient wants to hear that they weren't able to receive their treatment because someone in the sourcing organization ran out of a part… That's what drives me and my team.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

Multiple-parameter profiling of density gradient ultracentrifugation for characterization of empty and full capsid distribution in AAV preparations

Sebastijan Peljhan, Maja Štokelj, Sara Drmota Prebil, Pete Gagnon & Aleš Štrancar

Ultracentrifugation (UC) is a well-known technique for fractionating adeno-associated virus (AAV) capsids according to their density, which is mainly a function of their encapsidated DNA mass. Empty capsids represent the lowest density subpopulation. Full capsids represent the highest density subpopulation, sometimes accompanied by partially full capsids of intermediate density. Fractions can be collected after sedimentation for analysis but the practice is laborious and discourages application of multiple monitoring techniques that might provide deeper insights into sample composition. Anion exchange chromatography (AEC) also achieves fractionation of empty and full capsids for many AAV serotypes. The degree of separation varies among serotypes and does not correlate strictly with UC. This is not surprising since separation by AEC is highly influenced by capsid surface charge, which is independent of the amount of DNA packaged within the capsids. Chromatography methods however present a significant analytical advantage in the ease of monitoring the column effluent, including with multiple detectors. UV absorbance at 260 nm and 280 nm permits estimation of empty and full capsid proportions in any given peak. Intrinsic fluorescence enables estimation of relative areas of empty capsid peaks and full capsid peaks. Light scattering does the same and permits the further determination of capsid size and mass. In this report, we merge UC with an HPLC monitoring array to simultaneously analyze dual wavelength UV, intrinsic fluorescence, and light scattering through cesium chloride density gradient strata. Limitations of each monitoring method are discussed. UC results are compared with chromatography profiles to highlight distinction between separation methods.

Practical application of results for final product characterization is considered, along with potential to support development of better purification processes.

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INTRODUCTION

Density gradient ultracentrifugation (DGUC) is a well-known technique for fractionating adeno-associated virus (AAV) capsids according to the amount of encapsidated DNA they contain [1]. Empty capsids represent the lowest density subpopulation. Full capsids represent the highest density subpopulation, sometimes accompanied by partially full capsids of intermediate density. Fractions can be collected after sedimentation for analysis [2] but the practice is laborious and discourages application of multiple monitoring techniques that might provide deeper insights into sample composition. The concept of flowing density gradient-separated bacteriophage fractions through a UV monitor was demonstrated in 1978 and offers further potential for AAV [3]. The method known as Analytical Ultra-Centrifugation (AUC) pertains to a different technique that is also applied to AAV [4]. AUC does not exploit density gradients but relies instead on differences in the inherent sedimentation coefficients among sample components.

Anion exchange chromatography (AEX) also achieves fractionation of empty and full capsids for many AAV serotypes [4–15]. The degree of separation varies among serotypes and does not correlate strictly with DGUC. This is not surprising since separation by AEX is highly influenced by capsid surface charge, which is independent of the amount of DNA packaged within the capsids. Chromatography methods however present a significant analytical advantage in the ease of monitoring the column effluent, including with multiple detectors. Calculating the ratio of UV absorbance at 260 nm to absorbance at 280 nm permits estimation of empty and full capsid proportions in any given peak [4,5,15]. Intrinsic fluorescence enables estimation of relative areas of empty capsid peaks versus full capsid peaks [4,14,15]. Light scattering does the same and permits the further determination of capsid size and mass [14–16].

In this report, we present an expanded DGUC method for characterization of empty and full AAV capsid content in cell culture harvests, lysates, and chromatography fractions. The contents of post-DGUC tubes are pumped through an HPLC monitoring array to measure UV absorbance, intrinsic fluorescence, and light scattering across cesium chloride density strata. Conductivity is measured as a surrogate indicator of cesium chloride density. Signal integration produces a multi-parameter DGUC ‘centrifugram’ that corresponds in many respects to the chromatograms produced by chromatography methods. DGUC results are compared with chromatography profiles to highlight distinctions between separation methods. Practical application of results for final product characterization is considered, along with potential to support development of better purification processes.

MATERIALS & METHODS

AAV8 lysates produced from Sf9/BEV cells were obtained from the University of Nantes, INSERM UMR 1089, Nantes, France. AAV8 was chosen because AEX is documented to separate empty and full AAV8 capsids [9,12,14,15] and thereby facilitate comparison of empty/full separation by AEC and DGUC. Initial AAV purification was performed by

cation exchange chromatography (CEX) on a 1 mL CIMmultus® SO3 monolith (BIA Separations). CEX columns were equilibrated to 50 mM formic acid, 200 mM sodium chloride, 1% sucrose, 0.1% Poloxamer 188, pH 3.5, eluted with a linear gradient to 50 mM formic acid, 2 M sodium chloride, pH 3.5, then cleaned with 2 M sodium chloride plus 1 M sodium hydroxide. Volumetric flow rate on 1 mL monoliths was 5 mL/min (5 column volumes [CV]/min). AEX fractionation of CEX-purified AAV was performed on a CIMmultus® QA monolith (BIA Separations). The column was equilibrated with 50 mM bis-tris-propane, 2 mM magnesium chloride, pH 9.0; eluted with a linear salt gradient to 50 mM bis-tris-propane, 2 mM magnesium chloride, 200 mM sodium chloride, pH 9.0; then cleaned with 2 M sodium chloride plus 1 M sodium hydroxide.

Density gradient fractionation was performed on a Sorvall™ WX 90+ ultracentrifuge (Thermo Scientific) using 11.5 mL polyethylene UltraCrimp® centrifuge tubes (Thermo Scientific) in a T890 fixed-angle rotor. Samples containing about 1E+11 vector genomes (vg) according to ddPCR as described in [17] were mixed with concentrated cesium chloride to obtain an AAV sample in 3 M cesium chloride. Empty capsid sample volumes/concentrations were estimated based on the relative size of the empty and full capsid peaks from AEX. Centrifugation was performed at 53,500 RPM for 24 h at room temperature. The tube was then fixed in a stand and pierced near the top with a hypodermic needle (23 gauge, 70 mm, B Braun) extending to bottom-center (Figure 1). Another was inserted for venting with the tip remaining at the top of the tube to prevent air bubbles from mixing gradient strata during aspiration of the contents. Tube contents were pumped from the bottom of the tube directly through the monitor array of a PATfix™ LPG HPLC system (BIA Separations). This evacuated the tube in order of decreasing density. UV absorbance was monitored at 260 nm (solid red trace) and 280 nm (solid blue trace). Intrinsic fluorescence was monitored at an excitation wavelength of 280 nm and an emission wavelength of 348 nm with a fluorescence detector (Shimadzu, solid brown trace). Light scattering was monitored at a 90° angle with a DAWN® HELEOS II multi-angle light scattering detector (Wyatt Technology, solid black trace). Cesium

FIGURE 1

Schematic representation of density gradient ultracentrifugation with secondary stratigraphic analysis through an HPLC-based monitor array.

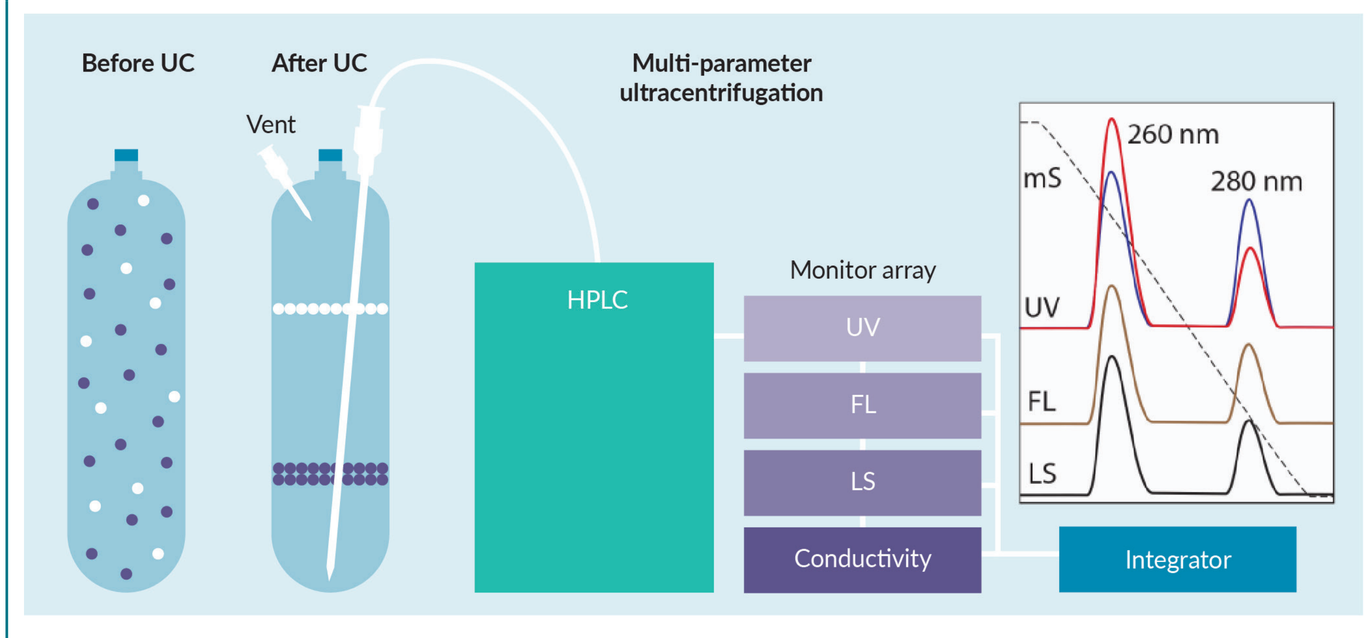
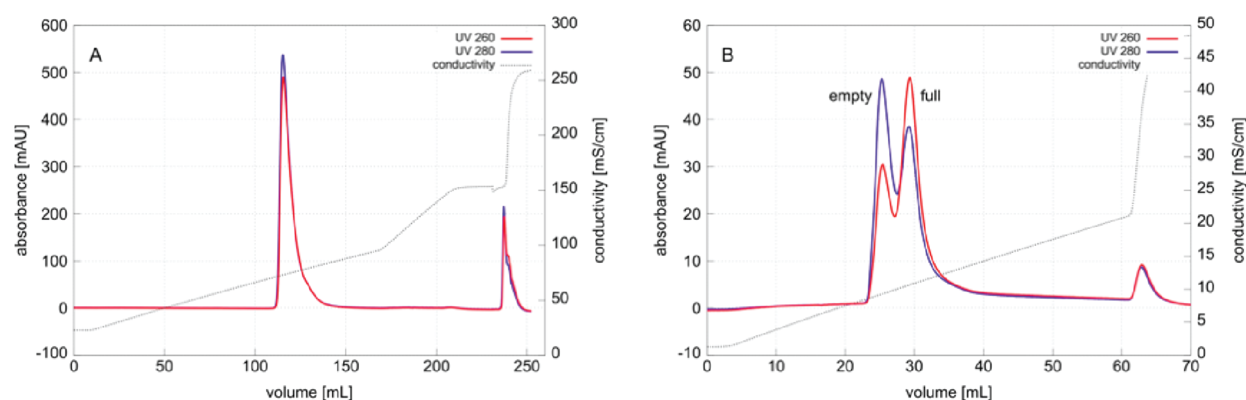


FIGURE 2

Chromatograms from initial CEX purification of AAV8 (A) and subsequent separation of empty and full capsids by AEX (B).



The red and blue lines represent UV absorption at 260 nm and 280 nm, respectively. The dashed black line indicates conductivity.

chloride density is represented by the conductivity profile (dashed black trace). The higher the conductivity, the higher the density of the cesium chloride.

The system was washed with water between samples. The discontinuity of refractive index between the water in the HPLC tubing and cesium chloride in the next sample created heavy signal noise at the beginning of the method. To properly zero the baseline, 1 mL of fluid was passed through the system to equilibrate the monitors to cesium chloride before the system was zeroed and data collection begun.

RESULTS & DISCUSSION

Figure 2A illustrates the initial CEX purification chromatogram. Mixed empty and full capsids eluted from CEX in a single peak. **Figure 2B** illustrates the AEX chromatogram showing separation of empty and full capsids after cation exchange capture. **Figure 3** illustrates a centrifugram developed with 1.4+E11 vg of CEX-purified AAV capsids. Monitor profiles are shown in two frames (A, B) so that overlap does not confuse interpretation. The 260/280 UV absorbance ratio of 1.41 at 4.5 mL confirmed its contents as full capsids (A). The ratio of 0.61 for peak at 7.3 mL confirmed its contents as empty capsids. Light scattering and fluorescence profiles (B) revealed a population of intermediate density capsids from 6 mL to 7 mL that might indicate partially filled AAV particles or incomplete capsids.

The UV absorbance ratio was unfortunately unable to provide more definition because of the increasing baseline across the profile. This is an artifact created by changes in refractive index across the density gradient, but also note that it affects the 260 nm baseline more than the 280 nm baseline. This complicates calculation of wavelength ratios and ultimately limits sensitivity. Samples containing fewer capsids can be detected by increasing monitor sensitivity but higher sensitivity also increases relative baseline slope. This puts 1E+11 vg close to the lower limit of capsid numbers required for UV monitoring. Baselines for intrinsic fluorescence and light scattering were flat, which means that sensitivity can be increased without compromising measurement accuracy. Present results suggest that running the method with 1E+10 vg or fewer capsids will likely produce useful intrinsic fluorescence and light scattering data.

Previously published results suggested the high density peak at 2.75 min might correspond to mispackaged plasmid DNA [2]. The present results suggest that population

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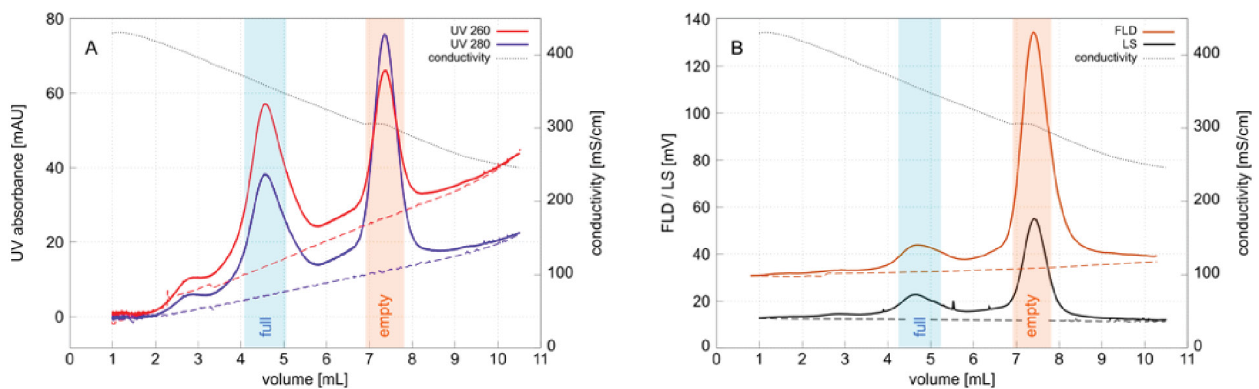
FIGURE 3

Centrifugram of CEX purified AAV.



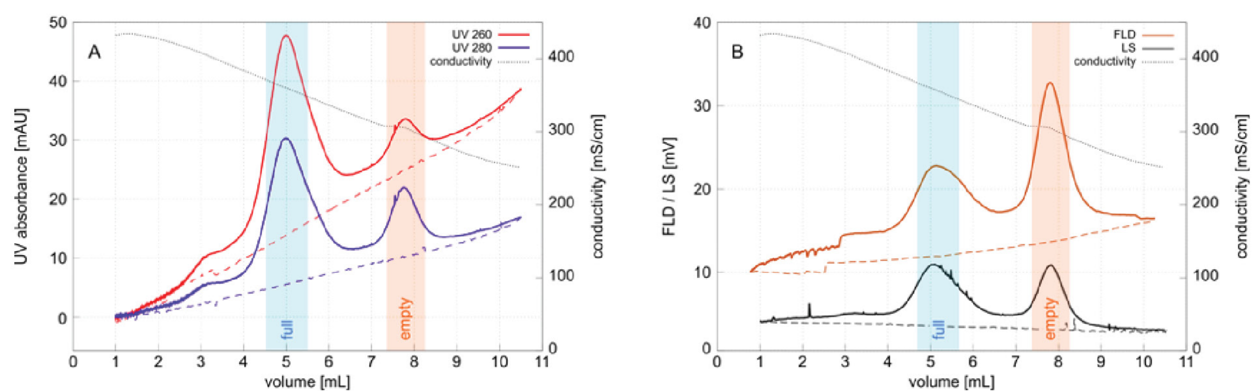
(A) UV absorption signals at 260 nm (red) and 280 nm (blue), **(B)** intrinsic fluorescence (brown) and light scattering (black) signals. Solid and dashed lines are representing sample response and the corresponding baselines, respectively. The dashed black line indicates conductivity as a surrogate indicator for cesium chloride density.

may rather represent dis-packaged DNA. The 260/280 ratio of about 1.4 is similar to full capsids but does not explain why they would exhibit higher density. Refractive index dependency of the UV baseline compromises precise estimation of DNA to protein ratio based on UV, but this population is clearly DNA-rich and intrinsic fluorescence confirms the presence of capsid proteins. Light scattering intensity unfortunately does not indicate how large the particles might be. Particle size and particle concentration both contribute to light scattering intensity and knowing one is required to interpret the other [16].

One hypothesis that is consistent with both the published results and the experimental data is that this population may represent aggregated full capsid debris created by exposure to cesium chloride under the high shear stress of ultracentrifugation. AAV capsid instability during DGUC has been noted by others who recommended inclusion of 10 mM magnesium ions to stabilize them [18,19]. Unpurified harvests and lysates sometimes exhibit

FIGURE 4

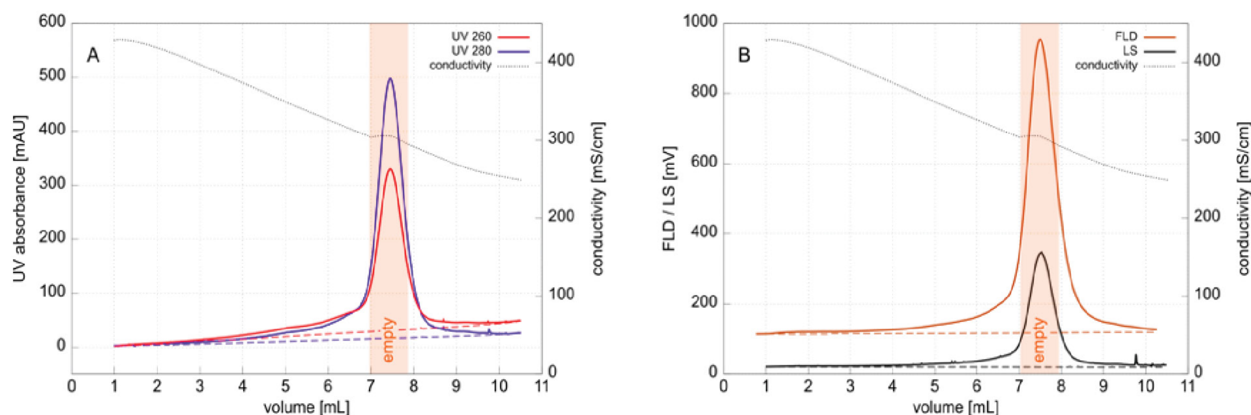
Centrifugram of the full capsid peak from AEX after AEX of CEX-purified AAV.



(A) UV absorption signals at 260 nm (red) and 280 nm (blue). **(B)** Fluorescence (brown) and light scattering (black) signals. Solid and dashed lines represent sample response and the corresponding baselines, respectively. The black dashed line indicates conductivity.

FIGURE 5

Centrifugram of the empty capsid peak from AEX after AEX of CEX-purified AAV.



(A) UV absorption signals at 260 nm (red) and 280 nm (blue). (B) Fluorescence (brown) and light scattering (black) signals. Solid and dashed lines represent sample response and the corresponding baselines, respectively. The black dashed line indicates conductivity.

a larger proportion of high density material (not shown) that suggests residual chromatin heteroaggregates might contribute to this population. Chromatin heteroaggregates exist as highly condensed structures that range in size up to 400 nm and are known to persist in 2 M sodium chloride [20]. Chromatin cannot be entirely ruled out as a contributor to the high density population in Figure 3 but it seems unlikely to be a major contributor because cation exchange has been shown to remove the majority of it [15]. AEC reduces chromatin levels further [20] yet the high density population is also observed in the full capsid peak from AEC (Figure 4), again pointing to degradation during DGUC.

Figure 4 illustrates the centrifugram of the full capsid peak obtained by AEX of the capsid peak from CEX (Figure 2). According to UV peak areas, the full capsid peak at 4.5 mL represents about 80% of total capsids, followed by an empty capsid peak at 7.7 mL apparently representing about 20%. Intrinsic fluorescence indicates that the proportion of empty capsids is closer to 50%. The discrepancy exposes a hidden distortion in UV measurement of relative peak size. In brief, the larger extinction coefficient of DNA, compared to capsid proteins, inflates the full capsid peak area [4,15]. Calculations can be applied to factor out the bias [21] but they suspend the simplicity of comparing peak areas. Light scattering provides a more conservative estimate of full capsid peak area than UV but the greater mass of full capsids can still inflate the area of full capsid peaks [22]. Light scattering measurements are also affected by refractive index [23]. Intrinsic fluorescence supports the most objective representation of relative peak areas [4,14,15]. It is unbiased by extinction coefficients, capsid mass, or refractive index. This leaves the comparison based on relative amounts of protein capsids, regardless of their contents.

Figure 5 illustrates the centrifugram of the empty capsid peak obtained by AEX of the capsid peak from CEX (Figure 2). The sample is heavily dominated by empty capsids at 7.3 mL, as indicated by the dominance of the UV trace at 280 nm. Identity of the species beginning to appear at about 2.0 mL and ramping up gradually to 7.0 mL is uncertain. Accounting for the contribution of refractive index suggests that UV absorbance at 260 nm and 280 nm appear to be roughly equivalent across this entire zone, which suggests the presence of both DNA and protein. Light scattering tracks with UV and suggests the presence of particles but does not necessarily suggest they represent fully assembled capsids. Recent studies have reported that capsid

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composition frequently varies from the ideal virus protein ratios of 1 VP1 to 1 VP2 to 10 VP3 [24], and that post-translational deamidation can substantially alter capsid surface charge [25]. The ramp region might contain such variants. It might also be populated by incomplete or damaged capsids. Whatever their origin and composition, they highlight the ability of DGUC to focus attention on a population that co-elutes from AEX with full capsids.

CONCLUSIONS

Characterizing DGUC profiles with multiple monitors provides valuable new perspectives for characterization of empty and full capsid distribution in AAV preparations. It is orthogonal to separation of empty and full capsids by AEX and enables more accurate interpretation of AEX chromatograms. This information can be used to better guide development of purification processes. It can also be used to guide development of density gradient formulations that may better conserve capsid stability during DGUC. Further characterization of the technique with capsids from other serotypes, lysates, harvests, chromatography fractions under various conditions and with differing abilities to separate empty and full capsids, all represent important opportunities to determine the full potential of the technique. Its performance with iodixanol and other density gradient media also promises to be interesting.

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Breaking viral vector bioanalysis barriers with centrifugal force

Patricia Ahrweiler

Advances in viral vector gene delivery systems, particularly adeno-associated virus (AAV) and lentivirus (LV), have accelerated the development of new cell and gene therapies. Regulatory programs for accelerated review have added to the demand for the manufacture of viral vectors, exceeding capacity and creating backlogs. Improvements in analysis speed, accuracy, precision, and dynamic range are potential targets for accelerating production timelines. Plate-based enzyme-linked immunosorbent assays (ELISAs), commonly used in analysis of viral vector titer, purity, and potency, have laborious and time-consuming manual processing drawbacks as well as long processing times and poor precision. A novel automated microfluidic, compact disc (CD)-based immunoassay format that uses centrifugal force to precisely control the flow of sample and reagents has been notably effective in accelerating bioanalysis of antibody-based therapeutics with high-precision results. One-hour assay run times and wide dynamic ranges accelerate workflows, and 10 µL sample requirements minimizes consumption of limited production material. These dramatic immunoassay improvements are expected to alleviate the analytical delays in viral vector manufacturing.

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Cell and gene therapies have brought the promise of effective, even curative, therapies for acute or chronic genetic diseases where no treatment or only long-term symptomatic treatment exists. Over the past decade, advances in viral vector delivery systems, along with programs facilitating development of gene therapies and immunotherapies, have fanned the flames of an already active field [1,2]. Programs being actively promoted by regulatory bodies such as the Medicines and Healthcare Products Regulatory Agency (MHRA) and the United States Food and Drug Administration (FDA) include expedited development and review, fast-track designation, accelerated approval, and breakthrough therapy designation, all focused on accelerating therapies treating unmet medical

needs. This further availability of shortened development timelines and financial benefits has attracted pharma and biotech companies to invest in the development of genetic therapies with rigorously scheduled development plans.

These incentive programs have also had a noticeable effect on the expansion of the gene therapy pipeline – in just the last 2 years, the number of cell and gene therapies in clinical development has grown from 289 to 362, or a 25% increase [3]. In addition, the number of gene therapy clinical trials is expected to continue its meteoric rise from 775 in 2019 to >4,000 ongoing or completed in 2020 [4], projected to be nearly 11,000 by 2026 [5,6]. Lentivirus (LV) and adeno-associated virus (AAV) vectors are the most successful delivery systems for cell and gene therapies, and recent regulatory approvals utilizing these vectors for generation of chimeric antigen receptor (CAR) T cell cancer immunotherapies include: Tecartus™ (brexucabtagene autoleucel, Kite, a Gilead company) for treatment of mantle cell lymphoma, Yescarta® (axicabtagene ciloleucel, Gilead Sciences, Inc.) for treatment of relapsed or refractory large B-cell lymphoma, and Kymriah® (tisagenlecleucel, Novartis) for treatment of B-cell acute lymphoblastic leukemia.

AAV vectors have risen in popularity for systemic delivery of genetic therapies primarily because of their small size, tissue tropism, and low immunogenicity. Recent AAV approvals include Luxturna® (voretigene neparvovec-rzyl, Spark Therapeutics) for *RPE65* mutation-associated retinal dystrophy, and Zolgensma® (onasemnogene abeparvovec-xioi, AveXis, Inc) for the treatment of pediatric patients with spinal muscular atrophy (SMA).

BREAKING THE MANUFACTURING BOTTLENECKS

The popularity of AAV and LV vectors, along with the recent swell in the development pipeline for cell and gene therapies, has outstripped manufacturing capacity and created backlogs in the bioreactor production, along with compressed development and production timeline pressures [5,7].

Analytical characterization of viral vector identity, potency, purity, safety, and stability during manufacturing can contribute significantly to the overall timeline for vector therapeutic production [8]. Physical titer determination is an integral component of process monitoring from culture growth to downstream purification, and for quality control (QC) testing of product attributes of potency and purity. These assays may be included as critical quality attributes (CQAs) identified by the manufacturer as "a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality" as defined by the FDA [9].

THE NEED FOR IMPROVED ACCURACY, SPEED & LOW VOLUMES

Improvements in analytical techniques, specifically in analysis speed, accuracy, and sample volume consumption, have been identified as a target for helping to meet compressed timeline demands for the production of viral vectors [10–12].

Data quality

As the number of clinical studies for AAV and LV-based gene therapies grows, the FDA increasingly emphasizes the importance of vector titer assay reproducibility and the measurement of full:empty AAV capsid ratios to facilitate dose comparison between clinical programs. In a recent workshop, a target of ≤15% precision for measurement of empty AAV capsids was set as reasonable starting with early phase studies in order to compare clinical study efficacy and adverse events between studies. The discussion during the workshop indicated that improvements in

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the reliability of analytical methods for viral vector titer or a switch to newer technologies may be needed to reach this goal [10,13].

Speed

Compressed timelines for gene therapy manufacturing intensify the need for faster analytical approaches to characterize the therapeutic to verify quality and titer [7]. Many existing methods are time-consuming, producing results long after the time window for adjustment of growth or purification conditions has passed. Long assay times also add to the bottleneck in the development and production of new products [5,8].

Sample volumes

Regulatory demands have increased the number of analyses required for characterization, putting an even higher premium on analytical techniques requiring less sample. While suspension-adapted cultures of HEK293 or insect cell (Sf9) baculovirus expression system cultures facilitate process scale-up, the complex process of 3-plasmid transient transfection (AAV rep and cap genes, adenovirus helper genes, and therapeutic transgene) continues to hinder batch size, batch yields of under 100 mg of virus typical [14]. It was estimated by one CMC specialist that almost half of the viral vector production batch may be consumed during QC bioanalysis steps [10]. Any increases in viral vector production for preclinical or clinical studies will be a noticeable improvement.

MEETING IMMUNOASSAY CHALLENGES WITH (CENTRIFUGAL) FORCE

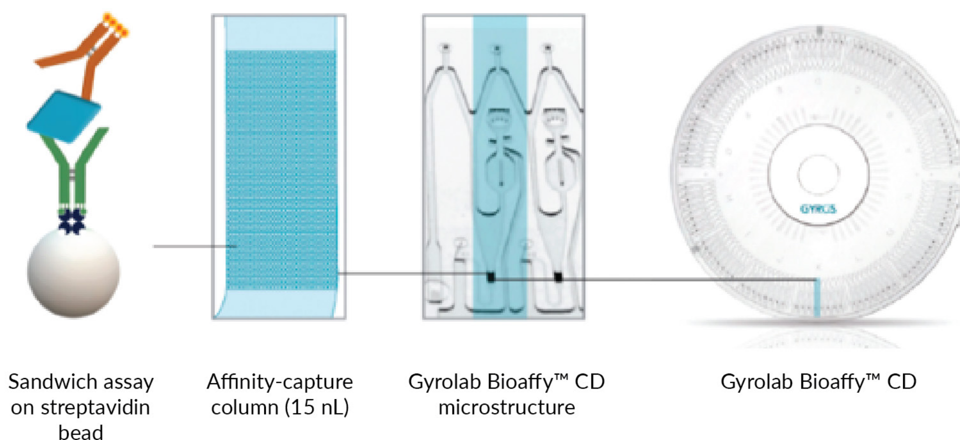
Immunoassays have long been an integral component of preclinical and clinical biotherapeutic development, as a well-established method for detection and quantitation of antibody-based therapies in pharmacokinetic (PK) and anti-drug antibody (ADA) immunogenicity. Immunoassays are also routinely used analytical assays during vector manufacturing and bioprocessing for titer, empty/full capsid ratio, and process impurity analysis [15]. Traditional plate-based immunoassay formats such as ELISAs are fraught with drawbacks of extensive manual manipulations, long incubation times, and high sample and reagent usage all stemming from the assay design – adhering the capture reagent to the bottom of flat well microplates and the addition or removal of assay reagents and wash buffers to or from the top of the well.

Immunoassay technologies that go beyond the microplate look to address these drawbacks. A microfluidic, CD-based immunoassay format that uses centrifugal force to precisely control the flow of sample and reagents to automate assay steps has been notably effective in accelerating bioanalysis of antibody-based therapeutics. The microfluidic technology integrated in Gyrolab® platform (Gyros Protein Technologies) with 96 or 112 flow through streptavidin-coated bead-based affinity columns for parallel assay automation, eliminates the need for lengthy incubation times (Figure 1). Laser-induced fluorescence (LIF) data is collected from each microcolumn during the run, to complete automate immunoassays with data production in about one hour. Control and analysis software designed for 21 CFR Part 11 compliance ensures that assays can be transferred to good laboratory or good manufacturing practice (GLP, GMP) environments.

This automated, microfluidic format with an affinity flow-through column format facilitates high binding capacity, resulting in a large dynamic range and shortens sample-contact time, minimizing assay susceptibility to matrix interference. These substantial improvements in immunoassay speed (4x faster), dynamic range (1-2 log expansion of dynamic range), and sample volume (20x less) are summarized in Table 1.

FIGURE 1

Gyrolab® Bioaffy™ CD-based microfluidic immunoassay design utilizing a 15 nL affinity capture column, streptavidin beads, and microstructures in a circular array for precise, automated liquid movements using centrifugal force.



Parallel processing of Gyrolab CD-based immunoassays on streptavidin beads within the affinity capture column uses centrifugal force and capillary action to precisely control the flow of reagents and samples over the column. On-column laser-induced fluorescence results are read automatically, and results are ready to analyze at the end of the run. The short contact times minimize matrix interference and dramatically shorten assay times.

VIRAL VECTOR TITER ANALYSIS: BREAKING BARRIERS

Total LV vector titer is typically monitored in-process during production and purification steps by quantitation of free p24 antigen, a component of the LV capsid (Figure 2). Advances in immunoassay p24 titer dynamic range, speed, and sample consumption have been made with the availability of Gyrolab p24 Titer Kit. Immunoassays measuring total LV vector titers using Gyrolab CD-based platform have been shown to cover a broad analytical range (0.2–1000 ng/mL), with 96 data points collected in 80 minutes, requiring less than 10 µL of sample. Intra- and inter-run precision run in duplicate in six runs on four instruments by three operators of ≤5.3 %CV [16] demonstrated an extremely robust assay as shown in Table 2.

Quantitation of AAV vectors during downstream processing can be complicated by the presence of empty and partially filled capsids and the need for serotype-specific assays [17]. Several analytical methods are routinely used for vector quantitation, all with distinctive drawbacks. The most widely used, quantitative real-time polymerase chain reaction (qPCR) is hampered by amplification

TABLE 1

Performance of Gyrolab® AAVX capsid titer immunoassay exceeds ELISA performance and suitability for bioprocessing development.

	ELISA	Gyrolab
Sample volume required	100-200 µL	8 µL
Number of hands-on steps	5	1
Total assay time	4 hours	1 hour
Dynamic range	1-2 logs	>3 logs

When compared to ELISA kits, Gyrolab microfluidic immunoassays greatly reduce the sample volumes, hands-on time required, and overall assay time, while expanding the assay dynamic range. These dramatic improvements in assay performance and sample consumption meet the demands for advances in vector titer bioanalysis required by gene therapy compressed production timelines and limitations on batch yields.

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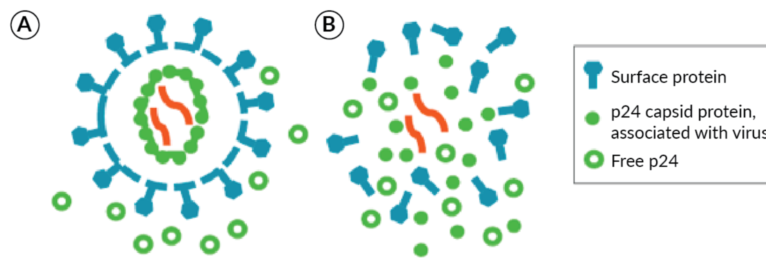
FIGURE 2

Lentiviral titer measurement using p24 immunoassays requires lentiviral particles A) to be disrupted with Triton X-100 detergent B) producing free p24 capsid proteins.



inefficiency, inhibitors, and a standard curve requirement [18] although the increasing adoption of droplet digital PCR (ddPCR) eliminates the standard curve requirement and is less subject to sample inhibition and amplification inefficiency. ELISAs detecting assembled AAV capsid proteins (of various serotypes) are commonly used for total AAV capsid quantitation but have the typical plate-based assay drawbacks of narrow dynamic range, long assay times, and involve many manual interventions.

AAV total capsid titer quantitation using the microfluidic format Gyrolab AAVX Titer Kit immunoassay has been shown to significantly improve data quality over plate-based methods by expanding the dynamic range, from 1.5 logs for a commercially available ELISA AAV2 kit to 3 logs for the Gyrolab AAVX Titer Kit (Figure 3) [19]. Functional aspects of the Gyrolab AAVX Titer Kit were also found to be highly beneficial to analysis workflows: assay time was shortened 4-fold, with one-hour assay times, and the volume required of limited AAV batch samples was shown to be reduced 10-fold to under 10 µL. Matrix-tolerant Gyrolab flow-through immunoassay formats have also demonstrated robustness towards bioprocess samples in host-cell protein characterization [20], AAV titer using commercially available ELISA kit reagents on Gyrolab platform [21], and using the Gyrolab AAVX Titer Kit (customer feedback, data not shown).

TABLE 2

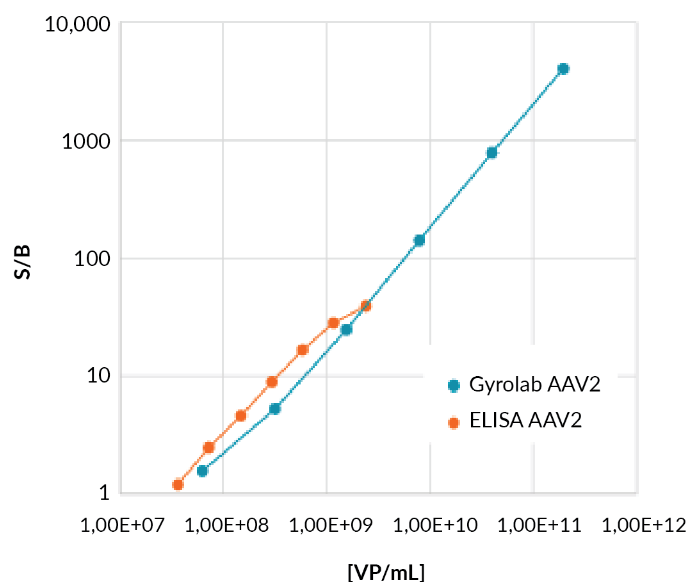
Intra- and inter-run precision for the Gyrolab® p24 Titer Kit standard curve samples.

	Expected conc (ng/mL)	Average measured conc (ng/mL)	Intra-run[1] CV (%)	Inter-run[2] CV (%)
Blank	0			
Standard 1[3]	1250	1250	3.6	3.1
Standard 2	250	251	2.3	1.9
Standard 3	50	50	2.8	2.7
Standard 4	10	10	2.9	2.8
Standard 5	2	2	1.7	2.1
Standard 6	0.4	0.4	2.0	1.8
Standard 7	0.08	0.08	5.0	5.3

Data for standard curve samples over the assay working range were run in duplicate in six runs on four instruments by three operators. (Six duplicate runs were performed on four different instruments, or N=12 per standard concentration). The intra- and inter-run precision was well under 10% (1.7–5.3%), demonstrating an extremely robust assay. The microfluidic design, flow-through affinity column, and automated assay all contribute to the reproducibility of assay results from run to run.
[1]Intra-run CV (%) = standard deviation of response divided by mean response from one run performed in duplicates.
[2]Inter-run CV (%) = standard deviation of means from six runs performed in duplicates divided by mean response for the six runs.
[3]Purified recombinant p24 standards diluted in assay buffer.

FIGURE 3

The dynamic range of AAV2 capsid titer immunoassay is expanded into higher concentration ranges with Gyrolab® AAVX Titer Kit compared to ELISA.



The broad dynamic range of Gyrolab AAV immunoassays reduces the frequency of sample re-runs and dilutions. Expansion of Gyrolab AAV immunoassay ranges to almost 2 logs higher concentrations is especially useful for high-titer AAV batch productions. Gyrolab AAV2 immunoassay was performed per the Gyrolab AAVX Titer Kit instructions. ELISA was performed per kit instructions (PROGEN). Duplicate data points were collected for AAV2 standards (Sirion Biotech GmbH) diluted 1:5 from 2.0E11 VP/mL (Gyrolab) and 1:2 dilutions from 2.4E09 for ELISA. (S/B, signal/background; VP/mL, viral particles per milliliter).

Gyrolab AAVX Titer Kits provide a single reagent set solution for most AAV serotypes, compared to the need for different detection reagents for each serotype in commercially available ELISA kits. Resolving the need to source multiple detection antibodies for different AAV serotypes, the Gyrolab AAVX Titer Kit incorporates Thermo Scientific™ CaptureSelect™ anti-AAVX (ThermoFisher Scientific) ligand that has binding selectivity and affinity for a range of AAV serotypes, with specificity towards assembled capsids. The biotinylated CaptureSelect anti-AAVX ligand as capture reagent and Alexa Fluor® 647-labeled CaptureSelect anti-AAVX as detection reagent was shown to be suitable for quantitation of AAV serotypes 1–8 and rh10 (Figure 4).

Combining qPCR or ddPCR techniques to measure vector genomes with ELISA for total capsid titer to determine AAV full-to-empty capsid ratios has been shown to be an effective and higher throughput method over transmission electron microscopy (TEM) and analytical ultracentrifugation [22] although ELISA has generally been considered to be an imprecise approach and limited to single AAV serotype assays. Gyrolab immunoassays for total capsid titer overcome these limitations can provide complete and rapid quantification of full-to-empty capsid ratios for accurate titer analysis, enabling data-driven decision-making relevant to production and bioprocess timelines.

IMPURITY ANALYSIS WHERE SPEED & DATA QUALITY COUNT

Process-related impurities present in biologics are highly regulated because of their immunogenic potential and associated risks to product safety, efficacy, and quality. Assays for measuring complex heterogeneous mixtures of culture-related host-cell proteins (HCPs) need to be robust and reproducible to meet regulatory

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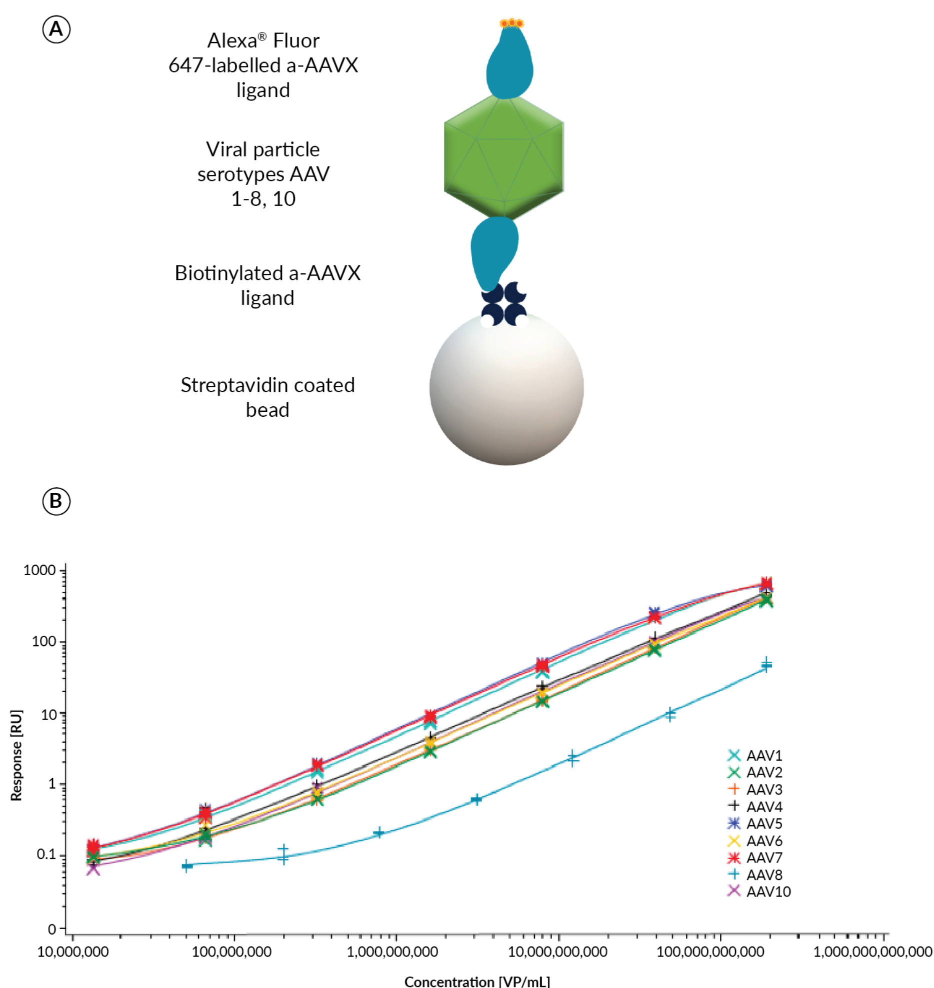
requirements for analysis of samples throughout manufacturing and bioprocessing.

Human embryonic kidney (HEK) 293 or HEK 293T adherent or suspension cell lines often are used to produce AAV and LV vectors for cell and gene therapies and for viral vector vaccines. ELISAs are the most commonly used method for HCP characterization of HEK 293 AAV or LV bioprocess samples but have drawbacks of high assay variability and low productivity. Thus, repeat analyses are common, delaying project decisions and lot approvals. Gyrolab immunoassays in a sandwich assay format using anti-HEK 293 HCP antibodies (Cygnus Technologies) as capture and detection reagents have been developed, delivering data for HCP analysis over a broad working range of 2–10,000 ng/mL. The broad assay range minimizes sample repeats, and the 1-hour assay time allows higher throughput

FIGURE 4

A) Gyrolab® AAVX Titer Kit with one set of capture and detection reagents were used B) for standard curves of AAV serotypes 1–8, and AAVrh10.



One of the challenges of measuring AAV capsid titer using ligand-binding assays has been the need for specialized detection reagents directed towards individual serotypes, and the difficulty in sourcing and evaluating these reagents for use. A) The Thermo Scientific™ CaptureSelect™ anti-AAVX (ThermoFisher Scientific) ligand incorporated into a sandwich immunoassay format as both capture and detection reagent provides binding and detection of 9 AAV serotypes (AAV1-8 and AAVrh10) in a single assay format with one set of reagents. B) Standard curves using commercially sourced AAV standards (Sirion Biotech GmbH) diluted in buffer for serotypes AAV1-8 and AAVrh10 demonstrated a 3.5 log dynamic range. Gyrolab AAV titer immunoassays were performed using the Gyrolab AAVX Titer Kit with duplicate data points according to the kit instructions.

analyses to keep up with the large number of samples generated during bioprocessing, with up to 960 datapoints/day.

SUMMARY

Improvements in bioanalysis approaches are urgently needed to meet the demands of compressed development timelines for gene therapies and backlogs in manufacturing pipelines. Immunoassays, traditionally using plate-based methods, are an attractive target for improvements in assay time, dynamic range, and sample volume requirements. Significant advances in these areas have been made by the microfluidic, nanoliter-scale Gyrolab immunoassay platform, producing assay data in one hour with a wide dynamic range, and consuming under 10 µL of sample. These dramatic immunoassay improvements are expected to alleviate the bioanalysis timeline delays in viral vector manufacturing.

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AUTHORSHIP & CONFLICT OF INTEREST

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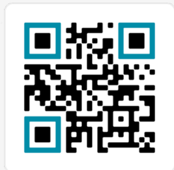
Faster Results - More Data - Less Sample

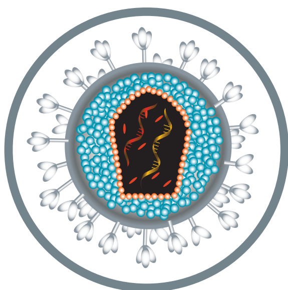
Gyrolab® Immunoassay Solutions for Cell and Gene Therapy Development



Gyrolab® AAVX Titer Kit

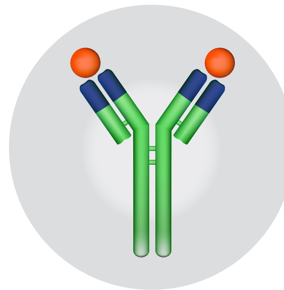
Miniaturized and automated immunoassays for viral vector bioanalysis

Gyrolab immunoassays deliver more data using 10x less sample in about an hour. Automated, microfluidic immunoassay formats increase assay dynamic ranges, improve matrix tolerance, and eliminate incubations and manual interventions that are common in plate-based assays. Gyrolab kits to measure AAV and lentiviral vector titer provide time-relevant data for process changes during vector bioprocess steps.



AAV and Lentiviral Titer Determination

Bioprocessing Impurity Analysis



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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Exploring the capabilities of a versatile, novel, automated closed system for cell and gene therapy manufacturing

Sarah Daoudi & Premkumar Jayaraman

As more cell and gene therapies move toward clinical trials, and into commercialization, new trends and challenges are emerging. Technologies and processes are rapidly evolving, and it can be challenging for manufacturers to select the best tools for their unique needs. Focusing on cell therapy manufacture in particular, there is a lack of specific equipment and products, and as such the resulting manufacturing workflows can be highly labor-intensive, often involving open processes and manual manipulations. Closed manufacturing systems, in combination with digital connectivity, can offer a solution to some of these challenges, as these systems enable repeatable, trackable, and GMP-compliant manufacturing processes. This article will discuss the benefits of moving towards modular, closed-system technologies designed for scalable and cost-effective manufacturing, with a focus on the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System – a revolutionary closed benchtop system which offers exceptional flexibility for cell washing, concentration, and separation by size.

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CHOOSING THE RIGHT TOOLS FOR THE JOB

Current challenges facing the cell and gene therapy industry include the management of supply chains and logistics, the redundancy of cGMP manufacturing, the adoption of advanced analytics tools and processes, and ensuring quality control (QC) throughout the entire process. Ensuring regulatory compliance, having the right documentation and

support, as well as clinical trial risk management, are also critical factors. When it comes to speed to market, there is a need for consistency in both quality and performance, as well as a consideration of the significant costs associated with bioprocessing scale-up and scale-out.

Although the cell and gene therapy market is dynamic and rapidly evolving, in some areas, such as autologous T-cell therapy, there is currently a lack of cell therapy-specific equipment and products. Cell therapy manufacturing is also highly labor-intensive, with many manual manipulations that introduce an increased risk of error. Many of the processes utilized are open and involve a variety of different types of bottles, tubes, and biosafety cabinets, which are potentially hazardous to the end product. There is currently no in-line monitoring, and QC release testing is a lengthy and expensive process which can be responsible for almost 30% of total costs in the manufacturing process. With multiple vendors and tools available, selecting the best option can prove complex and time-consuming.

THE GIBCO™ CTS™ ROTEA™ COUNTERFLOW CENTRIFUGATION SYSTEM FOR CLOSED & AUTOMATED MANUFACTURING

The Gibco™ CTS™ Rotea™ Counterflow Centrifugation System is a closed cell processing system offering exceptional cell recovery, flexible input and output volume capability, and high throughput rates, making it ideal for cell separation, concentration, and washing **(Figure 1)**. With a compact footprint and process flexibility, the system can scale from research through to commercial manufacturing of cell therapy products. The system is comprised of a compact, multipurpose instrument, a sterile single use-kit with multiple input and output ports, and a fully user-programmable interface.

The CTS™ Rotea™ instrument is manufactured according to ISO 13485, and is not considered to be a medical device. The single-use kit has a regulatory support file available, and the kit is also manufactured under ISO 13485, in an ISO 7 grade cleanroom. The software component of the system is able to communicate using OPC-UA and can be connected to the user's 21 CFR Part 11 compliant system. Different user levels can be assigned for manufacturing as admin, basic or full, and the software can also collect and manage batch record data.

It is also a modular system, so once a process is completed, the system is free to begin the next step. Another critical consideration in closed-system cell processing is the tubing compatibility of single-use bags and GMP-grade ancillary reagents for cell culture. Two cell therapy system products have also been launched, a CTS DPBS and CTS AIM V media in 2 liter bag formats, with the option to customize different cell volumes from 100 mL to 10 liters. These bags are intuitively designed for aseptically connecting to different single-use cell processing devices.

The Rotea™ instrument

As seen in **Figure 2**, the Rotea™ instrument utilizes counterflow centrifugation: cells enter the chamber through a narrow tube at a certain flowrate, and this high fluid entry velocity ensures that the cell exits the tip of the tube into the chamber. At the same time, the chamber will spin at an opposing g-force, preventing the cells from exiting the chamber. When the flowrate and g-force are in equilibrium, a fluidized bed will form in the chamber. At this stage media exchange, cell washing, and cell separation by size and concentration can be performed. The gentle fluidized bed supports low-shear processing, enabling over 95% cell recovery while maintaining cell viability.

The conical shape of the chamber increases the fluid velocity, and forces smaller cells out through the tip of the chamber first. Using the system software, the user can adjust the g-force and flowrate settings to separate

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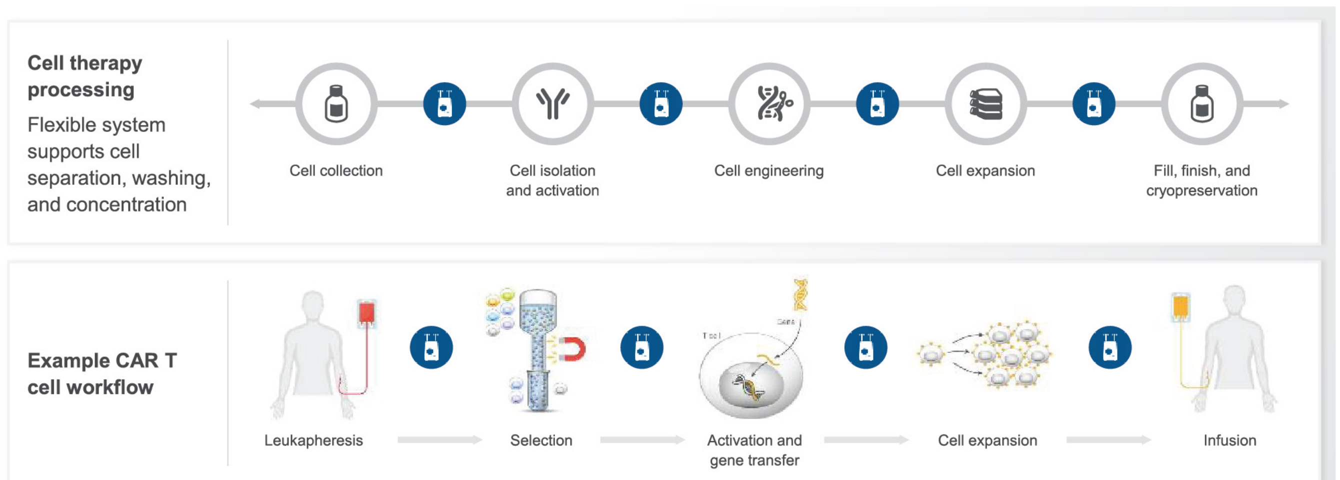
FIGURE 1

Common applications of the Rotea system.



The CTS™ Rotea™ Counterflow Centrifugation System is a highly versatile tool that is suitable for washing and concentration of cells between cell therapy processing steps.

smaller cells, in a process known as elutriation. For example, when loading in a leukopak, tiny platelets will leave the chamber while peripheral blood mononuclear cells (PBMCs) are retained inside. Therefore platelet depletion can be achieved simply by loading the sample, in a single step. The elutriation process can achieve higher purity of large cells or monocytes, and be used to collect smaller cells such as lymphocytes.

Retaining cell quality & composition

The system enables high recovery and viability, and output volumes of as little as 5 mL of concentrate. As seen in **Figure 3**, an average viability of 96% and a recovery of 94% was achieved in T-cell washing and concentration. When inputting a larger quantity of cells, a low output, along with high viability and recovery, can be achieved. Finally, the CTS™ Rotea™ system can achieve approximately 89% PBMC recovery from a leukopak, using cell lysis buffer across multiple different donors.

In regards to T-cell quality and composition, the Rotea does not impact cell population pre-and post-processing. When looking at $CD4^+$ and $CD8^+$, T-cell populations are comparable, demonstrating that the instrument can retain the quality and composition of the initial input.

THE ROTEA SYSTEM'S CELL PROCESSING CAPABILITIES: CASE STUDIES

The CTS™ Rotea™ is a highly flexible system that can be integrated into multiple aspects of the manufacturing process. The following case studies demonstrate the Rotea system's capabilities in cell isolation, cell selection by size, small to medium to large-scale cell processing, cryopreserved cell wash and medium exchange, formulation for cryopreservation, RBC lysis and depletion, and viral vector clarification.

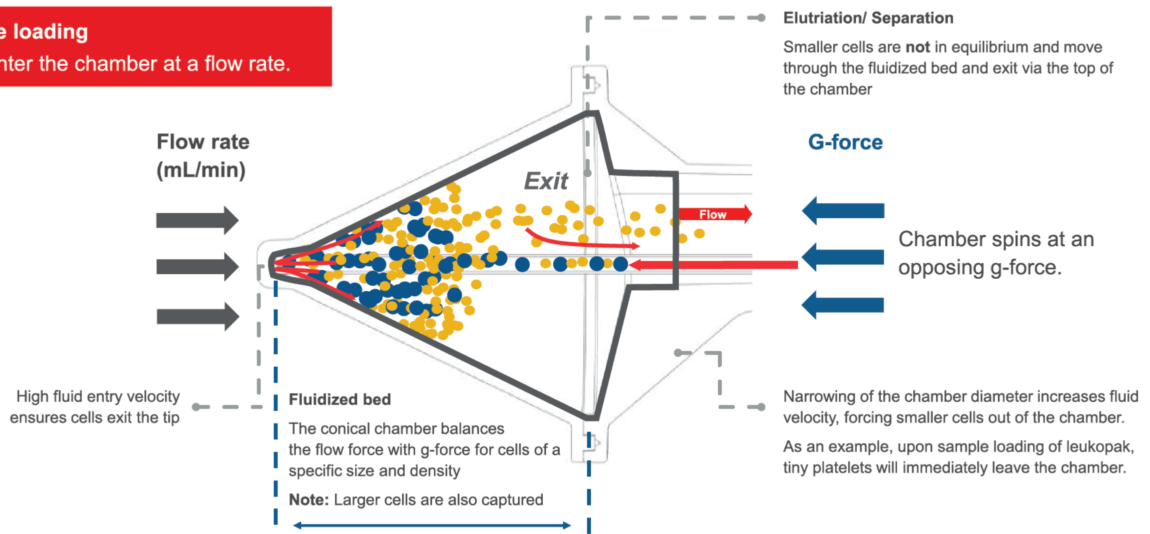
Case study: T-cell therapy workflow

PBMC isolation

In the T-cell therapy workflow, the first step in the entire vein-to-vein process is PBMC isolation from a leukopak or apheresis sample. Typically, the Rotea system can be used to isolate PBMCs by lysing away the red blood cell (RBC) from the chamber, without the

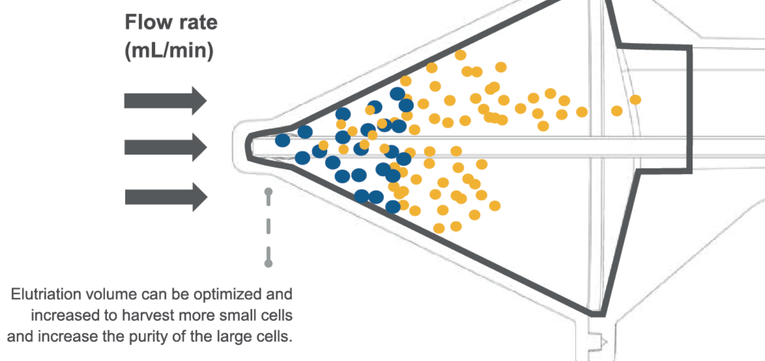
FIGURE 2

How counterflow centrifugation works.



Flow rate and g-force settings can be adjusted to elutriate and separate smaller cells.

In this example, g-force is lowered. At the same flow rate, the larger cells stay within the chamber while small cells are elutriated.



need for Ficoll separation. More importantly, Rotea's counterflow centrifugation technology allows separation of monocyte and lymphocyte populations after PBMC isolation. In addition, RBC elutriation can be performed.

On loading a leukopak into the Rotea, the plasma and platelets are readily washed away into the waste bag due to their smaller sizes. Following the sample loading step, lysis buffer is introduced into the chamber to perform in-chamber lysis, followed by wash and removal of lysed RBC debris.

The Rotea can drastically reduce the platelets and RBCs present, therefore increasing the percentage of white blood cells, including T-cells and other cell populations.

In this case study, a single donor leukopak was split into two, and the efficiency of PBMC isolation using manual Ficoll protocols versus a closed automated Rotea separation was compared.

As shown in Figure 4, the Rotea can isolate PBMCs in a closed process within less than 30 minutes, with equal end performance to Ficoll separation. The data also demonstrates that the RBC lysis process in the Rotea does not have any impact on the cell population recovery or viability of the cell.

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Separation of lymphocytes & monocytes

Following PBMC isolation using RBC lysis in the Rotea system, the lymphocyte and monocyte fractions were separated by optimizing g-force and flowrate. A fresh leukopak sample was used, and in-chamber lysis was performed, followed by monocyte and lymphocyte separation.

Before RBC lysis, the fresh leukopak sample had a total of 3 billion white blood cells as input, with approximately 22% monocyte and 55% lymphocytes. Following the in-chamber RBC lysis, the total PBMCs of the leukocyte fraction isolation was more than 90%, and over 67% of the monocytes were separated in the monocyte harvest fraction.

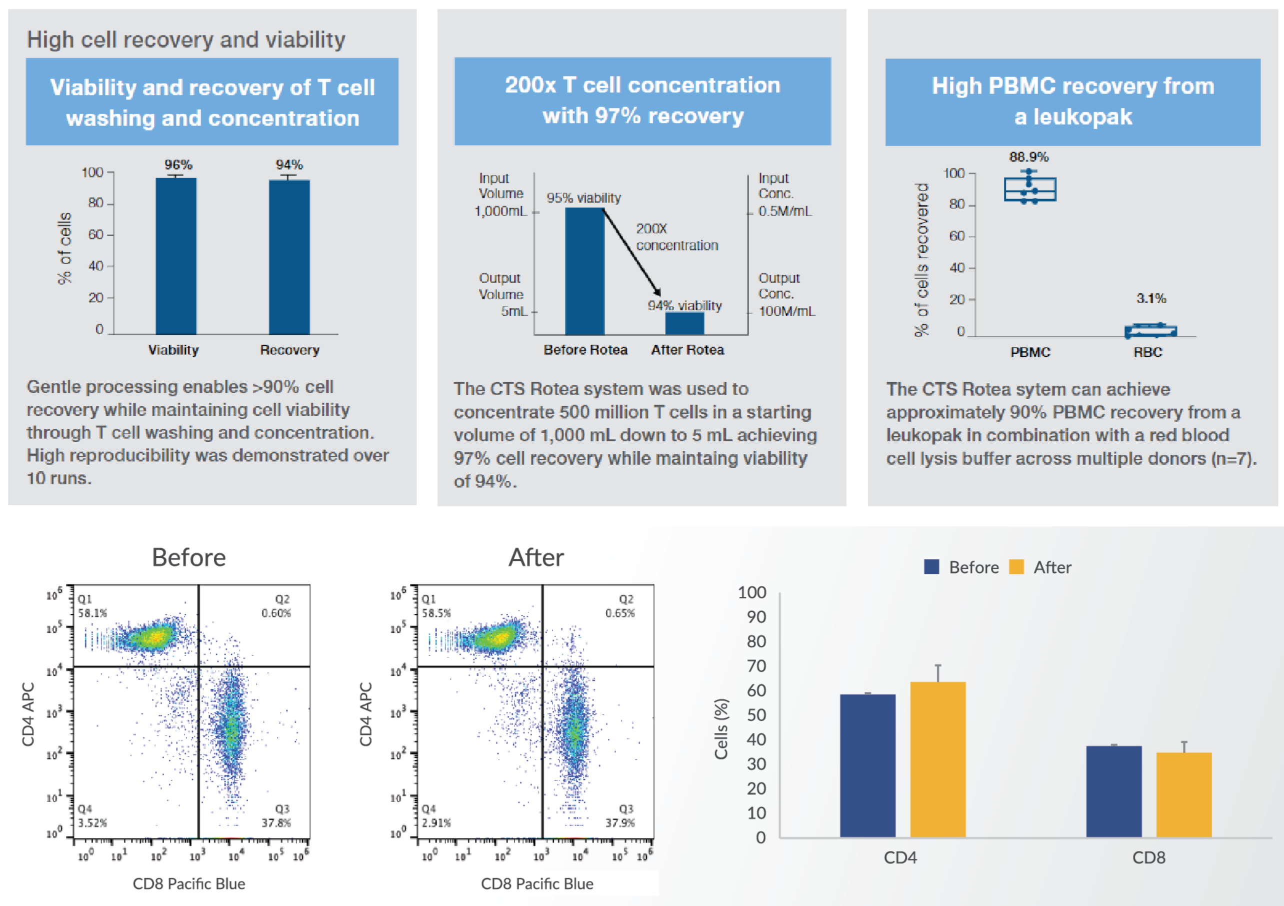
Depending on the starting donor's PBMC composition, the separation efficiency will be varied. However, by adjusting the g-force and the flowrate setting, based on Rotea's inbuilt protocols and process model feature, it is possible to optimize the maximum enrichment for each of the cell populations.

RBC elutriation

Another key capability of counterflow centrifugation is the ability to separate cells and particles by sight. RBCs tend to be dense and varying in size, therefore some RBCs will overlap in size with the lymphocyte fraction,

FIGURE 3

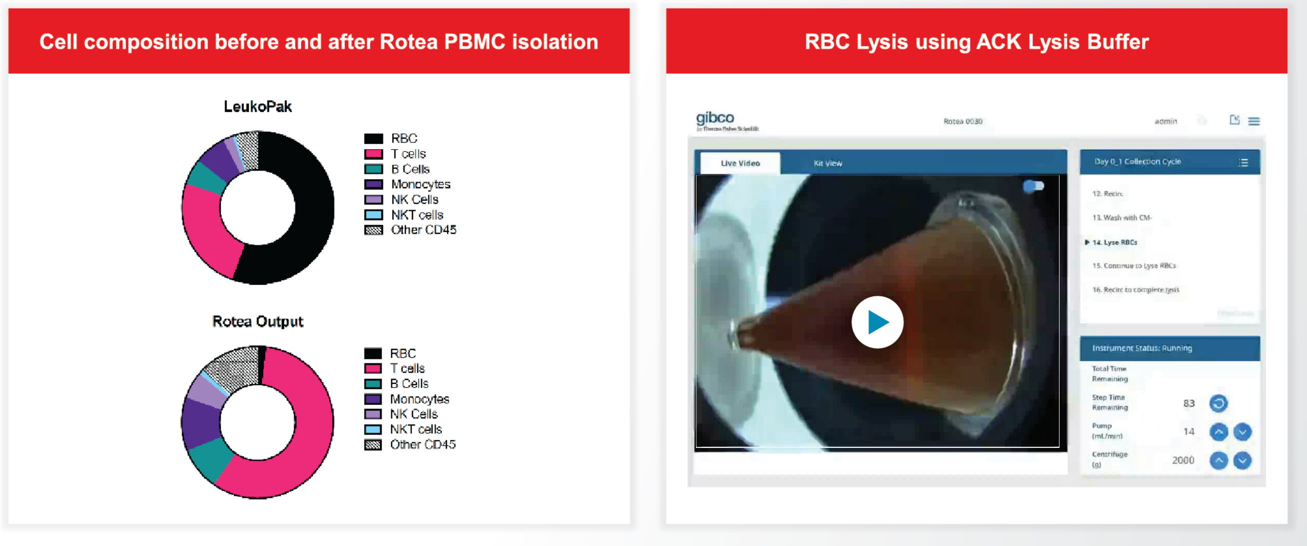
The Rotea system enables high recovery and viability without impacting the cell population.



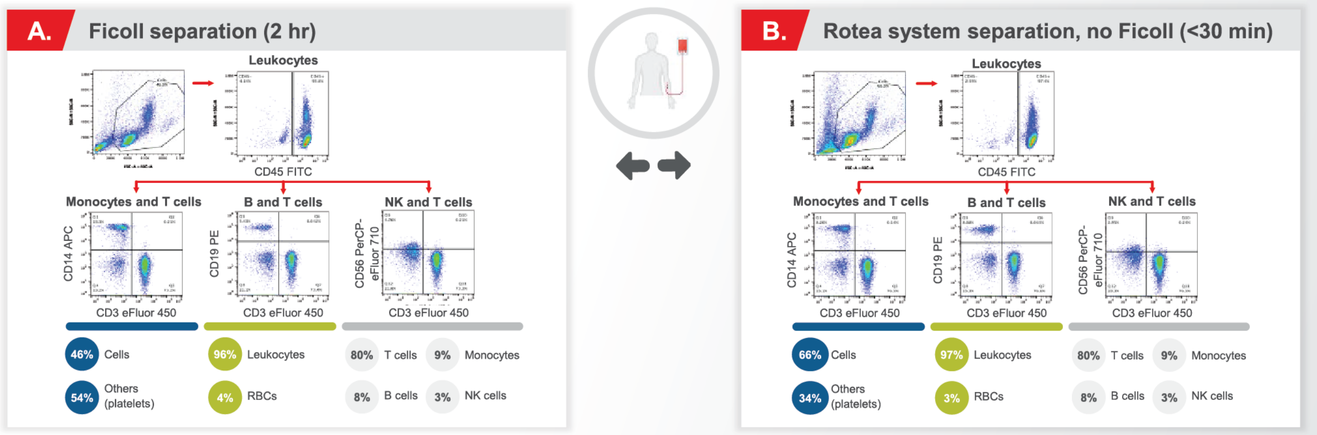
Flow cytometry data showed that CD4⁺ CD8⁺ T cell composition within CD3⁺ total T cells remains the same after processing with the CTS Rotea system.

FIGURE 4

The Rotea system can isolate PBMCs in a closed process within less than 30 minutes, with equivalent performance to Ficoll.



Experimental setup: Single-donor leukopak was split into two, and PBMCs were separated using (A) Ficoll polymer or (B) CTS Rotea system without Ficoll polymer.



and cannot be elutriated without using the RBC lysis process. However, a good proportion of smaller RBCs can still be depleted by the elutriation process alone.

An RBC elutriation was performed using a mobilized peripheral blood apheresis sample (Figure 5). As can be seen in the image on the left of Figure 6, RBCs are elutriated into the waste bag by washing the chamber with approximately 150 mL of the buffer. The RBC elutriation can be visualized on line A, which is red in color where the RBCs are exiting the chamber into the waste bag.

The image on the right of Figure 6 shows elutriation line A clearing after washing the chamber with the buffer. In addition to the 3-fold depletion of RBCs, cell viability of more than 95% was achieved after the process was completed.

Post-expansion processing

One of the last and most critical steps in the T-cell therapy workflow is to harvest the expanded cells, and formulate in a cryopreservation medium.

The image in Figure 6 shows the expanded T-cells accumulated in the chamber, which has a maximum capacity of around 5 billion non-modified T-cells. For users who wish to

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process over 5 billion T-cells, the Rotea can continuously load and harvest cells from the chamber multiple times, until the entire batch is processed.

The data on the right of Figure 6 demonstrates that PBMCs isolated from the Rotea instrument have comparable expansion rates and phenotypic changes as T-cells processed from a manual Ficoll-based separation.

Case study: natural killer (NK) cell wash & concentrate

Similar to T-cells, the system can also wash and concentrate freshly-expanded NK cells, using a propriety NK expansion prototype media. Almost 650 million NK cells were loaded into the chamber, and pre-and post-Rotea viability did not shift, remaining at around 97%. The total recovery was around 84% during an initial run, without any optimization performed. The Rotea was able to deplete debris and small-sized dead cells, providing consistently high cell viability and recovery.

In addition to the purity of NK cells measured by $CD56^{+}$, the cells showed a similar range of about 82% for both pre and post-Rotea functions. This data makes it clear that when working with highly sensitive cells such as T and NK cells, the Rotea is gentle and retains the cell population well.

Case study: iPSC spheroid processing

The Rotea instrument also has the capability to process cellular spheroids and aggregates without disruption to their morphology. The goal for this study was to separate a heterogenous iPSC spheroid population of around 40–200 µmin size from a polymer of roughly 20 µm.

The iPSC spheroids were captured in the chamber because of their larger size, while the smaller polymers were washed away from the chamber using the unique counterflow centrifugation technology. Different g-force and flowrate settings were used to optimize conditions and obtain high cell recovery and viability. At a g-force of 350xg and a flow rate of 40 mL per minute, more than 86% cell recovery was achieved, with the morphology of iPSC spheroids intact and healthy.

Case study: cryopreserved hMSCs: wash & formulate

FIGURE 5

Visualization of RBC elutriation.

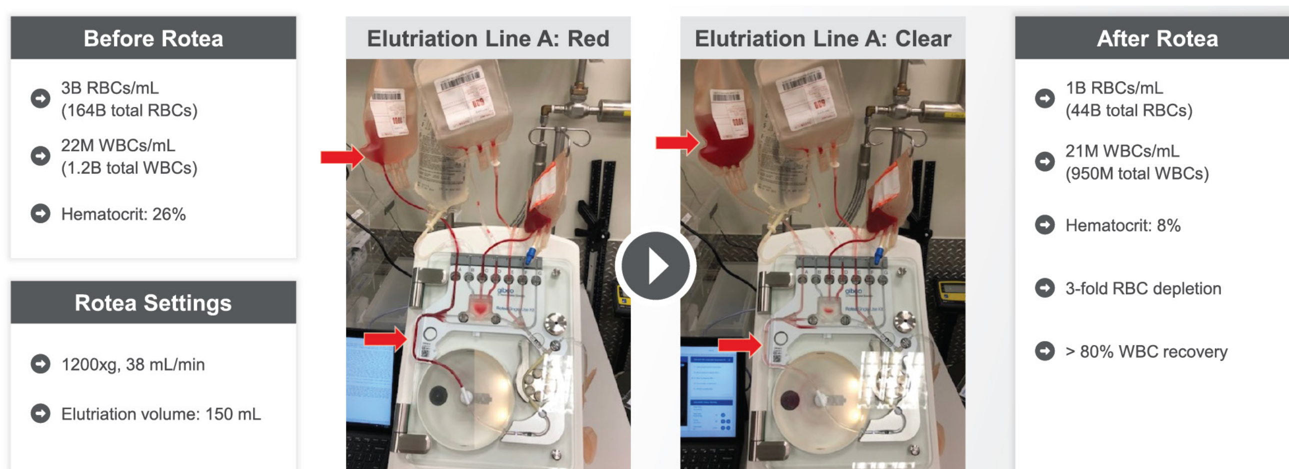
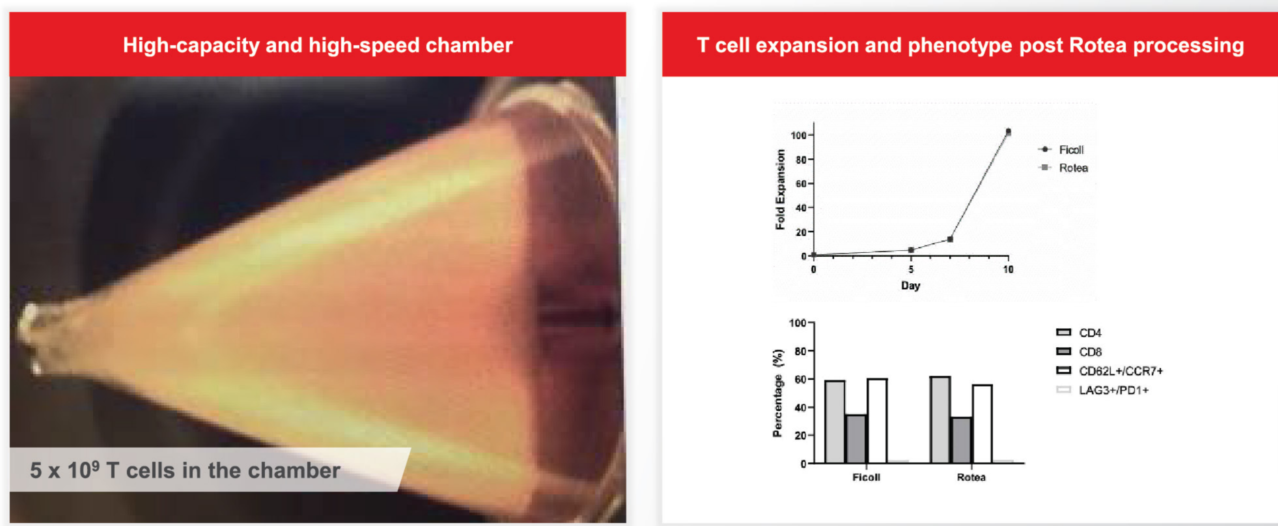


FIGURE 6

The Rotea drastically reduced platelets and red blood cells and increased the % of T-cells.



After processing, the PBMC isolated from Rotea has nearly the same expansion rate and phenotypes as compared to cells from traditional Ficoll separation.

An experiment was carried out for washing cryopreserved patient-derived human mesenchymal stem cells (hMSC) with a lactate ringer's solution, and formulation with injectable saline. Up to 100 million cells were loaded into the chamber, and post-thaw viability was around 90%. Manual centrifugation achieved 50% recovery, whereas the Rotea managed to achieve an average of 75% recovery, and more than 80% cell viability. It should be noted that this experiment was performed with patient-derived MSCs, which could be more sensitive to thaw and cell washes. Typically, more than 95% cell viability and recovery of freshly expanded cells are achieved.

Additionally, with one-time dilution of cells and one-time wash, residual bovine serum albumin (BSA) was reduced by more than 200-fold. With one dilution and two washes, this was increased to more than 3,000-fold.

Case study: lentiviral vector downstream processing

The Rotea system can also be flexibly incorporated into viral vector manufacturing. In upstream processing, the Rotea can be used for continuous bioprocessing and scale-up. During downstream processing, the system can be used for harvest and viral vector clarification.

This case study involved an experiment for lentiviral vector (LV) clarification of high density, suspension-adapted HEK293 cells. Around 300 million viral production cells were loaded in the chamber, forming a fluidized bed, and the lentivirus-containing supernatant was collected in a bag for concentration and purification. The titration flow data of the clarified supernatant demonstrated comparable or slightly better transduction efficiency of around 42%, compared to about 40% using a manual centrifugation method.

PROCESS FLEXIBILITY FOR CELL THERAPY

The Rotea process is highly flexible, and offers a multipurpose system for hMSC therapy manufacturing. It can be introduced into the MSC manufacturing workflow, aided by either a 2D monolayer or 3D microcarrier-based suspension culture.

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Starting from a working cell bank, the Rotea system can be used to thaw cryopreserved MSCs, wash media, exchange with fresh media, and transfer into a cell factory or bioreactor directly for expansion.

The Rotea can be used for volume reduction, and washing and concentrating harvested cells, either for further passaging or for formulation for cryopreservation. Specifically for microcarrier-based cultures, the Harvestainer™, a microcarrier separation system, can be utilized upstream of the Rotea to separate the cells from microcarriers.

The Rotea can also be used for processing small, medium and large-scale cultures up to 20 liters, but depending on the robustness of the cells, the instrument can also be used as a continuous cell processing device by harvesting directly from single-use expansion systems.

Small-scale process optimization typically involves processing cultures of less than half a liter, with the harvest line directly connected with syringes for easier evaluation of cell counts. In some cases during process optimization, it is possible to have sampling ports on the recirculation line to quickly evaluate the Rotea's performance. For processing medium or large-scale cultures, the Rotea can be connected directly to cell factories or bioreactors, or the cells can be transferred to larger bags which can then be attached for processing.

CONCLUSION/INSIGHT

The Gibco™ CTS™ Rotea™ Counterflow Centrifugation System is a versatile instrument with multiple applications, and offers a scalable, flexible tool that can be applied across a range of cell types and viral vectors used in cell and gene therapy manufacturing. By improving throughput and robustness of key steps in the cell and gene therapy workflow, the Rotea system can help manufacturers streamline their transition to GMP manufacture in a cost, time, and space-efficient manner.

Q & A

Sarah Daoudi
Field Application Scientist, Cell and Gene Therapy (North America West), Thermo Fisher Scientific

Premkumar Jayaraman
Regional Field Applications Scientist, Cell and Gene Therapy (Asia Pacific and Japan), Thermo Fisher Scientific

Q Can the Rotea be used in both autologous and allogeneic cell therapy development?

SD: The Rotea is optimal for autologous cell therapy applications. It can be used for allogeneic processes to an extent, but it is optimal for more autologous applications. One of

the key reasons for this is that allogeneic development has such high concentrations and high volumes.

Q Does the Rotea support other cell types and applications not covered in your presentation today?

PJ: I have covered most of the cell types and applications that are currently being used in the cell and gene therapy manufacturing workflow. However, the opportunities are currently limitless, and the list of applications are growing. We are partnering with many cell therapy developers and their innovative products.

Based on the inputs we obtain, we prepare for a demo. Our team of application scientists can go in and help to support and develop optimized protocols to transfer the existing manual workflow protocol into an automated closed processing system using a Rotea. We currently have over 10 existing standard protocols for processing a variety of cell types, and applications in each step of the cell therapy process.

Q The device is rated for research use only. How could the device be used in a commercial setting?

SD: We do have companies that do work in a commercial setting with the Rotea. It can be used for isolation of PBMCs instead of using the manual Ficoll process; so more larger scale.

It can be used with cell factory systems, again for a larger scale. So it started in research, however we do have individuals using it in large GMP facilities, as well as using it in a more commercial setting. It is completely versatile for upscaling as well.

Q Are there currently any published documents citing the Rotea in terms of commercial manufacturing or clinical trial use?

PJ: We just launched the product last October, but we have given early access to some adopters. Currently, we have more than 60 adopters globally, and there is growing interest in the market to adopt Rotea into the existing workflow because of the versatility and modularity.

Currently our customers span across translational research institutes, early clinical phase biotech companies, and big biopharmas who are in the process development and early commercialization stages. So naturally, the data being generated using Rotea for their INDs is not publicly disclosed.

However, we have a publication from our early adopter, Dr Rebecca Lim from the Hudson Institute of Medical Research. This was published in late 2019 as a peer reviewed journal article in [Jove bioengineering. This gives a look at what the features are, and the overall workflow that has been shown for MSC processing.

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Q Is the Rotea compatible when working with input volumes larger than 20 liters?

SD: We do get questions about volume quite often. The system is completely designed around concentration of cells, rather than input volume. So if you have 20 liters and for example, only about 5 billion cells in your 20 liter process, it is able to process volumes that are even larger than that.

20 liters is something that is possible within the instrument. If there are volumes that are a lot larger than that the system can be looped, in order to process multiple batches at the same time using the same instrument and the same kit.

Q Following on from that, is there a realistic expectation that the Rotea could effectively be scaled up to support a larger volume production of 100+ liters?

PJ: What we typically recommend for the Rotea is a maximum of a 20 liter volume. But again, it is a very flexible instrument, and you can run it in loops and multiple batches to discharge the harvest and then reload the cells and process.

It really depends on how robust your cells are, and how long the process can be done for. We have done demonstrations of around 20 liters and typically it will take around 2–3 hours to process cell types. This depends on what the cell types are, and how robust they are, so we can optimize the process for larger volumes.

Q What kind of regulatory support documentation do you have for the Rotea, and the single-use kit?

PJ: The single-use kits have a certificate of analysis by lot numbers. They are gamma sterilized and ISO 13485 compliant. We provide particulate testing, and we have done up to 20% DMSO for cryopreservation as well.

We provide extensive validation and qualification testing for the single-use kit. For the instrument itself, it is again manufactured under the same ISO 13485. It is not considered a medical device, and it is specifically designed for cell and gene therapy manufacturing, starting from research up to commercial production.

If you look into the software itself, it has been enabled by an OPC UA interface, which would allow you to connect to a third-party or users' own 21 CFR part 11 compliant system.

With the purchase of Rotea, customers have access to a very in-depth regulatory support file, the master file, and the validation guide to streamline their workflow qualification.

Q What is the approximate process time to go from 1000mL to 5mL with the Rotea?

PJ: It depends on the density of the medium you are working with, and the number of cells you are processing.

Typically, the maximum flowrate that the Rotea can process for a standard kit is 110 mL per minute, and the maximum g-force it can go up to is 3,000 xg. These are very high settings. Typically we look for a very conservative setting of running at 2,600 xg and 30 to 40 mL per minute. At 40 mL per minute, it would take you around 20–25 minutes to process and harvest the final sample.

Q Is there then a maximum number of cells that can be processed in at one time?

PJ: The chamber volume is 10mL, and it can fit in up to 5 billion smaller T-cells, so the size of the cells plays a role.

For example, if you are using adult stem cells like MSCs, typically 16–30 micron in size, those are bigger cells so it can only fit in 2–3 billion cells per run. But again, it can be run in a loop, so you can take in 2 to 3 billion cells, harvest out, take in the next batch to load in, and then do a multiple discharge.

Q Are there any future steps planned to make the Rotea suitable for later stage and commercial processes?

PJ: The system itself is designed for process development in a research lab setting. Once you optimize, then you transfer the protocol into a commercial process.

It is suitable for GMP manufacturing. The protocol builder has a unique feature where you can optimize the settings while you are doing the process optimization. Once you are done and you are ready to move into commercial manufacturing, the software can allow you to lock the protocols and allow individual users to be given access. You then cannot modify the protocols, so this qualifies into the GMP manufacturing, along with the other regulatory documentation we provide.

Q You mentioned having flexibility in terms of volume for the output sample, but can you also prepare the sample at a specific concentration?

SD: There is flexibility when it comes to volume. However when it comes to specific concentration, that is really going to depend on the cell type, as well as the viability.

For example, our recoveries right now are over 95%. Let's say you know that you are going to have a 95% recovery, and you know the concentration of mLs you want to a specific concentration. You can actually program the software to provide an output based on the specific concentration you want.

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So if you know you have 100 million cells and you want it in 5 mLs, you can just output 5 mLs of the cells, and you would have that specific concentration that you wanted.

However there is no calculation internally that specifies the concentration – you would have to put in what the output volume is based on doing your own calculation.

Q How involved is the support for developing a process, and can you help design the process used for specific user cells?

SD: Yes – the process is relatively involved. We have field application scientists such as myself and Prem, as well as an entire global support team that can help with designing a program.

Once a customer purchases an instrument, we go in and help create new processes and protocols that are compatible with people's cells.

However, it does go back to the customer as well for them to do their own process development. We can only support as much as we can, but we are here throughout the entire process. You are never really left to design things on your own; we try and help as much as possible.

Q Have you tried using the Rotea system for different AAV serotypes?

PJ: What Rotea can specifically do for viral vector manufacturing is essentially do upstream processing, where you can thaw your cryopreserved cells in a closed manner, and put them into a bioreactor for expansion, and then you can do a perfusion-based transfer, which we are currently evaluating to improve your expansion process.

After that, once you have done your transfection, you can use the Rotea to separate your cells from the supernatant. For the lentiviral process, the supernatant contains the virus, so you use Rotea to pull the cells from the chamber and wash away the supernatant into the bag so you can take it to concentrate.

For the AAV workflow, typically you need the cells because the cells have the virus. So the clarification process can be done in Rotea, and you can take the cells and perform the lysis for the downstream process.

BIOGRAPHIES & AFFILIATIONS

Sarah Daoudi
Field Application Scientist, Cell and Gene Therapy (North America West), Thermo Fisher Scientific

Sarah Daoudi is a Field Application Scientist that provides solutions and consultation for Thermo Fisher Scientific's cell and gene therapy workflows and Gibco Cell Therapy Systems (CTS) brand of products. Sarah has expertise in CAR-T workflows, manufacturing, viral vector production (lentivirus and adeno-associated viruses), and drug development. Formerly a Scientist II at Thermo Fisher Scientific, she helped launch the CTS Rotea System by producing application data, writing and developing new protocols, as well as extensive troubleshooting and customer support. In her

previous role as Process Development Research Associate at City of Hope (Duarte, California), Sarah oversaw and worked on improving, troubleshooting, and development of Phase I CAR-T cell therapeutic drugs and workflows for over ten different IRBs. Sarah received her master's degree in Cell and Molecular Biology from California State University, Fullerton (California).

Premkumar Jayaraman
Regional Field Applications Scientist, Cell and Gene Therapy (Asia Pacific and Japan), Thermo Fisher Scientific

Dr Premkumar Jayaraman currently provides consultation and training for Thermo Fisher Scientific's cell and gene therapy solutions such as the Gibco Cell Therapy Systems (CTS) brand of products. Prem has over 7 years of expertise in synthetic biology utilizing gene-editing technologies, stem cell bioprocessing (pluripotent and adult stem cells), and viral vector production (lentivirus and adeno-associated viruses). In his previous role as a Research Scientist at the Bioprocessing Technology Institute (A*STAR, Singapore), Prem oversaw a variety of cell and gene therapy projects, from media optimization to upstream process development. Prem received his doctoral degree in Biotechnology from Nanyang Technological University (Singapore).

AUTHORSHIP & CONFLICT OF INTEREST

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FASTFACTS

Streamlining at-line and in-line concentration measurements with the Variable Pathlength Technology approach

Cell & Gene Therapy Insights 2021; 7(2), 170

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Traditional UV spectroscopy is used for multiple applications in pharmaceutical analysis, but poses a range of challenges to manufacturers. Slope Spectroscopy® enabled by Variable Pathlength Technology allows for automatic and fine control of pathlength resulting in an unparalleled linear range for the system, providing robust and reliable analysis without the need for manual manipulation of samples.

TRADITIONAL UV SPECTROSCOPY: THE CHALLENGES

Traditional UV spectroscopy utilizes a standard UV-Vis spectrophotometer that uses a 1 cm fixed pathlength. When analyzing samples that are out of the concentration range of the spectrophotometer, it is necessary to dilute and/or treat the sample in order to obtain a reliable reading. Careful manipulation of a sample can take from 30 minutes up to 3 hours and increases the risk of error in the final measurement.

REVOLUTIONIZING UV-VIS ANALYSIS USING VARIABLE PATHLENGTH TECHNOLOGY

In contrast to traditional UV spectroscopy, the SoloVPE and FlowVPE systems from C Technologies utilize Variable Pathlength Technology (VPT). Using this approach, the sample does not need to be diluted or treated, and instead the pathlength is varied with a precision linear stage. This removes the need to dilute a sample, measure it, then plot and calculate the results. The measurement is automated by computer controlled linear stage that automatically reads the sample and establishes the proper linear range. An R-squared output is also provided to ensure linearity of measurement.

Like traditional spectroscopy, Slope Spectroscopy is based on the Beer-Lambert law, which is expressed as:

$A = \varepsilon l c$

Where " A " is the measured absorbance, "ε" is the wavelength dependent molar absorption

Coefficient (extinction coefficient), "*l*" is the pathlength, and "*c*" is the sample concentration.

Unlike in traditional spectroscopy where the concentration is used as the variable, the pathlength is instead used, allowing the concentration to remain constant and eliminating the need for dilution. Multiple pathlengths are used during the measurement in order to quantify the concentration with high accuracy, rather than a single datapoint that could be lost due to error – or even due to a smudge on a cuvette.

AT-LINE AND IN-LINE GMP SOLUTIONS

The SoloVPE system is an at-line system with applications including protein concentration at A_{280}, which is widely used across the biopharma industry from early-stage development to product release. It can also be used for polysorbate analysis, integrity testing for filters, DNA and RNA analysis, antibody-drug conjugate analysis, and multi-component analysis for blood products.

At-line testing: SoloVPE System:

- The ability to remove or automate multiple steps
- Low sample volume required
- No estimations, dilutions, or manual calculations
- A wide dynamic range
- Reduced time to results
- Reduced production hold time
- Improved reproducibility
- Increased sensitivity

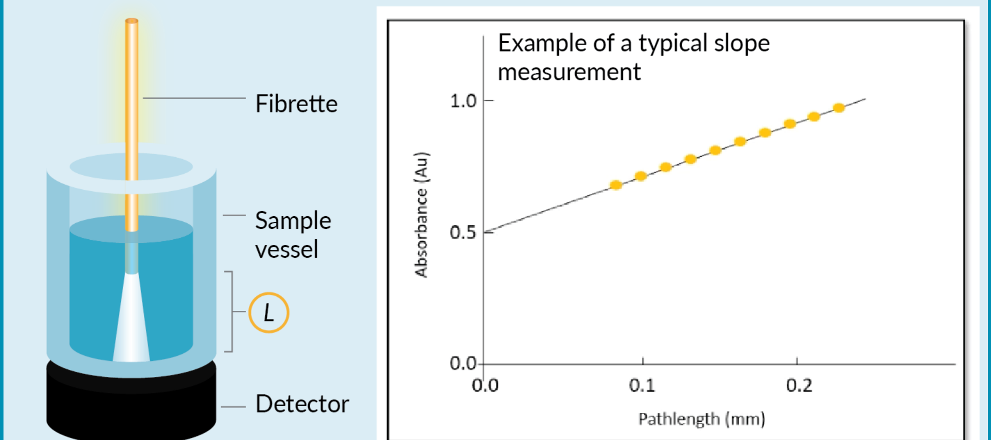
The FlowVPE system can be used in-line during downstream processing. It is particularly useful for ultrafiltration/diafiltration (UF/DF) and drug product analysis, but can also be used to measure high molecular weight species during the polishing step, or for load and elution during the capture step. It enables material usually used for sampling to be retained, which is of particular importance in the gene therapy field.

In-line testing: FlowVPE:

- The ability to obtain data immediately, without sending samples to other labs for analysis
- Actionable data to allow for quicker process understanding and DOE
- Reduced process development time
- A system which is easily transferable to later stage processes
- Reduced production hold time
- Multiple drug modalities, including proteins, DNA, vaccines and AAV vector

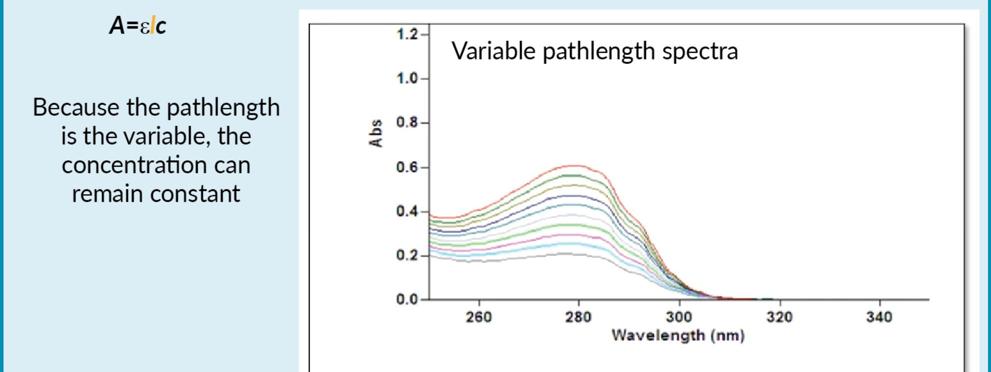
Slope Spectroscopy using VPT technology removes the multiple steps required for traditional UV spectroscopy, streamlining the process down to one simple step using SoloVPE, or no steps for the FlowVPE system. The result is built-in data quality, and an easily validated approach for GMP purposes. Variable Pathlength Technology can save time and reduce potential error in the gene therapy workflow, by enabling straightforward and robust concentration measurement in both process development and manufacturing.

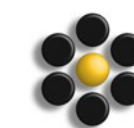
Figure 1. The Slope Spectroscopy method uses multiple measurements to calculate a slope regression. It is not an absolute single absorbance measurement system; the focus is on the change in absorbance from pathlength to pathlength to generate an accurate slope regression.



A=εlc

Because the pathlength is the variable, the concentration can remain constant





CELL & GENE THERAPY INSIGHTS

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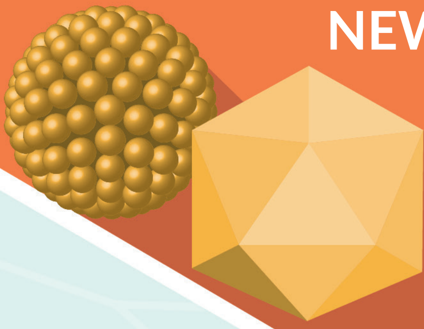

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
New vectors: update on emerging viral and non-viral delivery platforms

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Update on new viral vector strategies in gene therapy

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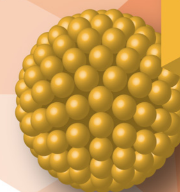

INTERVIEW

Update on new viral vector strategies in gene therapy

CLIVE GLOVER is the Director, Strategy at Pall Corporation where he leads Pall's cell and gene therapy business. Previously he was responsible for driving product development efforts around cell therapy at GE Healthcare and has also held positions in marketing and product management at STEMCELL Technologies. Clive holds a PhD in Genetics from the University of British Columbia.

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Q 2019 was marked by the market approval of Zolgensma®, an AAV-based gene therapy for inherited spinal muscular atrophy. The gene therapy field was under the impression that 2020 would be marked by the market approval of additional AAV based gene therapies. We are now in 2021, and no additional gene therapy was approved.

CG: There has been a great deal of excitement about adeno-associated viral vectors (AAV)-based gene therapies particularly with the approval of Zolgensma® by regulatory agencies in the US and Europe. AAVs are generally considered safe in comparison to other viral vectors such as lentivirus and retroviruses because they should not integrate

> "Gene therapy is starting to focus on diseases with a larger patient population, or which require bigger dose per patient."

into the genome of the host cell, but remain in episomal form in the nucleus of infected cells. However, there have been some recent findings that are challenging this idea.

A study was recently published analyzing the results of AAV gene therapy in dogs with hemophilia A after 10 years [1]. Nine dogs were treated with either AAV8 or 9 vectors expressing canine Factor VIII. After 10 years Factor VIII activity was still detected in all treated dogs. However, in two of those dogs, Factor VIII activity started to increase 4 years after the treatment and had tripled at 10 years post-treatment. Further analysis revealed that this increase in activity was due to clonal expansion of cells with integrated vectors. It was then revealed that in fact 6 out of the 9 dogs showed integration sites. While there was no reports of tumors or altered liver function, this study underscores the need for long-term monitoring of patients who have received AAV-based gene therapies to monitor for potential genotoxicity.

Genotoxicity, resulting in patient death has now been observed in the high dose arm of at least one AAV clinical trial. A recent analysis by Wilson and Flotte addresses dosage and toxicity observed with higher doses of AAV [2]. In numbers, currently infants are infused with up to 1×10^{14} genome copies (GC)/kg with Zolgensma®. In the case of the clinical study driven by Audentes Therapeutics, the 1×10^{14} GC/kg also led to encouraging results, but when testing a higher dose: 3×10^{14} GC/kg on three patients, it led to the death of two of the patients due to severe liver toxicity. This is not the only case, and this is why several trials were either put on hold (Audentes, Biomarin), or slowed down (Sarepta, SolidBio Pfizer, UniQure). While there was the initial impression that gene therapies targeting monogenetic diseases would proceed rapidly through the regulatory process, there is now precedence and regulatory agencies have a better understanding of advanced therapies and pain points that need to be addressed to make sure that these therapies can reach patients safely.

Q AAVs are one of the most promising vectors for human gene therapy. Keeping in mind the recent shortcomings, have we reached the limit of conventional AAV vectors and is it time to focus on developing and implementing the use of next-generation AAVs?

CG: We are seeing a general trend towards treating larger and larger diseases. Gene therapy is starting to focus on diseases with a larger patient population, or which require bigger dose per patient. As the diseases being targeted change, further innovation around the vectors is taking place. Tissue-specific promoters and/or enhancers are being used to boost transgene expression only in the desired tissue. Or capsids are being engineered to sidestep immune responses allowing for the patient specific dose to be decreased.

Combining the most mature single-use bioreactor system, the iCELLIS® with the gold standard in large scale transfection, PEIpro® from Polyplus-transfection, we've recently put together

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a general guidance for large scale viral vector production for gene therapy [3]. The combination of these two leading technologies addresses upstream industrialization challenges and makes a reliable and scalable platform for gene therapy manufacturing of AAVs as well as other types of viral vectors such as lentiviruses and virus-like particles (VLPs).

Q Lentiviral vectors currently in clinical trials are predominantly for *ex vivo* therapy. Is there a trend towards to use of lentiviral vectors for *in vivo* gene therapy?

CG: Contrary to AAVs, lentiviruses are usually used *ex vivo* direct administration of lentivirus *in vivo* has the possibility to allow for treatment of younger patients as the transgene could be stably integrated into the genome. The challenge here is the safe integration of the transgene into the genome, and produce higher amounts of lentivirus for direct systemic administration. Sanofi who acquired Bioverativ in 2018, is taking on the challenge to treat hemophilia with lentivirus as delivery vectors because they can carry more genetic information than AAVs. This means that one can package not only the corrected DNA sequence for the missing protein coagulation factor VIII, but also a biochemical switch to turn on or off expression of the corrected gene, and key genetic information to side-step immune responses. If they can find a way to stably and safely integrate the hemophilia gene in younger patient, than it is more likely to be a lifetime cure.

However, the challenges of using lentivirus for gene therapy has been underscored recently by the halting of the sale of Zynteglo®, Bluebird bio's *ex vivo* gene therapy for beta thalassemia, after two patients developed cancer several years after treatment. Interestingly, similar challenges have not been observed for Kymriah®, the other marketed *ex vivo* gene therapy using lentivirus. While the exact reason for this is not known, cell type and amount of lentivirus per patient likely differ and may explain this.

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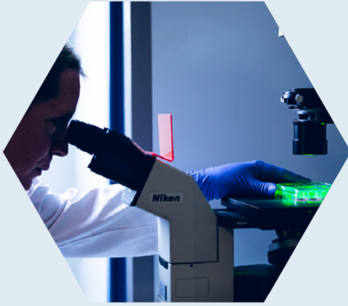

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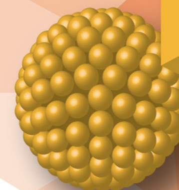
CELL & GENE THERAPY INSIGHTS

NEW VECTORS: UPDATE ON EMERGING VIRAL & NON-VIRAL DELIVERY PLATFORMS



CHANNEL CONTENT

INTERVIEW

Update on the *Sleeping Beauty* transposon system: current state-of-the-art & remaining challenges

ZOLTÁN IVICS received his PhD in molecular biology in 1994. After postoctoral studies at the University of Minnesota in the USA and the Netherlands Cancer Institute, he was appointed as a research group leader at the Max Delbrück Center for Molecular Medicine in Berlin, Germany. He was appointed as Head of Division at the Paul Ehrlich Institute in Langen, Germany, in 2011. Prof. Ivics' major scientific achievement is the molecular reconstruction of the *Sleeping Beauty* transposon and development of technologies based on *Sleeping Beauty* gene transfer for a wide array of applications involving genetic engineering of cells. Prof. Ivics has published >130 papers in peer reviewed journals, with a total Impact Factor of >800 and >7000 citations (h-index: 44), and is co-inventor on 12 patents. Since 2000 his research efforts were supported by >20 research grants from the German Research Foundation, the German Ministry of Education and Research, the European Commission, and the Volkswagen Foundation. He received recognition of the 'Molecule of the Year' in 2009 for developing a hyperactive *Sleeping Beauty* transposase that opened the door for clinical applications. He is a current member of the Board of the European Society of Gene and Cell Therapy (ESGCT), member of the Board of the German Society of Gene Therapy and member of the committee for 'Clinical trials and regulatory affairs' of the German Stem Cell Network. Prof. Ivics organized several international conferences, including the Annual Congress of the ESGCT in Berlin in 2017. He is an Elected Member of the Academia Europaea since 2018.

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Q What are you working on right now?

ZI: My field is non-viral gene engineering using the *Sleeping Beauty* transposon system. For me, the most exciting application for this particular gene delivery tool is therapeutic gene engineering, although there are other potential areas where this kind of tool can be used.

The main focus of my work is firstly to further develop the system with respect to efficacy and safety – the two fundamental pillars of any gene therapy methodology or genetic engineering system. And secondly, with the system we already have in hand, I am endeavoring to demonstrate proof of concept for hematopoietic stem cell-based gene therapy.

Of note, *Sleeping Beauty* is already in clinical use in the context of CAR-T cell applications – there are wonderful data out there in that field. However, hematopoietic stem cells represent a little bit more of a challenge for non-viral genetic engineering. Our work there is especially geared towards establishing robust and safe protocols in preclinical animal models, and then applying these methods to fix genetic diseases in the mouse system. Of course, once preclinical proof of concept has been established, the next step will be to move into first-in-human applications.

Q What *in vitro* models are you applying in support of your preclinical studies in mouse models? What approaches lend themselves to this particular system in that regard?

ZI: It is generally commonplace for any gene therapy-relevant protocol to do one's very first tests in cell culture or cell-based assays – in the context of our *Sleeping Beauty* vectors, we typically validate system components in genetically deficient primary cells that are derived from patients suffering from a certain genetic disease or defect. Typically, fibroblast cultures are available from these patients.

We can then apply our *Sleeping Beauty* transposon vectors, which are equipped with therapeutic transgene cassettes, and phenotypically convert these primary patient-derived cells. We can then show, through functional assays, that we can indeed convert these cells back into a wild-type or healthy phenotype, as a direct result of a therapeutic gene transfer into the genome of these cells.

Of course, whenever we use mouse models, the very first experiments we conduct also involve isolating fibroblast cells from the animals or, more specifically for diseases that affect the blood system, bone marrow-derived hematopoietic stem cells. We then use cell culture initially to show that the gene vectors we have built are functional and are doing the job we expect of them in a cell-based assay. Only once we have obtained that kind of evidence do we proceed to the next step: taking

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genetically engineered cells and transplanting them into a mouse in the hopes of obtaining therapeutic benefit in an *in vivo* animal model.

Q Non-viral delivery is clearly on the rise in cell and gene therapy in general – how do you view the field as a whole currently?

ZI: Firstly, I think it is really important to provide a definition of non-viral gene delivery.

I very often bump into people at conferences who either think a non-viral vector is just a straight plasmid, or who believe non-viral vectors are kind of synthetic, lipid-based transfection reagents that are actually used to complex nucleic acids in order to carry them into the cell. At least in my view, a non-viral gene delivery system is a combination of these two things.

Just to explain my point: a viral vector also has two main functional components – firstly, a nucleic acid component, which carries the therapeutic gene construct or gene of interest either into a cell, or in the case of an integrating viral system, into the genome of the target cell. The second important component is the viral capsid or envelope, which allows transduction or the transfer of that therapeutic nucleic acid into the cell. Similarly, I would define a non-viral vector system as consisting of the same two fundamental components: the nucleic acid we want to carry in, and another molecule, substance, or reagent that helps to shuttle that nucleic acid component into the cell.

In past years, it was generally the case that when you spoke with people who had been using classical viral gene delivery tools about non-viral gene delivery, you tended to encounter a skeptical expression. I must say, though, that this attitude has been shifting somewhat over the past 2–3 years. When I meet hardcore viral gene delivery people today, whether academic researchers or company representatives, they listen – they show interest and can see that the area is developing nicely and has potential. This is because the technology and the field as a whole have made a lot of excellent progress, including in the transposons area – however, major challenges do remain.

The first of these challenges – and a key reason why non-viral delivery has traditionally encountered skepticism from the viral vector field – is that non-viral vectors tend to lag behind with respect to efficiency. Whenever we talk about what fraction of the cells can be genetically engineered by a classical virus system versus any kind of non-viral gene delivery system, the latter still tends to be at a lower level.

A second related challenge relates to toxicity, or the side effects of the gene delivery step. As we just discussed, one component of a non-viral gene delivery system is a naked nucleic acid that needs to be shuttled somehow into the cell. With most of the non-viral delivery tools, the relative lack of efficiency I just mentioned results in the need to increase the amount of naked nucleic acids introduced into the therapeutically relevant cells. And for that, one pays the price of a significant level of toxicity.

I therefore think the next major step in terms of non-viral gene delivery platform tools is to come up with either a single system or a combination of different approaches that can match the efficiency of viral gene delivery, either *in vivo* or *ex vivo*, and by doing so, reduce or remove the toxicity issue.

The third challenge in front of us in the field of non-viral gene delivery is to be able to selectively target certain cell types. This is mainly relevant for *in vivo* application, where the ability to target specific cell types becomes so important. There are very elegant viral-based systems that can accurately target the therapeutic gene construct to a certain organ, for example deliver it to T cells in the blood, through modification of the capsid or the envelope of viral gene delivery vector systems. I think the key for non-viral vector systems area is to come up with a fully synthetic package – one where you have a synthetically produced nucleic acid component (DNA or RNA) and/or a recombinantly produced protein component mixed together in a complex with a synthetic, non-viral carrier function - then this entire complex is targeted *in vivo* to a certain cell type. This is possible to achieve – in fact, the first couple of examples of this type of approach are already out there.

I do truly believe the future of therapeutic gene delivery will be mainly based on non-viral gene delivery, and I'll tell you why.

First of all, at least in principle, all of the non-viral vector components, including the nucleic acids and the carrier molecules, can be produced fully synthetically. Even the nucleic acids can be produced outside of a living cell: *in vitro* transcription reactions are in place to produce messenger RNA completely enzymatically, without ever seeing a living cell. And the DNA component of a vector can also be produced fully synthetically in an enzymatic reaction, in a tube that only contains water, salt, buffer components, nucleotides, and an enzyme. A major advantage of a fully synthetic technology like this is that you have very significant control over manufacturing. The scale-up and GMP-related challenges that viral vector manufacturers face would not apply – you would be looking at a relatively straightforward, fully automated laboratory process. Additionally, the manufacturing timeframe would be significantly shorter than it currently is for viral vector production, which can also have a positive impact on the manufacturing timeframe of products that incorporate gene delivery in the bioprocess, such as CAR-T cell therapies – a type of product where time is a critical factor, of course. And thirdly, costs would be significantly reduced. There is a clear-cut economic advantage in manufacturing non-viral genetic engineering systems or tools versus viral ones – for example, it is cheaper to produce a plasmid, or an RNA, or a lipid nanoparticle in relatively large quantities. This becomes especially important in the *in vivo* gene therapy setting, where larger amounts of vector are generally required.

Q Can you go deeper on the chief regulatory obstacles that remain for non-viral delivery, as you see them?

ZI: This is a perfect question for me since the Paul-Ehrlich-Institut is both a research institute and a regulatory agency.

With non-viral vector systems, there are certain quality control requirements that can be bypassed, or at least expedited, that would otherwise be in place for viral gene delivery systems. For example, with a viral system, you would typically require an Environmental Risk Assessment, because you are working with an infectious viral particle. This is part of the procedure through which regulators assess a clinical trial application. Non-infectious, non-viral vector

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"Secondly, I believe that many in the field have yet to fully grasp that the transposon and what it does effectively connects the two different worlds of vector systems – viral and non-viral. *Sleeping Beauty* is non-viral because it is simply native nucleic acid. "

components, and their applications in patients, would clearly be associated with far simpler and easier documentation of their safety. Additionally, because manufacturing would mainly or entirely be comprised of completely synthetic production steps, quality control (QC) would also be relatively easier with respect to assessing the critical quality attributes and the release criteria for the reagents.

So, I actually foresee a certain future benefit of non-viral delivery systems not only in terms of the aforementioned technology requirements, but also from a regulatory perspective. I have to stress though that these potential advantages are probably limited to the quality attributes of the materials and manufacturing procedures for the drug product, and would not necessarily impact the preclinical efficacy and safety data or the clinical requirements that need to be met for a study.

Q Can you go into more depth on the *Sleeping Beauty* transposon system: what do you regard as the key next steps for this field?

ZI: It is very important to firstly clarify a couple of different aspects of *Sleeping Beauty*.

Number one – and going back to my initial definition of a non-viral delivery system – the second component that carries the therapeutic gene of interest into the cell must be introduced: it is not an inherent part of the *Sleeping Beauty* system. It can either be a lipid nanoparticle or electroporation technology, but some form of technology that can shuttle the nucleic acid into the cell must be adopted. (This is why, when I talk about the transposon, I am referring solely to the first component of the gene delivery system – the actual nucleic acid component).

Secondly, I believe that many in the field have yet to fully grasp that the transposon and what it does effectively connects the two different worlds of vector systems – viral and non-viral.

Sleeping Beauty is non-viral because it is simply native nucleic acid. You can incorporate a *Sleeping Beauty* transposon vector in a plasmid – or a so-called mini-circle, which is a smaller derivative of a plasmid – but nonetheless, it is just nucleic acid. It's just DNA. Classically speaking, plasmid DNA is non-integrating: you can inject or deliver it with whatever reagent, whatever technology you choose into a target cell population, and the plasmid DNA will hang around for a while, but it will not undergo genomic integration. However, *Sleeping Beauty* is an

> "Broadly speaking, the challenges facing *Sleeping Beauty* are the same as those … for the entire non-viral delivery field: matching the gene delivery efficiency of viral vector systems, and reducing the toxicity…"

integrating gene delivery tool. It incorporates an enzymatic step, which involves removing a therapeutic transgene cassette from the non-viral plasmid vector. This process then integrates the therapeutic transgene cassette into the genome of the targeted cell.

Therefore, at least from the genomic integration perspective, *Sleeping Beauty* represents kind of a meeting point between the viral and non-viral worlds. It's a non-viral gene delivery tool that does genomic integration – it is an important point to emphasize, particularly in light of the challenges that remain in this field.

Broadly speaking, the challenges facing *Sleeping Beauty* are the same as those I mentioned earlier for the entire non-viral delivery field: matching the gene delivery efficiency of viral vector systems, and reducing the toxicity associated with carrying the *Sleeping Beauty* transposon component into cells. However, the third key challenge is actually quite specific to *Sleeping Beauty*, and it relates to the fact it is a genomically integrating genetic engineering tool.

We have put in a lot of effort and published a number of papers in the past couple of years on characterizing the integration profile of this genetic tool, genome wide. In doing so, we have established certain advantages associated with the use of *Sleeping Beauty* over, for example, a retrovirus- or lentivirus-based vector system. For instance, retroviruses tend to prefer integration into expressed genes, or their transcriptional regulatory regions – the promoter and enhancer regions. Of course, this carries with it a certain risk of genome toxicity, or oncogenic transformation of that cell. By contrast, *Sleeping Beauty* is almost fully random in a genome-wide scale. Practically speaking, it can integrate anywhere. On the one hand, this represents an advantage over viruses, because this preferential integration into genes is clearly lacking with *Sleeping Beauty*. On the other hand, though, random integration is clearly not fully safe. Random integration can also result in insertion of a therapeutic gene cassette into a proto-oncogene, and thereby transcriptionally activate that proto-oncogene, or integration into a tumor-suppressor gene and thereby inactivating that tumor-suppressor gene. Overcoming this third challenge will require the field to establish technologies that allow us to actually guide or target the transposon integration to a certain site in the genome.

What progress have we made so far in addressing these three challenges? Regarding efficiency, I do believe that the current state-of-the-art available today (hyperactive *Sleeping Beauty* transposons) can match the efficiency of a lentivirus vector. This is a major area of focus for my lab, and although we are working on future modifications with the goal of engineering even more active variants of this system, I think what we have today is already a good option with respect to efficiency.

Turning to toxicity, this is not my personal area of expertise – I am kind of waiting for the field to come up with the nucleic acid delivery technologies (e.g. lipid nanoparticles) that can deliver naked nucleic acids in a very efficient manner into primary human cell types, either *ex vivo* or *in vivo*, and avoid pronounced toxicity on the cells.

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Regarding the third challenge, which is the question of how to avoid a negative impact on the cell from the integration of *Sleeping Beauty* transposon into the genome, this for me is the next step for the field, and another major point of focus for my research. We are currently seeking to put together programmable transposons and conditional transposition systems, which will allow us to integrate a therapeutic gene cassette very efficiently, and in a non-toxic manner, into one particular locus of the human genome. That is our vision for the further development of *Sleeping Beauty* transposon-mediated therapeutic gene delivery over the next five years or so.

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AUTHORSHIP & CONFLICT OF INTEREST

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Feb 2021

Clinical Trends

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Clinical Trends

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COMMENTARY

2021: a key year for allogeneic cellular cancer immunotherapy?

Sven Kili

175–181

CELL & GENE THERAPY INSIGHTS

Clinical Trends

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COMMENTARY

2021: a key year for allogeneic cellular cancer immunotherapy?

Sven Kili

Perhaps *the* key trend in 2020 for the cellular immunotherapy field was the emergence and advancement into the clinic of a new wave of novel allogeneic product candidates.

Allogeneic approaches offer clear advantages over autologous cell therapies, ranging from a less complex, more 'pharma-friendly' supply chain and less variable, healthier manufacturing starting materials, through to reduced wait manufacturing bringing Cost of Goods efficiencies, and potentially lower price points for a healthcare sector under growing financial constraints. However, there are some imposing obstacles to be addressed relating to demonstrating both safety and efficacy in the clinic, as well as overcoming scalability issues. In this month's *Clinical Trends*, we dissect the development plans and ongoing clinical studies of some of the key industry players.

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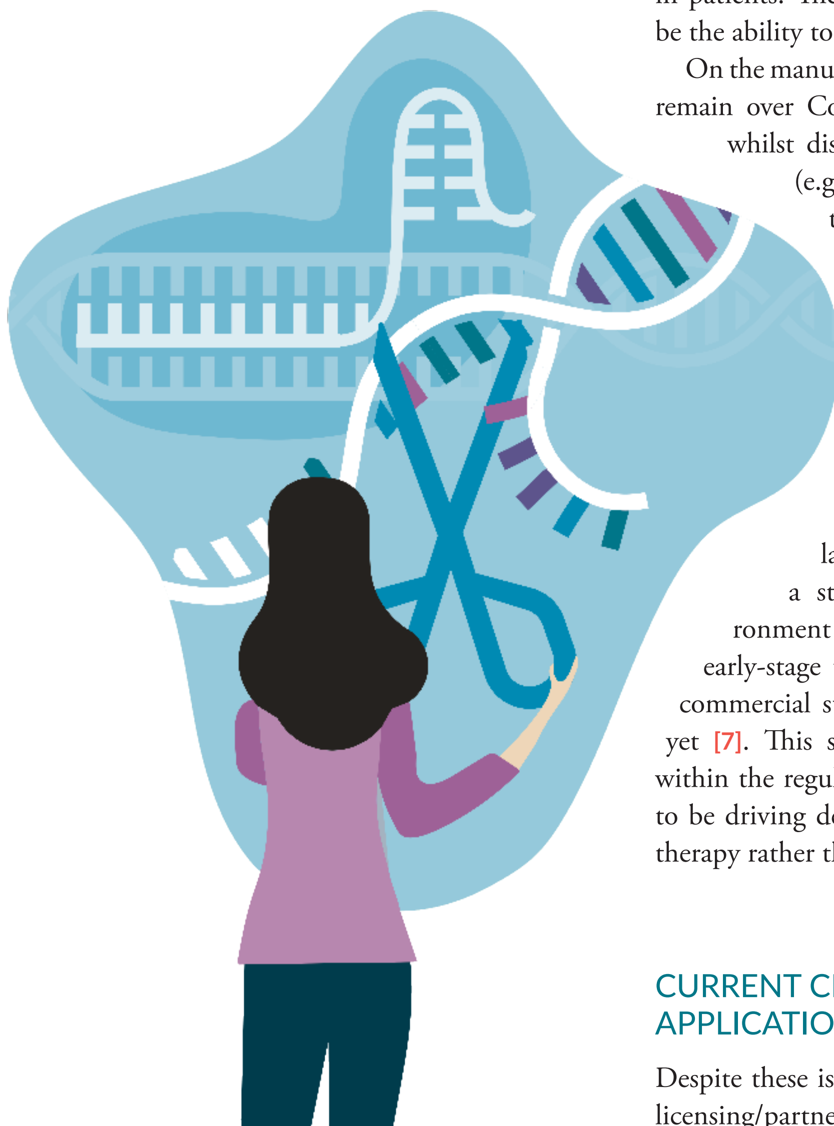
BARRIERS TO SUCCESS FOR THE ALLOGENEIC CELLULAR IMMUNOTHERAPY FIELD

Safety concerns remain a key obstacle for allogeneic cell therapy developers. In the T cell immunotherapy space, a number of strategies are being employed to address the triggering of graft-versus-host disease (GvHD) in patients, including targeted lymphodepletion prior to administration, HLA-matching of donor

materials, and either the application of gene editing or selection of non-alloreactive T cells to induce immune tolerance. Other developers are utilizing innate immune system cells (e.g. NK cells, γδ T cells) to reduce or remove the risk of GvHD [1,2]. However, further safety concerns (e.g. oncogenic events, off-target edits) relating to the use of gene editing platforms and universal cell sources will remain until these fledgling technology areas obtain more clinical data [3,4]. Furthermore, toxicity events common to the broader cellular immunotherapy field, such as cytokine release syndrome (CRS) and neurotoxicity, are also potential challenges for the allogeneic cell therapy space.

On the efficacy side, Allogene reported a short-term response rate of 63% in lymphoma patients at ASCO 2020 – an encouraging figure from such early data with very encouraging safety data [5]. However, at ASH 2020 last November, an early data readout in multiple myeloma was less well received (although that was likely due in part to a death reported among the patient cohort, which was linked to disease progression and the conditioning regimen used) and the announcement resulted in a dip in Allogene's share price [6]. Clearly, it remains to be seen if allogeneic approaches will be able to deliver efficacy data that is sufficiently close to that obtained by autologous approaches to both offset safety concerns and allow the aforementioned advantages of allogeneic cell therapy to come into the equation. One key issue that needs to be addressed by the field in this regard is improving the persistence of allogeneic cells in patients. The counter to this would even be the ability to re-dose [4].



On the manufacturing side, question marks remain over Cost of Goods and scalability, whilst disruption of the supply chain (e.g. in cell collection) caused by the COVID-19 pandemic is also not trivial, and likely to be ongoing for some time to come (although of course, such issues are not unique to the allogeneic cell therapy field).

Last but not least, the related issues of a current lack of standardization and a still-evolving regulatory environment – standard obstacles for any early-stage technology area - mean that commercial success may be some way off yet [7]. This said, there is clearly interest within the regulatory community to be seen to be driving development of allogeneic cell therapy rather than hindering it [8,9].

CURRENT CLINICAL APPLICATIONS

Despite these issues, a vibrant financing and licensing/partnering environment and the

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"...it remains to be seen if allogeneic approaches will be able to deliver efficacy data that is sufficiently close to that obtained by autologous approaches to both offset safety concerns and allow the ... advantages of allogeneic cell therapy to come into the equation.."

buy-in of major industry players [10,11] continue to drive rapid progress and advancement towards and into the clinic of allogeneic cell therapy product candidates.

Mirroring the autologous CAR-T cell therapy field, early clinical-stage allogeneic products to date have been largely aimed at hematological malignancies, with the first solid tumors indications just starting to enter the clinic [2].

On the National Cancer Institute (NCI) website, no fewer than 71 clinical trials using therapeutic allogeneic lymphocytes are listed [12]. In addition to studies being conducted by some of the world's foremost academic cancer research centers (e.g. MD Anderson, Dana-Farber, Fred Hutch, Baylor College of Medicine) plus the NCI itself, a number of industry trailblazers' ongoing studies are described, which speak to the variety of cell types, cell sources, engineering platforms, and trial designs that are now being applied in the clinical setting:

- Part of the Cancer Moonshot QUILT series of studies, a Phase 2b trial of NantKwest's t-haNK NK cell therapy in combination with Anktiva (ImmunityBio's human IL-15 superagonist), and Pfizer's PD-L1 checkpoint inhibitor, avelumab (Bavencio®) is ongoing across a wide range of tumor types.

- Based upon its proprietary ARCUS gene editing platform, Precision Biosciences is conducting 3 separate Phase 1/2a trials in relapsed/refractory ALL, B-cell NHL, CLL/SLL, and multiple myeloma. Data released in December 2020 by the company from the 27-patient dose escalation and dose expansion study of lead candidate, PBCAR0191, reported no cases of GvHD, no cases of Grade ≥3 CRS, and no cases of Grade ≥3 ICANS (Immune effector cell-associated neurotoxicity) [13].

- As previously mentioned, Allogene Therapeutics' early data read-outs were among the most hotly anticipated and carefully analyzed in 2020. Ongoing studies by the company include:

  - The ALPHA study of ALLO-501 anti-CD19 allogeneic CAR T cells in adults with relapsed/refractory large B-cell or follicular lymphoma, following a lymphodepletion regimen comprising fludarabine, cyclophosphamide, and ALLO-647 (a monoclonal antibody targeting CD52 antigen). Of the 19 (of 22 total) patients evaluated for efficacy prior to the ASCO 2020 presentation, there were 7 complete responses and 5 partial responses. Higher response rates were observed in CAR T naïve patients with an overall response rate in that population (16 patients) of 75% and a complete response rate of 44%. 9 of the 12 patients who had a response (75%) remained in response at the data cut-off. A higher conditioning dose of ALLO-647 was also associated with higher complete response rates.

- A further Phase 1 trial for a next-generation version of ALLO-501 (ALLO-501a) is now underway in adults with relapsed/refractory large B-cell lymphoma, following a lymphodepletion regimen comprising fludarabine, cyclophosphamide, and ALLO-647 (the ALPHA-2 study). Updated ALPHA and initial ALPHA-2 results are planned to allow preparation for a potential pivotal study initiation of ALLO-501A in 2021.

- A Phase 1 of ALLO-715 (BCMA-targeted allogeneic CAR T cell therapy) with or without SpringWorks' investigational gamma secretase inhibitor, nirogacestat, is underway in adults with relapsed or refractory multiple myeloma (the UNIVERCELL study).

- CRISPR Therapeutics, a trailblazer in the therapeutic application of CRISPR Cas9 gene editing, initiated the CARBON study – a single-arm, open-label, multicenter Phase 1 study trial to evaluate CTX110 in patients with relapsed/refractory B-cell malignancies. Top line results released by the company in October 2020 (from 11 of 12 enrolled patients) reported an acceptable safety profile and dose-dependent efficacy and response rates that were comparable to those for the early autologous CAR-T cell therapy trials. This and further studies (of CTX120 in liquid cancer and CT130 in solid tumors) are currently enrolling patients.

- One of the true pioneers of allogeneic cellular therapy, and well known for its

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proprietary TALEN gene editing platform, Cellectis' clinical development story has seen the FDA place clinical holds on 2 studies following patient deaths. Firstly, in September 2017, a hold was placed on the initial trial of lead candidate, UCART123, in AML and blastic plasmacytoid dendritic cell neoplasm (BPDCN) following the death of a patient from severe CRS. The hold was lifted the following December upon acceptance of an amended protocol that including a lowered dosing of UCART123 [14]. However, the study was then terminated in June 2019. A new, replacement IND was subsequently sought and granted, and the first patient was dosed in this new UCART123 study in January 2020 [15].
A Phase 1 open-label, dose escalation and expansion study of UCART22 in patients with relapsed/refractory CD22$^+$ B-cell ALL (BALLI-01) reported promising initial data at ASH 2020, albeit in a small patient population [16]. However, a third current study of UCARTCS1A targeting CS1 in patients with relapsed/refractory multiple myeloma the (MELANI-01 trial) underwent a clinical hold imposed by the FDA in July 2020 following the death of a patient due to cardiac arrest. This clinical hold was subsequently lifted the following November following adjustments to the clinical protocol designed to enhance patient safety.

- Fate Therapeutics' has perhaps the deepest clinical pipeline of any current allogeneic cellular immunotherapy company which is dominated by off-the-shelf, iPSC-derived NK cell therapy candidates (although the first iPSC-derived CAR T cell therapy, FT819, is entering the clinic shortly). Lead candidate, FT500, is being studied both as a monotherapy and in combination with immune checkpoint inhibitor antibodies in subjects with advanced solid tumors. The company had a busy second half of 2020, variously announcing a partial response reported with FT596 monotherapy at first dose level in refractory DLBCL, the initiation of enrollment for a study of FT596 in combination with rituximab for B-cell lymphoma, an IND clearance for FT538 (the first CRISPR-edited, iPSC-derived cell therapy in the clinic) for AML and multiple myeloma, an IND clearance for FT819 in advanced B-cell leukemias and lymphomas, and a collaboration with Janssen to develop novel iPSC-derived CAR NK and CAR T-cell product candidates.

- Celyad Oncology's IMMUNICY-1 trial is a study of the safety, activity, and cell kinetics of CYAD-211 in patients with relapsed/refractory multiple myeloma, following a lymphodepletion regimen with fludarabine and/or cyclophosphamideage. The first patient was dosed in December 2020.

- Artiva Biotherapeutics' AB-101 – another off-the-shelf NK cell therapy – is being studied in a Phase 1 as a monotherapy and in combination with rituximab in patients with relapsed/refractory B-cell non-Hodgkin lymphoma

CONCLUSION

2021 promises to be another intriguing year for the allogeneic cellular cancer immunotherapy field, with the release of more early clinical data set to provide an indication of precisely how near, or far, these potentially game-changing products are from providing a viable alternative to the autologous cell therapies currently on the market.

With the majority of autologous cell therapy trailblazers, including the likes of Kite Pharma/Gilead Sciences and Bristol-Myers Squibb/Celgene, already advancing next-generation allogeneic approaches into and through their preclinical pipelines, the familiar 'autologous vs. allogeneic' debate will gather pace. However, there will likely

be room for the two approaches to coexist long-term - for example, with allogeneic approaches providing a viable alternative for those patients whose own T cells are not sufficiently healthy to allow for autologous cell therapy [17]. For the meantime, all eyes will be on this fledgling but highly promising field as it continues to wrestle with safety-, efficacy- and manufacturing-related challenges.

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AFFILIATION

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SVEN KILI joined Antion as CEO in November 2020. He brings extensive, relevant clinical and industry expertise to grow Antion into a leading Cell and Gene Therapy company. He is currently also principal at Sven Kili Consulting Ltd. and will transition to a full time role at Antion over the coming months. He was previously the Head of Development for the Cell and Gene Therapy division of GSK Rare Diseases where he led teams developing and commercializing *ex vivo* Gene Therapies for a variety of rare genetic disorders including Strimvelis®, the first *ex vivo* gene therapy to be approved for children with ADA-SCID; Wiskott-Aldrich syndrome (WAS); Metachromatic Leukodystrophy (MLD) and Beta-Thalassemia. Prior to this, he was Senior Director, Cell Therapy and Regenerative Medicine for Sanofi (Genzyme) Biosurgery where he led the clinical development, approval and commercialization activities of the first combined ATMP approval in the EU for MACI®. He and his team also prepared and submitted Advanced Therapy regulatory filings for Australia and the US, including health technology assessments and he was responsible for late-stage developments for Carticel® and Epicel® in the US. Before joining Genzyme, Sven led the cell therapy activities and oversaw all UK & Irish regulatory functions, and was the QPPV for pharmacovigilance for Geistlich Pharma. Sven is also interim CMO of Bone Therapeutics, sits on the boards of CCRM in Canada, Xintela (a Swedish Stem Cell company), and the SAB for LGC Corporation, and is the chair of the CGTAC as part of the UK BIA and a Board member of the Standards Co-ordinating Body for Regenerative Medicine. Most recently, Sven was appointed as Chair of the UCL course "Masters in Manufacture and Commercialization of Stem Cell and Gene Therapies" steering committee. Additionally, he still maintains his clinical skills in the UK NHS and serves as an ATLS Instructor in his spare time.

AUTHORSHIP & CONFLICT OF INTEREST

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