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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Raw and Starting Materials

CELL & GENE THERAPY INSIGHTS

Volume 8, Issue 2

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FOREWORD

Raw & starting materials for cell and gene therapies

GARY C DU MOULIN, PhD MPH, RAC retired as Vice President, Quality Operations at Genzyme Biosurgery and Senior Director of Quality Aseptic Control for Genzyme (A Sanofi Company) after a 20 year career in the development and execution of quality systems for Genzyme's products including biologics and the FDA approved cell based therapeutics, Carticel and Epicel. Previously, he served as Vice President, Quality Operations at Cellcor, a pioneering company in the field of somatic cell immunotherapy based upon the infusion of T cells that have been activated ex vivo using a combination of previously generated cytokines and an anti-CD3 monoclonal antibody. Before entering the field of cell therapy he began an academic career on the faculty of Harvard Medical School and has 160 publications in the areas of microbiology, epidemiology, and the regulation and quality control of living cells as a therapeutic modality. Dr du Moulin received his graduate degrees from Northeastern University and Boston University School of Medicine. Dr du Moulin has served on US Pharmacopoeia's Gene Therapy, Cell Therapy, and Tissue Engineering Expert Committees and chaired the ad hoc advisory panel for fetal bovine serum. He currently serves on the Modern Microbiological Methods Expert Panel. He has served on the editorial board of Regenerative Medicine and is RAC certified and past Chairman of the editorial board of the Regulatory Affairs Professionals Society Magazine, RAPS Focus. Dr du Moulin was appointed to the Grants Review Working Group of the California Institute for Regenerative Medicine (CIRM). He is retired from the US Army Reserve at the rank of Colonel after 38 years of service. He has held academic appointments at Harvard Medical School, Boston University School of Medicine, Northeastern University, and most recently has completed a teaching assignment at the Massachusetts College of Pharmacy and Health Sciences University where he taught graduate pharmacists a course entitled, "Principles of Quality Assurance and Quality Control in a Regulated Environment". Dr du Moulin continues to consult in the cell and gene therapy sector and helps develop STEM programming for youth-based non-profit organizations.

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The cell and gene therapy industry is facing a highly complex and rapidly evolving discovery path very different from conventional drug or biologic development. Engineering biology is reshaping translational medicine yielding an opportunity to address hundreds of disease areas. As a result, thousands of clinical trials are underway within this new paradigm. The Alliance for Regenerative Medicine's 2022 state of the industry address reported that at present there are 2261 global clinical trials underway with six new regenerative medicine products approved by US and international regulatory bodies in 2021 [1]. The raw and starting material supply chain needed to support these trials is an enormous challenge but is critical if patients are to have access to these therapies, safety is to be ensured and if efficacy of these therapeutic modalities is to be validated.

Gene therapy manufacturing processes are complex and may be comprised of multiple biological manufacturing processes producing such critical components and starting materials as media, plasmids and viral vectors. The gene therapy product involves the convergence of these processes with the drug product manufacturing process. Ultimately, the production of a cell and gene therapy product can comprise hundreds of raw and starting materials, a multitude of manufacturing steps including open and closed manual or automated operations performed by many personnel in a variety of manufacturing establishments. Quality control analytics supporting these processes also requires a host of media, reagents, and consumables. Significant biological variability is pervasive throughout these manufacturing processes. Moreover, patient specific therapeutic programs accentuate process variability which can impact the drug potency due to a patient's unique medical condition. Finally, multiple organizations involved in producing the final therapeutic product could exhibit significant compliance or GMP deficits that ultimately affect the product's quality and patient safety. The FDA's compliance actions with a number of these organizations is already a matter of record.

Reliance upon a consistent supply of raw materials is critical for developing successful cell and gene therapy products. While the cell and gene therapy industry have made significant strides to ensure regulatory compliance and patient safety, the challenges surrounding scalability and consistency of raw materials remains, now taking place in a world facing a global pandemic. Often, GMP-compliant raw materials either do not currently exist or are only available 'off the shelf'. In house modifications to these materials increase the possibility of user error and adds complexity when scaling up the manufacturing for complex processes such as plasmid and viral vector production. Box 1 presents a partial list of common raw and starting materials commonly used in the production of cell and gene therapy products.

The sponsor's quality assurance systems are tasked with responsibility and control of the immense challenge of selecting and qualifying these raw materials. Patient cells and tissues as starting materials of biological origin only add to the level of scrutiny and qualification required before they can be confidently used in manufacturing. Phase appropriate GMP clinical investigation mandates that all raw and starting materials are sufficiently qualified to ensure safety and potency prior to the onset of human clinical trials [2–5]. Mitigating the variability and managing the consistency of these materials at an early product development cycle is a huge responsibility fraught with great risk if not well managed.

REGULATORY CONSIDERATIONS

US FDA regulatory guidance, European Union's directives, and ISO standards, ICH and PIC/S Annex 1 GMP guidance's provide an important baseline for developers establishing the manufacturing processes for plasmids, viral vectors, or cell and gene therapy products. Moreover, the US and European Pharmacopeias have published multiple general chapters and monographs defining steps in creating a robust quality management risk

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BOX 1

Partial list of common raw and starting materials that may express inherent variability in cell and gene therapy product manufacturing

- Media components (animal component free)
- Glucose
- Glycerol
- Salts and minerals
- Amino acids
- Yeast extract
- Animal derived materials
- Human albumin
- Exchange chromatography resins
- Plasmids
- Cytokines
- Monoclonal antibodies
- Cell lines (animal, insect or human derived)
- Viral vectors
- Buffer components
- Transfection reagents
- Purines and pyrimidines
- Polyethyleneimine (PEI)
- Polyethylene glycol (PEG)
- Detergents (SDS, Triton X-100)
- Dimethyl sulfoxide (DMSO)
- Antibiotics
- Tangential flow filters
- Growth factors
- Digestive enzymes
- Recombinant proteins
- Lipid nanoparticles
- Single use disposables
- Antibody-coated beads

strategy with qualification criteria for a number of critical materials. Available US and international guidelines that address raw and starting materials are listed in Box 2.

DEVELOPMENT TO COMMERCIALIZATION: MATERIAL QUALITY RISK MANAGEMENT CONSIDERATIONS

The principles of Quality by Design In accordance with ICH Q8-Q10 should be applied. A risk based approach should be used when performing process validation studies. Materials used in the manufacturing process can represent a significant source of risk that may impact the product's Critical Quality Attributes (CQAs). In order to mitigate risk the overall raw material control strategy needs to be assessed. By evaluating types of risks the necessary control to ensure robustness of the process and safety of the product can be defined. Issues such as scalability is one consideration when you design the manufacturing process. At early points in the product development process the Quality Target Product Profile (QTPP) must be determined that defines the desired product characteristics and sets goals for all developmental phases. For example, what raw and starting materials, reagents and consumables would be required to dose 200 patients/year with 10^{14} viral genomes (vg) / patient. Thorough understanding of the Product's CQA and Critical Process Parameters (CPP) must be determined. Granularity of this process can only serve to better define the quality attributes one hopes to build into the product. This could include, for example, compatibility of product contact with process equipment related leachables (PERLs) required to qualify plastic components such as single or multiple use systems. Risk assessment tools such as those described by the US Pharmacopeia General Chapters <1043>, <665> and <1665> or the Raw Material Sourcing, Quality and Volume Team within the Cell and Gene Therapy Phorum [6–9]. In fact, the Regulatory Governance Team in BioPhorum recently published a document entitled, "BioPhorum approach to the registration of innovative raw materials using Quality by Design principles" which introduces the terminology, "Target Material

BOX 2

Gene therapy regulatory guidance that address materials used in manufacturing human cell and gene therapy products

- FDA: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs): Guidance for Industry, January 2020.
- USP: General Chapters <1046> Cell Therapy Products, <1047> Gene Therapy Products, <1043> Ancillary Materials for Cell, Gene, and Tissue Engineered Products, <1024> Bovine Serum, <90> Fetal Bovine Serum – Quality Attributes and Functionality Tests
- EU: The Rules Governing Medicinal Products in the European Union, EudraLex Volume 4, Part IV. Guideline on Good Manufacturing Practice Specific to Advanced Therapy Medicinal Products, Directive 2001/83/EC, Ph.Eur. Chapter 5.2.12, Raw materials of biological origin for the production of cell-based and gene therapy medicinal products, Specific monographs, e.g. insulin, CSF, etc., EMA/CAT/852602/2018 31 Jan 2019 Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials.
- PIC/S: GMP Annex 2A Manufacture of ATMPs for Human Use
- ISO: ISO/DIS 20399 Biotechnology-Ancillary Materials present during the production of cellular therapeutic products and gene therapy products (Draft International Standard)
- ICH: ICH Q7A: Good Manufacturing Practice for Active Pharmaceutical Ingredients (Principles important although document excludes cell and gene therapy products)
- Note: US FDA regulatory guidance, EU directive and ISO standards are consistent about the definition of raw materials (also called ancillary products): 'Material that comes in contact with the cell or tissue product during cell processing but is not intended to be part of the final product formulation'.

Profile" or TMP consistent with QbD principles that would systematically assess the intended use, quality, safety and other criteria for raw materials used in cell and gene therapy manufacturing[10].

In February 2021, regulatory guidance from the European Medicines Agency was issued providing interpretation of the principles of Good Manufacturing Practice for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacture of ATMPs [11]. While not requiring a GMP certificate for manufacture and testing sites, compliance with the principles of GMP are considered mandatory by the ATMP manufacturer, sponsor, marketing authorization holder or importer to the European market.

In selecting raw and starting materials, application of the principles of GMP reflect a level of flexibility based on a Risk Based approach. It is inherent upon developers, especially at early stages of CGT development to inculcate a risk based decision making process that is well established and operational by trained staff within their establishments. Without these procedures in place GMP flexibility cannot be adequately quantified or defended. Quality, safety, and efficacy of the finished pharmaceutical dose can be jeopardized impacting the success of the entire developmental endeavor.

Risk factors that should be assessed when selecting raw and starting materials are identified in Box 3. If significant risks to the product are identified, measures must be taken to control risks with mitigation efforts defined and implemented.

It should be noted that the International Council for Harmonization (ICH) has recently issued a revision of the Q9 guideline, "Quality Risk Management", that aims to address the shortcomings of the current guideline [13]. Shortcomings of the current Q9 document have included, high levels of subjectivity in risk assessments and in QRM outputs, failure to adequately manage supply chain and product availability risks, lack of understanding as to what constitute formality in QRM work, and lack of clarity on risk based decision making. The ICH Q9 (R1) guideline was published on the European Medicine Agency (EMA) website on 16 December 2021, following ICH endorsement of the revision in mid-November. The document updates the original ICH Q9

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BOX 3

Partial list of risk factors for consideration when assessing raw and starting materials for a cell and gene therapy product

- Transmissible spongiform encephalopathy
- Potential for viral contamination and cross contamination with other vectors or other genetic material
- Replication competent virus (in case of replication-deficient viral vector). It should be demonstrated the absence of formation of replication competent virus at the level of the viral production system used.
- Potential for microbiological (e.g. Mycoplasma) or endotoxin/pyrogen contamination
- Potential, in general, for any impurity originating from the raw materials, or generated as part of the process and carried over
- Sterility assurance for materials claimed to be sterile
- Potential for any impurities carried over from other processes, in absence of dedicated equipment and/or facilities (for instance residual DNA (antibiotic resistance gene, residual DNA from potentially tumorigenic cell lines, etc.), substance of animal origin, antibiotic, etc.)
- Environmental control and storage/transportation conditions including cold chain management if appropriate
- Stability
- Supply chain complexity and integrity of packages

From EMA/246400/2021, Inspections, Human Medicines, Pharmacovigilance and Committees Division – 24 February 2021 [11,12].

guideline, which is now 16 years old. Plans are to issue the Step 4 guideline by June 2022. Cell and Gene Therapy developers should familiarize their organizations with the new guidance and incorporate its content in revised organization specific standard operating procedures.

LIST OF CONTENT & PARTICIPANTS FOR THE MARCH, 2022 ISSUE

This month's *Bioinsight Spotlight* is dedicated to a further deepening of the understanding of the complex issues surrounding raw and starting materials. We are fortunate to have notable authorities actively working in this industry agree to discuss these critical issues in much greater detail. Here is a brief summary of their contributions to this issue.

In their excellent Commentary, Lim and Goojar from the UK's Advanced Therapy Manufacturing (GMP) Unit of the National Institute of Health Research describes the challenges in qualifying raw materials in ATMP applications. These scientists provide a comprehensive and globally focused summary of the available guidance and risk management approaches developers can pursue to ensure the highest quality in raw materials required for ATMP production. There is a special focus on risk minimization in controlling raw materials of biological origin. There are three superb papers on the collection and qualification of cells required as starting materials. Melissa Carpenter, CSO of Elevate Bio describes their approach to produce GTP, GMP compliant clinical grade induced pluripotent stem cells (iPSC), a challenge for many developers requiring these cells as starting material. Barbara Seymour, Senior Director of Manufacturing at Generation Bio discusses some of the considerations and challenges they face with sourcing donor supplied human stem cells for use in allogeneic cell therapies. Joseph M Roig of BMS provides a critical assessment of the challenges in managing variability in autologous apheresis products that collect peripheral blood mononuclear cells (PBMCs) as a raw starting material. The interview with

Dennis Royal of NKarta describes his concerns in maintaining the supply chain for Phase 1 clinical trials in a post pandemic world. John Duguid, Senior Director of Research and Development at Vericel Corporation, also describes the potential for supply chain disruptions due to the COVID-19 pandemic and how the cell and gene therapy industry must develop, in addition to routine raw material qualification procedures, emergency response mechanisms to reduce risk and maintain an adequate safety stock. Biswarup Dasgupta provides his perspectives on ensuring GMP compliance as these novel AAV vector-based gene therapies move through the regulatory pathway. Dr Fouad Atouf, Vice President of Global Biologics at the U.S Pharmacopeia (USP) describes his role at the USP in providing scientists, developers, manufacturers, and regulators with document and reference standards to ensure the quality and development of novel biological advanced therapeutics. Gosse, et al, from Alcyone Therapeutics discusses in clear, concise terms plasmid DNA manufacturing and the evolving regulatory guidance depending upon the function of DNA plasmids as raw materials, intermediates, drug substances or products.

CONCLUSIONS

Manufacturers of cell and gene therapies must ensure that all components used are appropriately qualified and consistent with the Quality Target Product Profile (QTPP). Quality specifications for gene therapy raw materials should confirm the product's quality, patient safety, product efficacy, and process consistency.

Characterization of the gene therapy product early in development can serve to:

1. Improve manufacturing practices to increase productivity and process recovery,
2. Reduce variability and optimize analytics for characterization, quantitation, and functionality,
3. Assess and mitigate risks by analyzing failure modes and stability profiles,
4. Standardize ancillary materials to facilitate consistency in manufacturing of finished products (USP guidance),
5. Facilitate early scale up and engagement of commercialization partners,
6. Create a robust Quality Agreement with CDMOs and suppliers that will build trust and communication transparency, minimize risks and costs, and maximize yields and results.

REFERENCES

1. Lambert J, Nuwaysir E, Smith B *et al.*Cell and Gene State of the Industry Briefing. Alliance for Regenerative Medicine. Jan 10, 2022.
2. Parenteral Drug Association. Technical Report No. 56 (revised 2016). Application of Phase Appropriate Quality System and cGMP to the Development of Therapeutic Protein Drug Substance (API or Biological Active Substance). Parenteral Drug Association, Bethesda, MD.
3. du Moulin GC: Regenerative Medicine Advanced Therapies: Phase Appropriate Good Manufacturing Practices. In Phase Appropriate Good Manufacturing Practices for Biological Processes. 2018; Ed. Deeks T, Parenteral Drug Association, Bethesda, MD, pp 341-397.
4. Scott, M, Clarke D, Lipsitz Y *et al.* Transitioning from development to commercial: Risk-based guidance for critical materials management in cell therapies. *Cytotherapy*. 2020; 22, 669–678.
5. du Moulin GC: Considerations for performing virtual quality audits on manufacturers of gene therapy viral vectors: An auditor's perspective during the COVID-19 public health emergency. *Cell & Gene Therapy Insights*. 2021; 7, 199–210.
6. United States Pharmacopeia. General Chapter <1043> "Ancillary Materials for Cell, Gene and Tissue Engineered Products".
7. BioPhorum Operations group, Ltd. Raw material sourcing, quality and volume team (Cell and Gene Therapy Phorum). Perspectives on raw and starting materials risk assessment for cell and gene therapy (CGT) processes. October 2020.
8. United States Pharmacopeia. General Chapter <665>. Plastic components of systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances or products (To be official May 1, 2022)

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9. United States Pharmacopeia. General Chapter <1665>. Characterization and qualification of plastic components of systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances or products (To be official May 1, 2022)

10. BioPhorum Operations group, Ltd. Biophorum approach to the registration of innovative raw materials using quality by design principles. January, 2022.

11. European Medicines Agency. Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs. EMA/246400/2021.

12. Barone P, Wiebe M, Leung J, *et al.* Viral contamination in biologic manufacture and implications for emerging therapies. *Nature Biotechnol.* 2020; 38, 563–572.

13. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonized Guideline. Quality Risk Management – Q9 (R1) Draft version. Endorsed on 18 November 2021.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

EXPERT INSIGHT

Considerations for use of hematopoietic stem cells in allogeneic therapies

Barbara Seymour

This article reviews some of the considerations and unforeseen challenges with sourcing and preparing regulatory filings using donor supplied Hematopoietic stem cells (HSCS) in novel allogeneic cell and gene therapies. There are unique complications not seen with autologous therapies as developers must understand the regulatory requirements for donor sourcing, testing and isolation of $CD34^+$ cells for IND submissions. Finding the right partner for sourcing as well as lot release testing is discussed. Understanding the procurement requirements for the starting cells and the manufacturing process used is reviewed. This article covers some of the areas that organizations may miss when developing these types of cell and gene therapy products for the first time.

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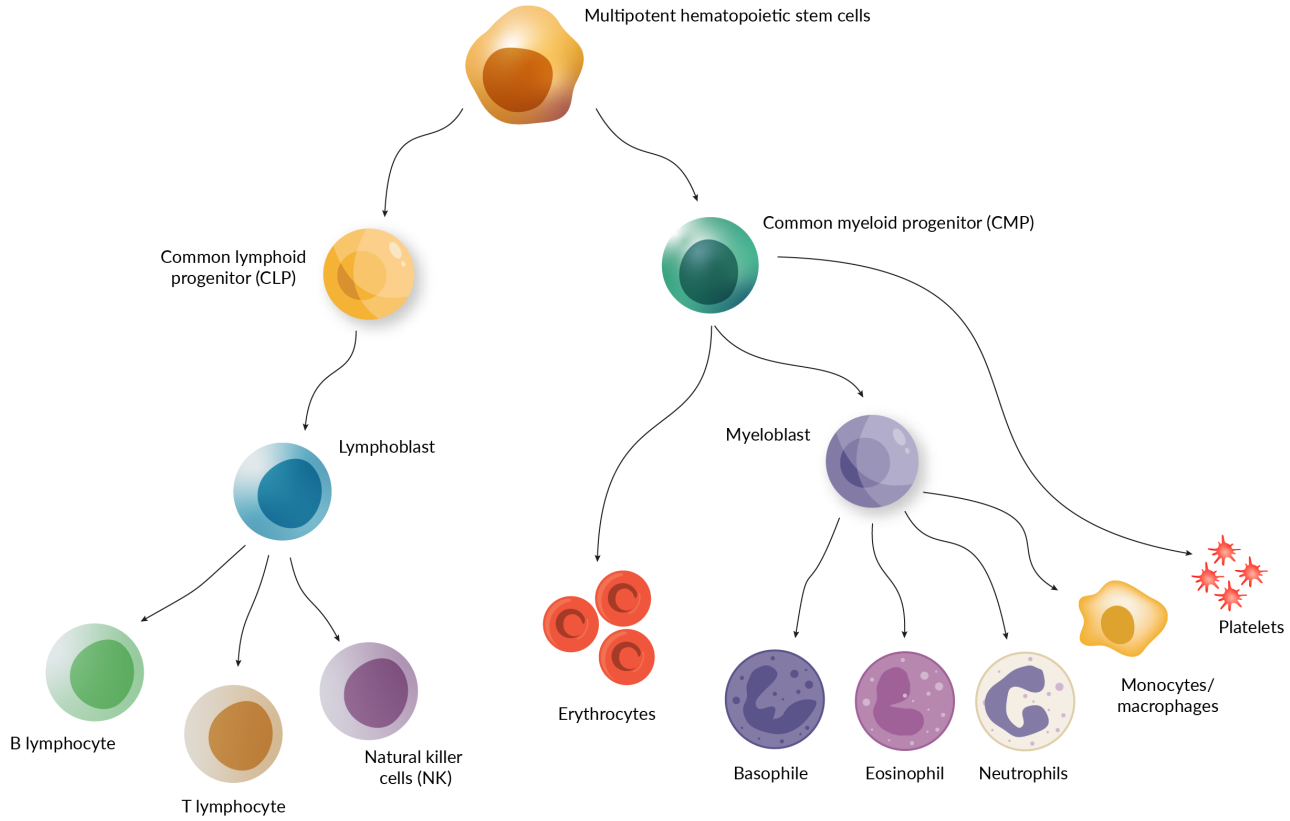
Hematopoietic stem cells (HSCs), discovered in the 1960s, have seen an increase in the number of indications for oncology and regenerative medicine due to the unique self-renewal potential and multi potent potential of these cells [1,2] (Figure 1). The World Marrow Donation Association matches more than 50,000 patients worldwide with transplants every year [3]. Beyond using HSCs for hematopoietic cell transplantation (HCT) after cancer therapy, many biotechnology companies have looked to capitalize on the use of donor HSCs for novel cell and gene therapy products. Use of

FIGURE 1

Hematopoietic stem cell differentiation.



Differentiation of hematopoietic multipotent stem cells into progenitor cells which then differentiate into distinct hematopoietic lineages [5].

an allogeneic starting material like HSCs assumes a cost of goods saving with large-scale manufacturing potential that makes these products very attractive for biotechnology companies. Understanding the procurement and regulatory demands of human cells, tissues, and cellular and tissue-based product (HCT/P) which are considered than minimally manipulated per FDA 2020 guidance [4] is essential for a successful clinical and subsequent commercial program.

DONATION TYPES

Developing successful products using donor-based HSCs for allogenic clinical applications must be strategically planned to meet regulatory requirements and eventual scale-up demands as products move into commercialization. There are three major sources of hematopoietic stem cells (HSCs); bone marrow harvested by aspiration from the cavity of the ilium (hipbone), peripheral blood obtained through leukapheresis, and umbilical cord blood (UCB) collected from the placenta after childbirth [6]. Bone marrow (BM) aspiration is a surgical procedure that takes place in a hospital operating room. A peripheral blood stem cell (PBSC) apheresis takes place at a donation center and requires donors to be treated with a cytokine and small molecule regimen to mobilize HSC from the BM into the peripheral blood. There are different types of mobilization schemes for a PBSC donation, the most traditional being daily injections of a recombinant human granulocyte colony-stimulating factor (rhG-CSF)

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for 5 days. This can also be paired with Mozobil a CXCR4 chemokine receptor which is shown to be the best regimen for obtaining the highest total nucleated count and $CD34^+$ percentage [7]. Side effects of mobilization are typically skeletal pain or flu like symptoms [8].

The source of the starting material should be evaluated by the team developing the product and be based on critical quality attributes. Use of UBC provides an alternative to the invasive extraction of BM or exposure to mobilizing agents prior to the collection of HSC from PBSC. While quantitatively limited, the collection of UCB takes nothing away from the neonate or the mother, therefore donor safety is a major advantage [9]. A study was done using the same donor to collect BM and then PBSCs after mobilization with glycosylated G-CSF for 4 days. The report found BM collections to contain a higher proportion of $CD34^+$cells (1.3% vs 0.7%, $P < 0.0001$) and a comparable proportion of $CD3^+$ cells (median 29% vs 26%, $P = 0.4$) however the absolute numbers of CD34+ and $CD3^+$ cells were several times higher in PBSC collections [10].

SOURCING STRATEGIES

Sourcing strategies must be considered early in the product lifecycle to ensure HSCs are available for development, clinical and ongoing commercial supply. The donor population of an allogenic starting material can be dramatically limited if a universal donor is required for the allogeneic cell product(s). Programs such as a red cell therapy may require an O negative blood type which limits the total pool to about 7% of the population. On average only 5% of the population is willing to donate whole blood, [11] and asking a donor to be mobilized in a multi-day procedure further reduces this population to approximately 4% [12].

Donors must then be evaluated for suitability by:

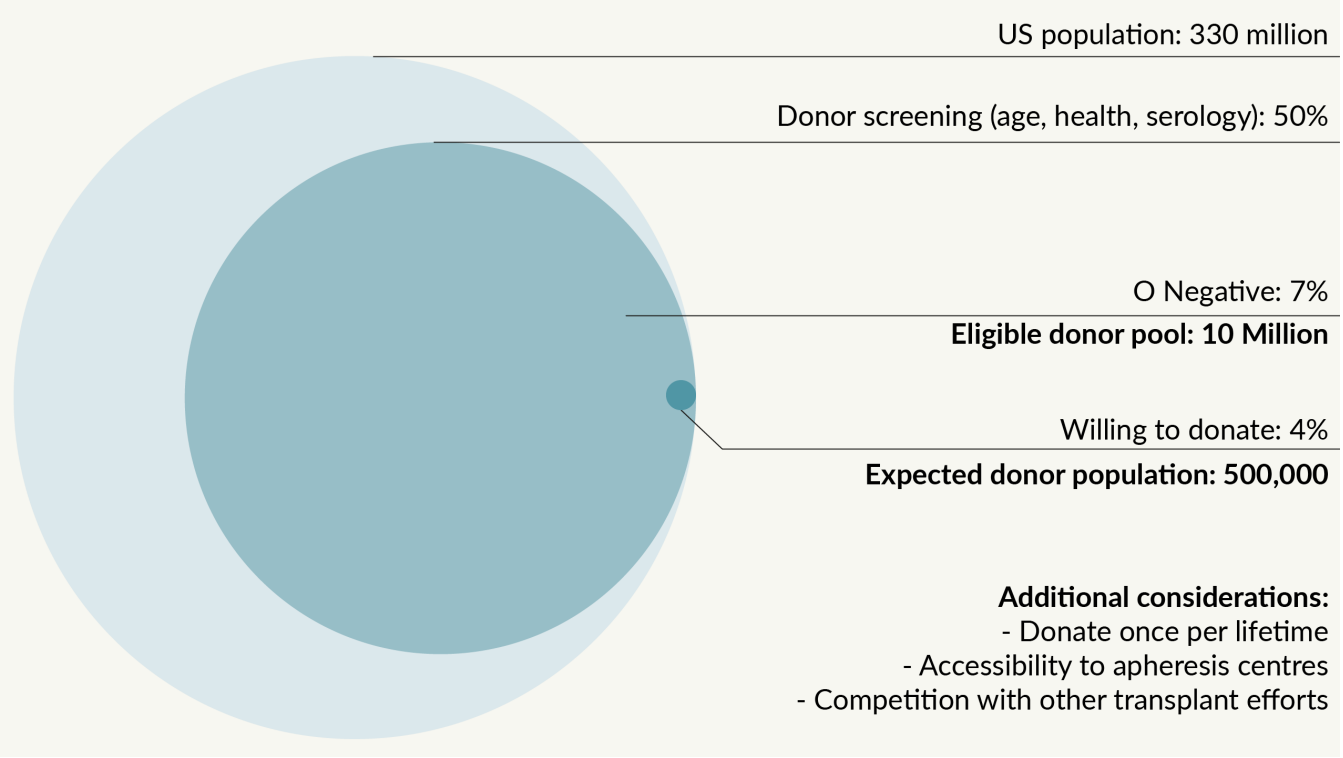
1. Detailed medical history, supported by a standardized questionnaire;
2. Physical assessment with special consideration of peripheral veins;
3. Electrocardiogram (ECG) at rest;
4. Ultrasound examination of the upper abdomen with measurement of spleen diameter;
5. Laboratory examinations including complete blood count with differential, clinical chemistry (liver enzymes, electrolytes, metabolic parameters, serum protein electrophoresis), urinalysis, infectious disease markers (hepatitis A/B/C, HIV, human T-cell lymphotropic virus type (HTLV) I/II, syphilis, toxoplasmosis, Epstein-Barr virus (EBV), cytomegalovirus (CMV), blood group/Rh testing, and pregnancy test in women of childbearing age (urine or serum)[7] Compounding requirements for age and health and CMV negative status further cuts this limited pool in half again, to less than 0.15% of the population [13] (Figure 2).

There are a number of centers across the U.S. who will manage the screening and collection of mobilized PBSCs from consented healthy donors (All Cells, Be the Match, Key Biologics, BloodWorks NW). The clinical sponsor will need an approved Internal Review Board (IRB) Collection Protocol for these sites to use and therefore are responsible for any adverse events to the donors. Many sponsors have limited a donor to one mobilized donation per lifetime.

The collection is considered part of the manufacturing process with the regulatory expectation that the IND will include the procedures for collecting, testing, shipping, and storing the apheresis starting material. The IND should also include the Informed Consent Form (ICF) used by the donation site. The sponsor is also responsible for qualifying the incoming starting material

FIGURE 2

Donor population estimations.



and demonstrating that donor testing is performed according to 21 CFR Part 1271, Subpart C [14], including a negative Zika virus test (Table 1). Requirements for a buccal swab for COVID 19 screening has been revised a few times over the past year and are currently not required by FDA as the donor must be in good health and meet all donor eligibility criteria on the day of donation (21 CFR Part 630, Subpart B [15]).

ISOLATION OF CD34⁺ CELLS

After the collection of the UCB, BM or PBSC, cells can be frozen prior to selection of the CD34⁺ cells or shipped to a location (manufacturing site) for isolation of the fresh cells. Transport of the UCB/BM/PBSC cells (if performed prior to isolation) must be in a validated container. A description of the facilities, equipment, and procedures that are in place to ensure aseptic processing of the product and to prevent contamination, cross-contamination, and mix-ups must be included in the IND. The sponsor will also be required to describe the procedures that are in place for environmental monitoring, product segregation and equipment / facility cleaning.

Time to transport and condition of transport has been documented in several publications but should be confirmed by the sponsor for the starting material and differentiating process they are running. Cord blood has been documented to show an increase in CD34⁺ cells after room temperature storage for 48 h [16]. Bone marrow should be stored at room temperature and processed within 24 h, [17] while data shows a mobilized apheresis collection should be stored at 4°C for up to 48 h [18,19].

The method used for isolation of the CD34⁺ cells will need to be described in the IND along with all equipment and reagents. If an automated system is used, the sponsor should obtain a letter of authorization (LoA) from the vendor to the Biologics Master File (BB-MF) of the instrument.

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TABLE 1

Infectious disease testing for HSC Donation*.

Test description	Acceptance criteria
Hepatitis B Surface Antigen (HBsAg)	Nonreactive
Hepatitis B Core Antibody (Anti-HBc)	Nonreactive
Hepatitis C Virus Antibody (Anti-HCV)	Nonreactive
Human Immunodeficiency Virus Antibody (HIV-1 /2)	Nonreactive
Human T-Lymphotropic Virus Antibody (HTLV-I/II)	Nonreactive
Syphilis	Nonreactive
Trypanosoma cruzi Antibody	Nonreactive
H Human Immunodeficiency Virus -1 NAT	Negative
Hepatitis B NAT**	Negative
Hepatitis C NAT	Negative
West Nile Virus NAT	Negative
Zika NAT	Negative
Anti CMV antibody	Negative

* Other infectious agents may be required as defined by local regulations

** NAT – Nucleic Acid Test

Some of the current reagents used for CD34⁺ isolation are of human (IgG, albumin) or murine origin (CD34 reagent kit) and will also need an LoA or a Humanitarian Device Exemption Number from the vendor included in the IND. Most teams will cryopreserve isolated CD34⁺ cells after isolation in aliquots for later use. The cryoprotectant should be in an animal origin free solution as much as possible. If the collection was frozen prior to isolation, the sponsor should show data that the additional cryopreservation step will not have a negative impact on the starting CD34⁺ cells. Following the isolation, the CD34⁺ cells must be qualified prior to use in the manufacturing process. Table 2 lists a standard set of specifications for these cells as a starting material.

USING ALLOGENIC HSCS FOR A GMP MANUFACTURED PRODUCT

Donor to donor differences will need to be addressed in the development of the manufacturing process. Having a consistent manufacturing process that does not require surface marker or growth-based decisions is preferred with contract manufacturing organizations who plan room allocations and staff on a day-by-day basis. A well-designed manufacturing process that takes into account differences between donors may require using more cells to start the run than a typical characterized cell bank. The starting material can also be prescreened prior to use on the manufacturing floor to determine the number of vials needed to initiate a run, thus saving cells and reducing waste.

Testing of an allogeneic product beyond the standard lot release testing for sterility, endotoxin, mycoplasma, potency, identify and purity must also include tests for the presence of the following human viruses to reduce potential risk to clinical trial subjects:

- Human immunodeficiency virus (HIV)1/2
- Human T-cell leukemia virus (HTLV)1/2
- Hepatitis C virus (HCV)
- Hepatitis A virus (HAV)
- Hepatitis B virus (HBV)
- Cytomegalovirus (CMV)
- Human herpes virus (HHV)6/7/8
- Herpes simplex virus (HSV)1/2
- Parvovirus B19 (B19)
- Epstein-Barr virus (EBV)
- West Nile Virus (WNV)

These tests can be carried out using Polymerase Chain Reaction technology by major

TABLE 2

Specification of CD34⁺ Cells.

Parameter	Specification
CD34+ Viability	≥ 70%
CD34+ Purity	FIO* (typically >90%)
CD45+ Viability	≥ 70%
Sterility	No growth
Endotoxin	FIO
Mycoplasma	Negative

* In early phases of a program, CD34⁺ purity after isolation is typically left as For Information Only (FIO) until sufficient runs are performed to allow a specification to be set.

testing labs such as Charles River, Eurofins, or BioReliance. These companies should be able to provide a testing panel capable of detecting a broad range of Herpesviruses is used, as Herpesviruses can remain latent in various cell compartments (for example Epstein-Barr Virus in B cells and CMV in hematopoietic cells). These panels can detect the following: Herpes Simplex Virus (HSV) 1, HSV 2, Epstein Barr Virus (EBV), Cytomegalovirus (CMV), Human Herpes Virus (HHV) 6, HHV 7 and HHV 8, among others.

All tests should be performed using either FDA licensed, approved or cleared test kits, and the tests should be performed in a CLIA certified or equivalent laboratory.

SUMMARY

The use of allogenic HSCs in developing novel cell and gene therapies has promise and potential to treat a variety of oncology conditions or as a regenerative therapy. The perceived cost of goods savings on production of large-scale batches of allogeneic products is slightly offset by the additional testing requirements of the donors and final product cells. Obtaining strategic partners to help source donors and perform the isolation of $CD34^+$ cells, as well as release testing, is essential for long term supply and success of the programs. Information of the starting process from donor selection onward must be included in the CMC section of the IND. The IND sponsor is responsible for all aspects of the trial including the health of mobilization of the donors (if performed).

TRANSLATION INSIGHT

The use of donor material as a starting source for small scale clinical runs can present a number of challenges to success for long-term, large scale commercial programs. Depending on the program and use of the starting cells, the number of runs possible from one donation can be large or small, but it will always be finite. Developing programs using stem cells from donors has limitations that were not present in the development of monoclonal antibodies where master cell banks and working cell banks could be created. Thinking about ways to generate alternate cell sources (such as iPSC) or looking at ways to increase the yields of the isolated $CD34^+$ cells or in expansion of these should be determined early in the development lifecycle. Making a change to a critical starting material after initial proof of concept may require additional arms in a clinical study for comparability purposes. Developing an immortalized HSC for use in therapeutic indications may be the benchmark, but there are very high hurdles to confirm these cells will be suppressed once used therapeutically and not contribute to the development of cancer.

REFERENCES

1. Müller AM, Huppertz S and Henschler R. Hematopoietic stem cells in regenerative medicine: astray or on the path? *Transfus. Med. Hemother*. 2016; 43, 247–254.
2. Wang, Xiuyan, and Isabelle Rivière. Genetic engineering and manufacturing of hematopoietic stem cells. *Mol Ther. Methods Clin. Dev*. 2017; 5, 96–105.
3. Donation: The Basics. *WDDA*. https://wmda.info/donor/basics-donation/ (Accessed Feb 2022)
4. Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use. *FDA*. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/regulatory-considerations-human-cells-tissues-and-cellular-and-tissue-based-products-minimal. (Accessed Feb 2022)
5. Alomari M, Almohazey D, Almofty SA, Khan FA, Al hamad M, Ababneh D. Role of Lipid Rafts in Hematopoietic Stem Cells Homing, Mobilization, Hibernation, and Differentiation. *Cells* 2019; 8, 630.
6. Barriga F, Ramírez P, Wietstruck A, Rojas N. Hematopoietic stem cell transplantation: clinical use and perspectives. *Biol Res*. 2012; 45, 307–16
7. Fruehauf S, Veldwijk MR, Seeger T et al. A combination of granulocyte-colony-stimulating factor (G-CSF) and plerixafor mobilizes more primitive peripheral blood progenitor cells than G-CSF alone: results of a European phase II study. *Cytotherapy*. 2009; 11, 992–1001.

DOI: 10.18609/cgti.2022.040

8. Hölig K. G-CSF in Healthy Allogeneic Stem Cell Donors. *Transfus. Med. Hemother.* 2013; 40, 225–235

9. Gupta AO and Wagner JE. Umbilical Cord Blood Transplants: Current Status and Evolving Therapies. Front. *Pediatr.* 2020; 570282.

10. Singhal S, Powles R, Kulkarni S *et al.* Comparison of marrow and blood cell yields from the same donors in a double-blind, randomized study of allogeneic marrow vs blood stem cell transplantation. *Bone Marrow Transplant* 2000; 25, 501–505.

11. Patel EU, Bloch EM, Grabowski MK *et al.* Sociodemographic and behavioral characteristics associated with blood donation in the United States: a population-based study. *Transfusion* 2019; 59, 2899–2907.

12. Ting CY, Lee YF, Lim C *et al.* Factors Associated with Intention to Donate Hematopoietic Stem Cells among Blood Donors. *Transfus. Med. Hemother.* 2021; 48, 188–195.

13. Cytomegalovirus Infections. *Medicine Plus* 2016. https://medlineplus.gov/cytomegalovirusinfections.html (Acessed Feb 2022).

14. 21 C.F.R § 1271. *Code of Federal Regulations*. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-L/part-1271/subpart-C?toc=1 (Accessed Feb 2022)

15. 21 C.F.R § 630. *Code of Federal Regulations*.- https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-630/subpart-B?toc= (Acessed Feb 2022).

16. Moldenhauer A, Wolf J, Habermann G, Genter G, Kiesewetter H, Salama A. Optimum storage conditions for cord blood-derived hematopoietic progenitor cells prior to isolation. *Bone Marrow Transplant*. 2007 Nov; 40, 837–42.

17. Hahn S, Sireis W, Hourfar K *et al.* Effects of storage temperature on hematopoietic stability and microbial safety of BM aspirates. *Bone Marrow Transplant* 2014. 49, 338–348.

18. Lazarus HM, Pecora AL, Shea TC *et al.* CD34+ selection of hematopoietic blood cell collections and autotransplantation in lymphoma: overnight storage of cells at 4 degrees C does not affect outcome. *Bone Marrow Transplant*. 2000 Mar; 25, 559–66.

19. Wiercinska E, Schlipfenbacher V, Bug G *et al.* Allogeneic transplant procurement in the times of COVID-19: Quality report from the central European cryopreservation site. *J Transl Med.* 2021; 19,145.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

COMMENTARY/OPINION

Regulatory & supply chain implications for plasmids as critical starting materials in the manufacture of viral vector gene therapy products

Melissa Gosse, Cameron Jones, Desyree Jesus & Susan D'Costa

Plasmid DNA is actively being used as starting material/intermediates, drug substance, and/or drug product in the manufacture of several therapeutics including DNA vaccines, viral-vector and non-viral-vector gene therapies, and mRNA vaccines. Since plasmids have a large range of uses, there have been various plasmid guidelines released from the different agencies. Specifically for gene therapy, recent guidelines have tried to provide clearer guidance, however there is still ambiguity around quality requirements. For example, the specifications for the release of plasmids from a plasmid manufacturer and the facility requirements for accepting these plasmids for use in viral vector manufacturing are not always aligned and can cause disconnect in the supply chain. For a sponsor managing plasmid manufacturing for use as starting material in viral vector manufacturing, it is important to be aware of these differences in requirements ahead of time. The current manuscript highlights these regulatory differences and calls out the importance for a sponsor to build a robust supply chain between the plasmid manufacturer and the viral vector manufacturer well in advance to minimize bottlenecks due to quality requirements and timelines.

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Plasmids are small, circular, extrachromosomal DNA that replicate independently of the host DNA. They occur naturally in bacteria like E. coli and provide the host a competitive advantage including antibiotic resistance and the ability to survive extreme environments [1]. Plasmids have become fundamental tools for recombinant DNA technology; given their smaller size and greater number than the host chromosome, they can be easily isolated in pure form. Additionally, they are stable and easy to manipulate and replace non-essential genetic material with therapeutic genes of interest [1]. At a therapeutic level, plasmid DNA (pDNA) is being actively used for DNA vaccines and gene therapies against several infectious-, acquired-, and genetic-diseases. In addition, pDNA is used as critical starting material for viral vector vaccines, mRNA vaccines, and viral vector gene therapies (Box 1) [2].

Plasmid DNA manufacture is critical when viral vectors are produced via transient transfection. Manufacture of these viral vector products involves the transfection of mammalian cells utilizing one pDNA containing the genetic elements including the therapeutic gene of interest to be incorporated into the viral vector, and one to three additional pDNAs containing the helper functions for viral vector production [3]. Manufacture of these plasmids poses regulatory challenges since plasmid DNA manufacturers maintain their own manufacturing processes and differ in their quality and testing approaches. These regulatory challenges are discussed further in the sections below. Guidances for plasmid manufacture focus primarily on their use as therapeutics [4–6]. However, plasmids also function as critical starting materials in viral vector production [7]. Given the increasing regulatory stringency in subsequent stages of clinical development, it is important to reconcile the production and procurement of pDNA by evaluating the different needs from a quality and testing perspective.

Depending on the type of plasmid use (whether utilized as starting/critical raw material, intermediate, drug substance, or drug product in drug development), not only are there different agencies pushing different guidelines, but there are also different specifications applicable to the release product, from manufacturer, to its acceptance downstream. This clearly causes a disconnect in the supply chain of these plasmids, as it will be discussed further in the sections below.

BOX 1

Therapeutic uses of plasmids.

DNA vaccines and gene therapies for:
- Infectious diseases
- Acquired diseases
- Genetic diseases

Critical starting material for:
- Viral vector vaccines
- mRNA vaccines
- Viral vector gene therapies

PLASMID DNA DESIGN & MANUFACTURE OVERVIEW

While individual plasmid producers utilize their own platform process for the design and manufacture of plasmid DNA, the basic principles are the same as shown in Figures 1 & 2.

At a high level, the backbone for each recombinant plasmid contains an origin of replication, an antibiotic resistance gene for selective pressure during replication, and optimized genetic elements (promoters, enhancers, helper genes, vector transgenes, etc.) needed for viral vector production (Figure 1). Recombinant plasmids are used to transform appropriate strains of E. coli. and clones of transformed, antibiotic-resistant E. coli are selected. The optimally producing clones are then expanded to produce master and working cell banks used for further plasmid production [8].

The manufacturing process utilizes high-density fermentation conducive to the production of supercoiled plasmid DNA (Figure 2). During downstream purification, the bacterial cells are pelleted, lysed, and processed through one or more chromatography steps to enrich super-coiled plasmid DNA away from host cell impurities, and linear,

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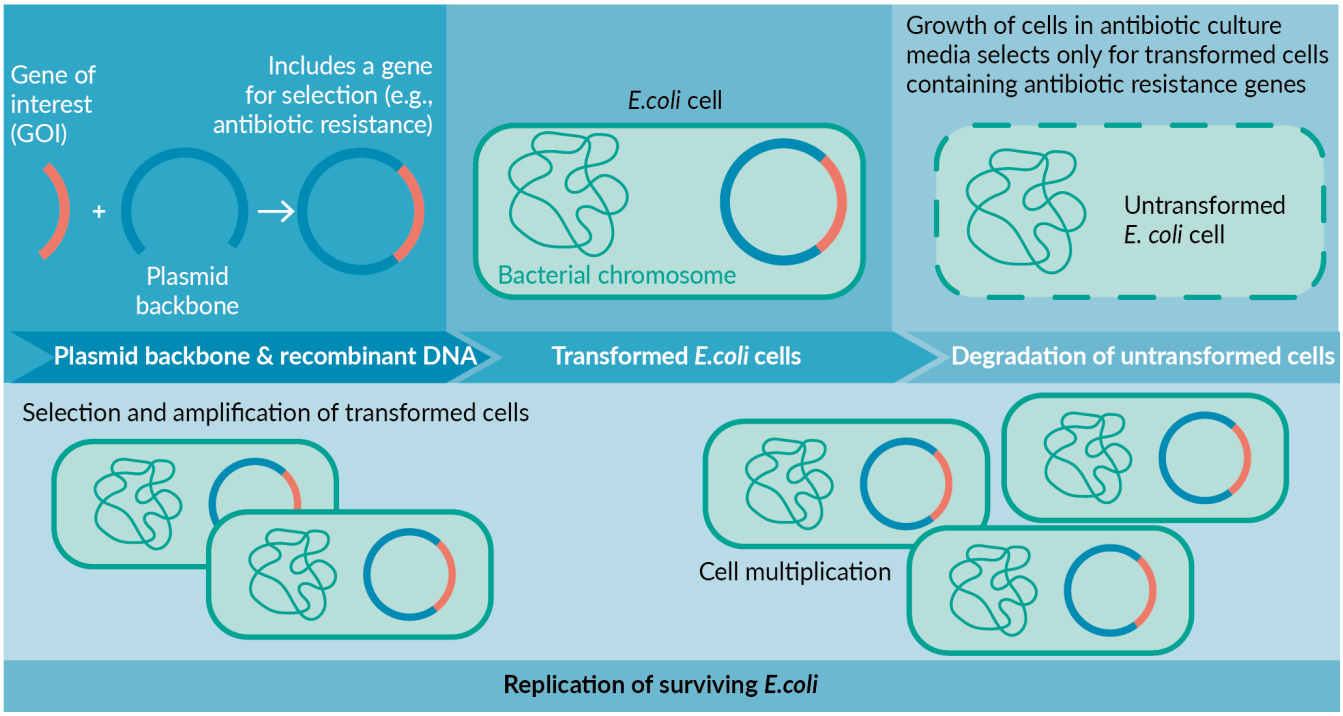
FIGURE 1

Plasmid DNA – design and optimization. steps involved in design, selection and optimization of E. coli containing recombinant plasmid.



nicked, and open/closed plasmid forms [8]. Regulatorily, there is gathering impetus to use single-use systems to produce plasmids used in viral vector manufacture.

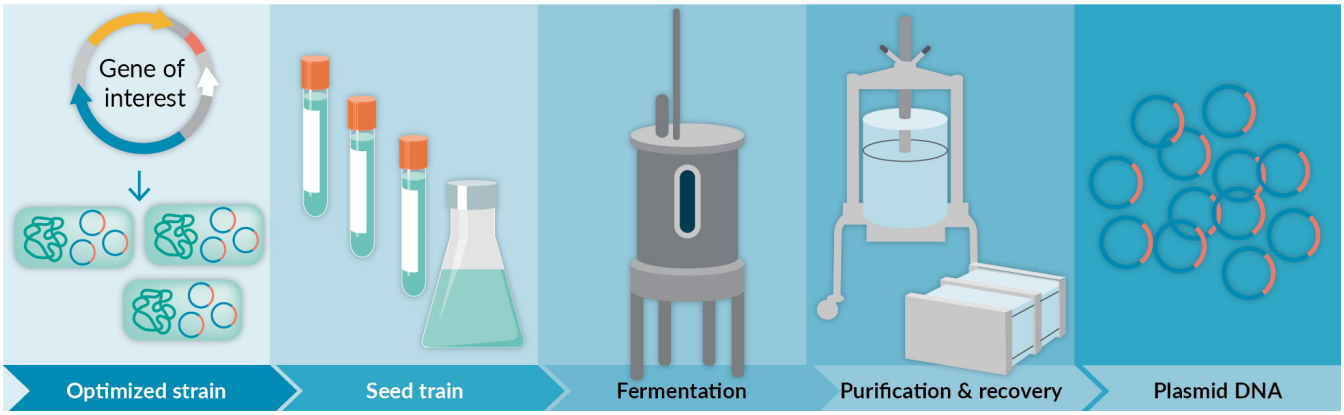
GUIDANCE FOR USE OF PLASMIDS IN VIRAL VECTOR MANUFACTURE USING TRANSIENT TRANSFECTION

Utilizing phase appropriate quality systems for the release of plasmid DNA in viral vector production is important, as the regulatory expectations around plasmids used for transient transfection in manufacturing viral vectors, continue to evolve. Plasmids for vector manufacturing are clearly defined as a starting material for advanced therapy medicinal products (ATMPs) (not as a raw material) via the Annex to the EU Directive 2009/120/EC, Part IV, Section 3.2 [9] and referenced in the EMA's (European Medicines Agency) 2018 guideline [10]. However, the FDA (United States Food and Drug Administration), has referred to these plasmids as intermediates in the 2020 FDA Chemistry, Manufacturing, and Control (CMC) Guidance for Gene Therapy IND (investigational new drug) applications [11]. An intermediate is defined in ICH Q7 as a material produced during steps of the processing of an API (active pharmaceutical ingredient) that undergoes further molecular change or purification before it becomes an API [12]. In comparison, ICH Q7 defines a starting material as a material that is used in the production of an API and is incorporated as a significant structural fragment into the structure of the API [12].

In April 2021, the EMA announced the availability of a Questions and Answers (Q&A) Guidance around the grades of plasmids required for the production of ATMPs [7]. Specifically, the EMA states that while a GMP (good manufacturing practice) certificate is not required for manufacturing and testing sites of starting materials for ATMPs, these sites should conform and comply with principles of GMP. The EMA also clarified that a QP (Qualified Person in the EU)

FIGURE 2

Plasmid DNA - Manufacturing Overview. Expansion of master cell bank, high density fermentation and downstream purification



release would not be needed. Table 1 is presented in the Q&A Guideline to help clarify the EMA's expectations around the level of GMP controls needed for different products using plasmid DNA. A gradient system is used to define the level of control: dark gray is used to denote where GMP manufacturing is applicable, and light gray is used to denote where GMP principles should be applied. As an example, for in vivo viral vector gene therapy ATMP manufacturing, plasmid manufacturing is shown in light gray, indicating GMP principles should be followed and full GMP procedures are not required.

The 2021 EMA Q&A Guideline also gives guidance on how to select the GMP principles to be followed using a risk-based approach. In summary, the starting material manufacturer should be qualified by the ATMP manufacturer. The ATMP manufacturer should have an agreement in place with the starting material manufacturer regarding procedures to

TABLE 1

Table from 2021 EMA Q&A on the principles of GMP manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs [7].

Example Products	Application of GMP to manufacturing steps is shaded in dark blue GMP principles should be applied where shaded in light blue Starting material – active substance – finished product →			
In vivo gene therapy: mRNA	Plasmid manufacturing and linearizion	*In vitro* transcription	**mRNA** manufacturing and purification	Formulation filing
In vivo gene therapy non-viral vector (e.g. naked DNA)	Plasmid manufacturing	Establishment of bacterial bank (MCB, WCB)	**DNA** manufacturing, fermentation and purification	Formulation filing
In vivo gene therapy viral vectors	Plasmid manufacturing	Establishment of a cell bank (MCB, WCB) and virus seed when applicable	**Vector** manufacturing and purification	Formulation filing
Ex-vivo genetically modified cells[3]	Donation procurement and testing of tissues/cells[1]	Establishment of a cell bank (MCB, WCB) and/or vector expansion and virus seeds when applicable	Plasmid manufacturing Vector manufacturing	**Genetically modified cells** manufacturing	Formulation filing

In the table above, the AMTP starting materials are underlined and the ATMP active substances appear in **bold**. The construction of the plasmid by in silico and molecular biology methods occurs before the plasmid manufacturing and is considered research and development. Therefore it is not under the scope of the current Q&A.

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TABLE 2

Analytical Tests and Example Methods for release of Plasmid DNA used in viral vector manufacture.

Analytical test	Example methods
Identity	Restriction Fragment Length Polymorphism (RFLP), Sanger Sequencing, Next Generation Sequencing (NGS)
DNA Concentration	UV spectrometry
Purity	UV spectrometry (OD 260/280), Agarose Gel Electrophoresis (% supercoiled DNA, total DNA), Capillary Gel Electrophoresis (% supercoiled DNA)
pH	USP<791>
Osmolality	USP<785>
Residual Kanamycin	qPCR/ddPCR
Residual host genomic DNA	qPCR/ddPCR
Residual host cell protein	ELISA, micro BCA
Residual RNA	RTPCR, reverse phase HPLC, Agarose gel electrophoresis stained with SYBR Gold
Bioburden/Sterility	USP<61>/USP<71>
Endotoxin	USP<85>
Mycoplasma	USP<63>
Appearance	Visual Inspection

follow. Those procedures should be proportionate to the potential impact of the starting material in the quality, safety, and efficacy of the finished medicinal product. In addition, the Q&A Guidance states that sterile or low bioburden starting materials which can be sterile filtered should follow relevant sterilization guidelines and aseptic manufacturing practices.

While this guidance helps clarify EMA's expectations, the latest from the FDA regarding plasmid controls comes from the 2020 guidance [11]. The FDA refers to plasmids used for transient transfection to manufacture viral vectors as 'intermediates' and specific GMP requirements are not defined. The 2020 guidance mentions the plasmid DNA should be made from qualified banks and the manufacturing procedures, reagents, and specifications should be listed in the IND. Testing suggested for the plasmids to include sterility, endotoxin, purity, and identity. See Table 2 for examples of release test methods for these assays. It is also noted that the MCB (master cell bank) for a bacterial bank of a plasmid intermediate may not be necessary for early phase, but that the details around the history and derivation of materials used to generate

TABLE 3

Analytical Tests and Example Methods for release of E. coli. master cell bank used in plasmid manufacture.

Analytical test	Example methods
Identity	Restriction Fragment Length Polymorphism (RFLP), Sanger Sequencing, Next Generation Sequencing (NGS)
Host Cell Identity	Confirmation of Species by API 20 Test Confirmation of Gram-negative rod form by Gram staining Colony Morphology Confirmation of Phenotype
Plasmid Retention	Confirmation of drug resistance by antibiotic typing
Viable Count	Microbial enumeration and counting
Purity Test	Microbial enumeration to detect absence of bacterial and/or fungal, and bacteriophage contamination
Plasmid Copy Number	qPCR/ddPCR

a bank should be described in the IND. The guidance also lists some recommended testing for bacterial cell banks used to manufacture the plasmid intermediate, including but not limited to, bacterial host strain ID, plasmid presence, cell count, plasmid ID by restriction enzyme (RE) analysis, plasmid sequencing and host strain purity (Table 3).

Considering the various recommendations given by the EMA and FDA, contract plasmid manufacturers must decide the level of controls they will put in place for their platform plasmid manufacturing process and their testing strategy. Plasmid manufacturers must consider the types of plasmid products they intend to manufacture, which include starting material plasmids for viral vector manufacturing, in addition to other plasmid DNA therapies and vaccines [4–6]. Since the guidelines put the responsibility on each manufacturer, plasmid manufacturers may leverage the ICH Q6A and Q11 guidance and may consider the manufacture of plasmid DNA as a bulk drug substance with more stringent quality controls rather than a starting material [13,14]. In this scenario, the plasmid would be filled into bulk drug substance containers as low-bioburden material due to the downstream steps occurring under non-sterile conditions (e.g., column purification and tangential flow filtration). A fill/finish step is generally omitted because:

1. Large volumes are needed of the plasmid when using it for viral vector manufacturing,
2. Fill/finish capabilities for these large volumes are not available or easily accessible and add unnecessary cost
3. The plasmid is not the final product being given to patients, but a starting material/intermediate for the viral vector product.

Filling into bulk drug substance containers could be considered sufficient in this context. In addition, as part of the viral vector manufacturing process, an additional filtration of the plasmids through a sterilizing grade filter should be leveraged to further reduce risk of contamination.

Alternatively, viral vector manufacturers have their own requirements for receiving plasmids into their facility for use in the drug substance manufacturing process. Most viral vector manufacturers require sterility as a plasmid release test to accept plasmid DNA into their facility. However, per ICH Q6A, bioburden, not sterility, should be used to release a low-bioburden bulk drug substance [13]. It is considered standard in finished product manufacturing that sterility is not imparted to a finished product because it has been tested for sterility. Sterility assurance comes from a combination of quality controls and demonstration of sterility through media fills and appropriate testing. Manufacturers of plasmids used as starting material, may be hesitant to test for sterility of the starting material to avoid any misnomer or representation of the same level of sterility control as would be expected for a finished product. Therefore, it is not uncommon to see a bioburden limit of <1 CFU/10 mL on plasmid bulk drug substance. This limit means the material is free of microbial contamination, however it cannot be claimed as sterile because it was not manufactured under the same level of quality controls and media fill demonstrations as would be expected with sterile finished product.

For a sponsor managing plasmid manufacturing and viral vector manufacturing at two different CMOs, it is important to be aware of these requirements ahead of time to reduce any delay of bringing the plasmid DNA into a viral vector CMO. These discrepancies could be overlooked at first glance.

CONCLUSION

Plasmids are a crucial starting material for viral vector manufacturing . It is important for a sponsor to build a robust supply chain between the starting material manufacturer and the ATMP/viral vector manufacturer well in advance to minimize bottlenecks in quality management and timelines. Additionally,

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there is gaining impetus for further harmonization of the guidance for plasmids as starting material/intermediates in the viral vector manufacturing process, and for manufacturer alignment on best practices and unifying standards [15].

REFERENCES:

1. Hayes F. Chapter 1 – The Function and Organization of Plasmids. In Casali N, Presto A (eds.). *E. Coli Plasmid Vectors*: Methods and Applications. Methods in Molecular Biology. Vol.235. Humana Press. 2003; 1–5.
2. Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, and Hou H. Role of Recombinant DNA Technology to Improve Life. *Int. J. Genomics*. 2016; 2405954.
3. Clément N & Grieger JC . Manufacturing of recombinant adeno-associated viral vectors for clinical trials. *Mol. Ther. Methods. Clin. Dev.*.2016; 3: 16002.
4. FDA Guidance for Industry, Recommendations for Microbial Vectors used for Gene Therapy. September 2016.
5. FDA Guidance for Industry, Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. November 2007.
6. United States Pharmacopeia (USP), Gene Therapy Products, USP <1047>, November 2020
7. European Medicines Agency, 24FEB2021, EMA/246400/2021, Inspection, Human Medicines, Pharmacovigilance and Committees Division, Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs.
8. Prather KJ, Sagar S, Murphy J & Chartrain M. Industrial scale production of plasmid DNA for vaccine and gene therapy: plasmid design, production, and purification. Enzyme Microb. Technol. 2003; 865–883.
9. Annex to the EU Directive 2009/12/EC, Part IV, 3.2 Specific requirements for gene therapy medicinal products.
10. European Medicines Agency, 22MAR2018, EMA/CAT/80183/2014, Committee for Advanced Therapies (CAT), Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products.
11. FDA Chemistry, Manufacturing, Controls Information for Human Gene Therapy Investigational New Drug Applications (IND), January 2020.
12. ICH Q7, Good Manufacturing Practices for Active Pharmaceutical Ingredients (APIs), November 2000.
13. ICH Q6A, Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances, October 1999.
14. ICH Q11, Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/biological entities), May 2012.
15. Would you like to influence plasmid release specifications for CGT? *BioPhorum* 2020. https://www.biophorum.com/would-you-like-to-influence-plasmid-release-specifications-for-cgt/ (Accessed Mar 2022).

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS

SPOTLIGHT

Managing variability in autologous apheresis collections

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"Prediction algorithms based on the patient's cell count that consider the apheresis device collection efficiency can be used to calculate the blood volume to process to collect a desired target cell yield"

EDITORIAL

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There is a growing interest in the Apheresis and Cell/Gene Therapy communities to standardize one of the main contributors to the variability of the manufacturing process of autologous cell & gene therapy products, most of them based on peripheral blood mononuclear cells as a raw starting material, which is the apheresis collection. Manufacturing processes, particularly for CAR T cell, have been designed in accordance with Good Manufacturing Practices to mitigate the impact of the variable content of apheresis products on the final drug product. For example, including a cell expansion step of the transduced cells or adjusting the quantity of viral vector used depending on the actual $CD3^+$ cell content. Even so, these manufacturing processes still could benefit from more homogeneous apheresis products. To ensure more homogenous and uniform starting material for genetic modification, T cell enrichment can be introduced in the CAR T manufacturing process. In the case of gene therapy products, the standardization of the $CD34^+$ cell content would for sure minimize manufacturing failures associated with a low cell dose starting material, given the fact that these manufacturing processes do not include a cell expansion step and the overall cell recovery is generally low.

Prediction algorithms based on the patient's cell count that consider the apheresis device collection efficiency can be used to calculate the blood volume to process to collect a desired target cell yield. Also, very reliable prediction algorithms based on the linear regression between the patient's cell counts and the number of collected cells per liter of blood processed can be easily built using retrospective data with the same purpose. Whereas these algorithms can be successfully applied to the majority of cell collections, however, some disease-specific co-morbidities that alter the physical or biochemical characteristics of blood may limit their usefulness. Of course, not every characteristic of the apheresis product can be standardized. There is very little that can be done regarding the quality of the cells collected from the very sick patients to be treated with CAR T cell therapy. Regulatory agencies' clearances given to these drug products (DPs) require that patients must have been treated unsuccessfully with other conventional therapies (e.g., autologous stem cell transplant, chemotherapy) prior to being treated with CAR T cell therapy. The use of very aggressive chemotherapy regimens that severely impact the $CD3^+$ cell health in terms of cell senescence & exhaustion make these $CD3^+$ cells less than optimal for the CAR T cell manufacturing process. A possible solution here would be to extend the washout period between the last treatment and the apheresis collection.

On a different note, apheresis collection centers are being increasingly overwhelmed by the number and diversity of apheresis collection protocols and audits proposed by the industry (either in the research, clinical or commercial stage), which end up consuming a disproportionally high share of their resources in terms of staff, time and money. At the same time this can easily cause confusion and undesired deviations given the number of differences between these protocols. The root cause is that the apheresis collection manuals developed by different companies have been written independently, and at the time of writing this editorial no standardization initiative has been implemented yet.

This may change in the relatively near future. In the recent 2021 Association for the Advancement of Blood & Biotherapies (AABB) Meeting there was a session specifically dedicated to the standardization of apheresis collections.

In one presentation, a British group named SAMPLE (Standard Approach to ATMP tissue colLEction) described the factors impacting the variability of the apheresis products, making an emphasis on the variation amongst manufacturer's requirements. They proposed a series of recommendations geared towards to reducing unnecessary complexity and variation in apheresis collections, increase harmonization and ultimately improving efficiency in apheresis collection to increase capacity within the apheresis collection centers.

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During a second presentation, a US group, the Standards Coordinating Body (SCB) with the Foundation for the Accreditation of Cellular Therapy (FACT), Parenteral Drug Association (PDA) and AABB as co-sponsors, presented the results of two surveys developed by a group of experts from the cell therapy industry, apheresis centers, accreditation bodies and others. The first survey was sent to the industry and the second one to apheresis centers. Questions were similar for both groups and included questions about audits, accreditation/registration, SOPs, required patient data, collection parameters, labelling & packing. The results of these surveys confirmed that apheresis centers are already overburdened, and that there is little consensus on basic parameters and procedures between apheresis centers and industry. Respondents agreed that the standardization of requirements and parameters to simplify audits and SOPs could address the biggest burdens to apheresis centers. The final recommendations from the SCB group reflected the respondents' opinion and highlighted the benefits of reducing the current burden for the apheresis centers.

More recently, the American Society For Apheresis (ASFA), with the support of FACT, AABB, ASTCT (American Society for Transplantation and Cellular Therapy), NMDP (National Marrow Donor Program) and ISCT (International Society for Cell and Gene Therapy) has written a guidance document for the cell therapy industry in white paper format that has been accepted for publication by *Cytotherapy* [1]. The document describes best practices for the cell therapy industry when writing an apheresis collection manual or protocol and identifies the same issues affecting apheresis collection centers described by the SAMPLE and SCB groups. The document goes in detail through all the steps from on-boarding and cell collection to quality assurance and auditing, proposing specific measures to ensure a significant degree of standardization in every step, particularly the cell collection. Among other key recommendations, the document highlights the importance of including a patient readiness or acceptance criteria based on hematological parameters, a collection target (preferably a cell dose), the optimal/suggested apheresis machine settings as well as allowing the apheresis center to take samples from the collected product to assess its quality and calculate the collection efficiency of the process. The utilization of prediction algorithms to facilitate achieving the collection target is mentioned throughout the document. Additionally, it is mentioned in the white paper that the NMDP launched a centralized quality system audit program, called the "Be the Match Bio Therapies Quality System Audit Program", to help reduce redundancy with the many audits that apheresis centers are required to host. The program, endorsed by ASFA, includes a standardized biennial audit whose results will be made available to the industry. The ASFA white paper further highlights the importance of identifying trained staff (including medical personnel, allied health staff, registered nurses) and continuously educating them with professional knowledge.

Based on ongoing research and published data, a list of recommendations has been presented by scientists to minimize the different sources of variability and to help standardize the processing of apheresis products from start to finish. This include fully documenting the apheresis collection process with patient, collection, and product data, using recommended apheresis collection device settings from one procedure to the other, and using prediction algorithms to determine the volume of blood to be processed. In summary, recognizing the issues associated with the lack of standardization of autologous apheresis cell collections for the cell & gene therapy industry, both apheresis centers, accreditation bodies and the industry are finally working together to identify and implement solutions.

REFERENCES

1. Liu HD, Su L, Winters JL *et al.*; on behalf of the Immune Effector Cell Therapies Subcommittee, Clinical Applications Committee, American Society for Apheresis. Immune Effector Cell Therapy Collections: A White Paper from the American Society for Apheresis. Cytotherapy 2022.

BIOGRAPHY

Joseph Roig obtained his BSc in Science of Chemistry in 1980 (University of Barcelona) and started his professional career at Coulter (Beckman-Coulter nowadays), where he worked in the blood cell counting and flow cytometry fields. He joined COBE BCT (currently Terumo BCT) in 1991 to help the company start operations in Spain, moving to the US to continue working for the same company in 2000. During the years that he worked for COBE/Terumo BCT he was involved with all existing apheresis variants (donor collections, leukapheresis, therapeutic apheresis) as well as cell processing. He joined the Terumo BCT Scientific Support group in 2012 and the Medical Affairs group in 2016, the same year he got a Masters in Transfusion Medicine and Advanced Cell Therapies (EMTACT) by the Autonomous University of Barcelona. He became an independent apheresis consultant in 2019 and consulted for several cell & gene therapy companies including CRISPR Therapeutics, BioNTech, BlueBird Bio, PACT Pharma, Editas and Aruvant among others. He started working for Bristol Myers Squibb in 2020 and he is still working there as the Associate Director of Apheresis for Global MS&T (Manufacturing Science and Technology).

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INTERVIEW

David McCall, Editor, Cell and Gene Therapy Insights, **speaks to Fouad Atouf**, Vice President, Global Biologics, United States Pharmacopeia

Standardization & analytical tool innovation in the cell & gene therapy field

FOUAD ATOUF is Vice President, Global Biologics, for USP. He leads all scientific activities related to the development and maintenance of documentary and reference standards for biologics and advanced therapies as well as the oversees biologics laboratories in USP–US and USP–India. Dr Atouf has been at USP for over 15 years and served in a variety of scientific leadership roles including being the regional champion for the Middle East and North Africa Region, where he helped facilitate, programs designed to enhance the understanding of the role of regulations and standards in the registration of medicinal products. Dr Atouf has a strong background and experience in the development and standardization of cellular and tissue-based products. Prior to joining USP in 2006, his research at the US National Institutes of Health focused on developing methods for the in vitro generation of cell-based therapies for diabetes. Dr Atouf is the author of numerous publications in peer-reviewed journals and a frequent speaker at national and international scientific conferences. Dr Atouf earned his Master's degree in Biochemistry and his PhD in Cell Biology from the Pierre & Marie Curie University, Paris, France.

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Q What are you working on right now?

FA: In my current role, I oversee the overall operations for biological standardization at the United States Pharmacopeia (USP). We are implementing a strategy that I helped launch in 2017, which covers the overall biologics landscape. Our focus is documentary standards aimed at advancing quality topics, and reference standards for measuring the performance of assays and processes. This type of work requires engagement with stakeholders, leading collaborations and partnerships.

In addition to advanced therapies, the scope of standards we cover includes other biologics like recombinant proteins, hormones, peptides, heparin, and vaccines. We are also looking at analytical tools for these products, and exploring cross-cutting issues such as impurities and sterility assurance.

Finally, regarding the early stage of development, we have launched a program on biomarkers and the type of assays and standards developed there.

Q As a former researcher who specialized in cell therapy, what are your reflections on the journey towards standardization the field has undergone?

FA: It has been interesting to see how the field has evolved over the past few decades. There has been progress on multiple fronts, including the implementation of novel technologies for both manufacturing and testing for advanced therapies. Progress has also been made in how we approach development and validation of analytical methods. For example, the flow cytometry has advanced phenomenally from when I used it 20 years ago. It is now widely used in QC environment to release products. This is also true of PCR-based methods, which are becoming more and more sophisticated as they move out to the QC environment.

The other part of the evolution relates to increased guidelines from regulatory agencies and pharmacopeias, to address challenges with the qualification of raw materials and methods. These aspects have advanced dramatically in the past 20 years. With that said, the challenge with standardization is the increased number and variety of products and processes. It makes it hard to set standards that fit the needs of each single product, especially when considering rare diseases and specific applications. This is why standards should focus on cross-cutting issues to address the analytical needs for the overall class of products.

Q How have regulatory guidance and the standards for cell and gene therapy (CGT) raw materials evolved recently, from the USP perspective?

FA: Cell-based products carry the risk of taking some of the raw material to the finished product. One of the important aspects to consider is how regulatory guidance

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can integrate better understanding of how these materials interact with cells. It is really remarkable to see this type of risk being captured in the evolving scientific and regulatory paradigms in support of a stronger advanced therapy field.

Another example in the gene therapy field is that both industry and regulatory agencies are looking into enhanced tools for characterization of the active substance, as well as the raw materials and other components used throughout the manufacturing process. The guidelines are evolving along with the development of new technologies with greater sensitivity, allowing for better characterization. For example, in adeno-associated viral (AAV) vector-based gene therapies, one of the critical elements currently being tackled is how to measure the ratio between full and empty capsid. It is a priority to accelerate these types of tools and methods so we can bring these therapies to the patient, which means it has become a major focus for standardization organizations to make those measurements more efficient. USP is actively working on these types of standards and new guidelines, and forming an expert panel to launch these efforts.

"The CGT field is constantly introducing new analytical tools with greater sensitivity to better characterize the raw materials and the finished products."

Q Where specifically do you see challenges for novel analytical tools being applied in the testing and qualification of CGT raw materials today?

FA: The CGT field is constantly introducing new analytical tools with greater sensitivity to better characterize the raw materials and the finished products. It is noteworthy that the field is leveraging some of the lessons from the biotechnology industry, including the development and manufacture of monoclonal antibodies and large molecules, and utilizing some of the same analytical tools. For CGT, those tools will have an impact on every aspect of production, because they will allow better upstream/downstream process development and product release.

One of the challenges relates to the qualification and validation of these methods for their intended use in CGT. Two examples come to my mind. One is the use of next-generation sequencing (NGS) to measure foreign DNA encapsulated in AAV particles. Working out how to qualify those methods for specific use and what kind of standards you can develop is not a simple task.

The other example relates to leveraging the lessons from biotechnology manufacturing and testing: the use of liquid chromatography–mass spectrometry (LC-MS) for the characterization and quantitation of host-cell proteins (HCPs). HCPs are a big challenge, because when you use cells to manufacture viral vectors, there is the risk that the viral vector may take some of the HCP proteins or DNA. LC-MS is a more sensitive method than ELISA,

and it has been adopted for the measurement of HCPs in monoclonal antibodies and other therapeutics. The validation and qualification of these methods for viral vectors are a work in progress right now.

Q Are there potential issues with disharmony between different bodies and jurisdictions in this arena, and are there any efforts afoot to counteract any challenges that may arise?

FA: Firstly, regulatory convergence has been the driver for a gradual and global alignment of regulatory expectations. This is an important step before considering harmonization, which is a much higher standard of success and requires jurisdictions to agree on implementing similar technical and regulatory guidelines.

Scientists and regulators need to engage in continuous dialogue on issues related to product development and analytical testing. The findings of those discussions and dialogues need to be published to help others starting out in the field, and to inform agencies, who can start incorporating those findings into guidance. The point is to further scientific dialogue, as scientific thought leadership is critical. There are many conferences and public workshops organized by either public sector agencies or pharmaceutical science organizations, which allow the kind of conversations that can lead us on the path towards convergence and ultimately harmonization. You need to start by agreeing on what the problem is, and then on the types of solutions. This makes the convergence and harmonization a much easier exercise. As a community of CGT stakeholders, we need to publish the findings from studies, sharing the lessons learned to benefit others.

Q What are the overriding challenges in driving increased standardization in the field moving forward, and how is USP planning to approach them?

FA: The major challenge is caused by very rapid growth in the number and variety of CGT products, which is remarkable in terms of addressing unmet need and treating conditions that have not yet been addressed by conventional treatment. The challenges that come with this fast growth are the growing pains across the value chain associated with bringing advanced therapies to market.

Standardization needs to cover the overall value chain to be effective, because you cannot set standards for one component of the value chain without the others. It takes a lot of engagement and commitment.

We can look at the CGT value chain from a couple of dimensions. In one dimension, we can see what the activities are. This means looking at the lifecycle of product development, including process development, development of regulatory strategy, manufacturing (whether it is in-house or outsourced), the introduction of new technologies, and packaging and distribution all the way through to the patient's bedside. If you view the type of standards

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required as the second dimension, then accreditation of a facility, best practices for the qualification of raw materials, manufacturing, and analytics all need to be addressed. The introduction of advanced manufacturing and looking at the analytics and the associated reference standard, is critical. So you must address both dimensions, and have the right type of stakeholders around so that people can cooperate and collaborate to bring the solutions together.

In general, USP tends to focus on tools that solve problems in analytical testing and reference material type. Beyond this, we are trying to expand the work we do to other aspects of the CGT value chain. For example, we want to be able to explore solutions to address standardization approaches to cover the chain of identity and the chain of custody for CGT. We also want to find out the kinds of toolkits we can bring to the public domain and help developers control their processes, and what kind of information we can extract from this. We recognize that setting a standard is a high bar, and we want to make sure we do it right and in a good sequence. We start with best practices, and then make sure everyone is comfortable before we get into a documentary and/or reference standard.

Q What are some of the key CGT-related goals and priorities over the next few years, both for yourself in your own role and for USP as a whole?

FA: The work we have been doing was a result of stakeholder feedback. This work is continuing, but I am also focused on supplementing it by developing a roadmap for USP's work in the CGT field over the next 5 to 10 years. We want a framework where we have identified the current problems we are trying to solve. We recognize that things evolve externally with industry, and we will adapt to those developments. The roadmap is critical for us internally, and we also want to be able to publish a version of that, to increase the transparency around the kinds of tools that might be coming out – this will in turn enhance dialogue with stakeholders.

In terms of a specific standardization effort that may see the light of day within the next 12 months, we have ongoing work in our laboratories and in collaboration with partners to release reference materials to support measurement of vector copy number for lentiviral-based therapies. Controlling the copy number of integrated viruses is a safety challenge and having materials that allow you to calibrate and measure how many copies integrate in a genome is important.

In addition, we have another project focused on the measurement of the ratio between full and empty capsid, for AAV applications. In collaboration with NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals) and NIST (National Institute of Standards and

"We also want to find out the kinds of toolkits we can bring to the public domain and help developers control their processes, and what kind of information we can extract from this."

Technology), we have a proof-of-concept study exploring the methods that can measure the ratio between empty and full capsids. This round-robin study should finish within the next 12 months and give us a good idea of the most suitable methods. In parallel to this, we are trying to develop a few reference standard ideas to support that measurement. And besides analytical tools and the associated reference standards, we remain committed to exploring some of the digital and software-based solutions to manage the product development lifecycle.

Another example is building on what the community, USP, the FDA, and other agencies, have done in trying to find an alternative to the sterility test and the rapid microbial test. We are looking into this area and assessing new technologies, which we will hopefully be available in the next few years.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

COMMENTARY

Challenges in qualification & management of raw materials in advanced therapy medicinal products

Shok Ping Lim & Sakina Gooljar

Raw materials play a vitally important role in the manufacturing of advanced therapy medicinal products. The ability of raw materials to directly impact the safety, purity and efficacy of the final product has led to the requirement that advanced therapy medicinal product manufacturers control the quality of raw materials in their manufacturing processes. However, some challenges exist in the raw material qualification process specific to the management of biological-derived materials, raw material grades and labels, qualifying suppliers and establishing robust supply chains. Therefore, a risk-based strategy is commonly employed to address these challenges in the management of raw materials for advanced therapy medicinal products.

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The clinical development and commercialization of advanced therapy medicinal products (ATMPs) have evolved rapidly in the past few years. An ATMP is a medicinal product that is either a gene therapy medicinal product, a somatic cell therapy medicinal product, or a tissue-engineered product [1]. The potential for ATMPs to meet the unmet medical challenges of various diseases has resulted in the growing global interest in ATMPs. The

advancement in ATMP manufacture contributes to the increasing complexity of materials used in the production processes.

Materials used in the ATMP manufacture can refer to starting materials, raw materials, ancillary materials, excipients, non-active substances, active substances, cell banks and others. In the European Union (EU), the terms 'raw materials' and 'starting materials' must be distinguished [2]. Starting materials such as human cells and tissues as well as excipients are not within the scope of discussion in this article. Raw materials are defined as reagents, solvents, substances or components used in the manufacturing process of a medicinal product but not intended to be part of the final product [3,4]. The term 'raw materials' will be used throughout this commentary to describe such material as it is not used internationally, and the terminology varies among regions. In the United States, the term 'ancillary materials' is commonly used [5] while 'raw materials' are used in the EU and International Conference on Harmonization (ICH) guidelines [3,4]. Examples of raw materials used in the ATMP manufacture include cell culture media and supplements, process buffers, cytokines, growth factors, cell isolation and separation reagents, and cryopreservation medium.

Over the recent years, raw materials used in the manufacturing process of ATMPs have been recognized to be critically important in controlling the overall quality and safety of the final products. This is due to the fact that raw materials come in contact with the products in manufacturing that will be eventually administered to patients, and also terminal sterilization is always not possible for the finished products [6,7]. Thus, raw materials need to be adequately qualified and controlled before being released to be used for ATMP manufacture to ensure the consistency of the product quality and ultimately the patient's safety. Nevertheless, challenges in selecting, qualifying and managing raw materials in ATMPs remain, as a consequence of a variety of reasons. This article will discuss the challenges in raw materials regulation, the difficulties in controlling materials of biological origin, the confusion in raw material grades and labels, as well as the management of suppliers and supply chains in ATMP manufacture.

CHALLENGES IN QUALIFICATION AND MANAGEMENT OF RAW MATERIALS

Regulatory challenges in raw materials

Currently, no legislation exists worldwide to specifically define the legal framework for the regulatory and quality requirements of raw materials used in ATMP manufacture. Nonetheless, guidelines are available from various national and international organizations, providing general guidance to control the quality and consistency of raw materials [6]. Regulatory guidance documents from different regions that can be referred to when qualifying raw materials in the ATMP manufacture are listed below.

Global:

- ISO Technical Standard 20399:2018 Ancillary materials present during the production of cellular therapeutic products
- ISO 9001: Quality management systems
- ICH Q5A/D: Quality of biotechnological products
- ICH Q7: Good manufacturing practice guide for active pharmaceutical ingredients
- ICH Q8 (R2): Pharmaceutical development
- ICH Q9: Quality risk management
- ICH Q10: Pharmaceutical quality system
- PIC/S GMP Guide Part 1: Basic requirements for medicinal products
- PIC/S GMP Guide Annex 2A: Manufacture of advanced therapy medicinal products for human use
- PIC/S GMP Guide Annex 13: Manufacture of investigational medicinal products

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- PIC/S GMP Guide Annex 20: Quality risk management

Europe:

- EudraLex Volume 4 Part IV: GMP requirements for ATMPs
- EudraLex Volume 4 Annex 2: Manufacture of biological active substances and medicinal products for human use
- EudraLex Volume 4 Annex 13: Investigational medicinal products
- EP General Chapter 5.2.12: Raw materials of biological origin for the production of cell-based and gene therapy medicinal products
- EMA Directive 2001/83/EC: Guideline on the risk-based approach according to Annex 1, Part IV, applied to advanced therapy medicinal products
- Commission Directive 2009/120/EC amending directive 2001/83/EC relating to medicinal products for human use as regards advanced therapy medicinal products
- Regulation (EC) No. 1394/2007: Advanced therapy medicinal products regulation
- British Standards Institution. PAS 83:2012 Guidance on codes of practice, standardised methods and regulations for cell-based therapeutics
- European Directorate for the Quality of Medicines

USA:

- USP Chapter <1046>: Cellular and tissue-based products
- USP Chapter <1047>: Gene therapy products
- USP Chapter <1043>: Ancillary materials for cell, gene and tissue-engineered products
- USP Chapter <90>: Fetal bovine serum quality attributes and functionality tests
- USP Chapter <92>: Growth factors and cytokines used in cell therapy manufacturing
- USP Chapter <1024>: Bovine serum
- 21 CFR 210 and 211: Current good manufacturing practice (drugs)
- 11 CFR 211.80: Current good manufacturing practice for finished pharmaceuticals: components and containers/closures
- 21 CFR 211.110: Current good manufacturing practice for finished pharmaceuticals: control of in-process materials
- 21 CFR 610.15: General biologicals products standards: constituents materials
- 21 CFR 1271.210: Human cells, tissues, and cellular and tissue-based products: supplies and reagents

Australia:

- Australian regulatory guidelines for biologicals (ARGB): Critical raw materials used in manufacturing

Japan:

- MHLW No. 210: Standards for biological ingredients
- MHLW No. 266: General principles for the handling and use of cellular/tissue-based products
- MHLW No. 0208003 and 0912006: Guidelines on ensuring quality and safety of products derived from processed cell/tissue
- MHLW No. 1314: Guidelines on ensuring the quality and safety of products derived from processed human stem cells
- MHLW No. 0327025: Points to consider on manufacturing and quality control

Although there are some consistencies between the national and international guidance listed above, geographical discrepancies still exist in the quality and safety requirements

for raw materials. There is no global standardization of critical quality attributes for raw materials used in the ATMP manufacture. However, it is near to impossible to write a single guideline to cover the regulatory requirements for all the raw materials used in ATMP manufacture, owing to the diversity and complexity of the materials as well as the specificity of the applications. Hence, the best practice for ATMP manufacturers is to communicate with their regional regulatory agencies on the qualification requirements for the raw materials used in their specific ATMP manufacturing processes.

Apart from that, the manufacturing sites of raw materials are usually not audited and licensed by any regulatory authorities. In addition, there are no legal requirements for raw materials to be manufactured under a specific quality management system or GMP standard. These deficiencies give rise to the challenges faced by many ATMP manufacturers in determining the compliances of raw material manufacturers with GMP principles and the robustness of the quality system they are using. As the manufacturing of raw materials is not regulated, ATMP manufacturers bear the ultimate responsibility for assessing the suitability and quality of the raw materials used in their manufacturing processes to produce consistent, safe and high-quality ATMP products.

Difficulties in controlling raw materials of biological origin

The use of raw materials of biological origin in ATMP manufacture is considered high risk attributable to the potential risk of transmission of adventitious agents and the introduction of biological impurities into the final products. USP General Chapter <1043> [5] and EP General Chapter 5.2.12 [8] outline a set of quality standards for the use of human or animal-derived materials, providing references for ATMP manufacturers. The best practice is to avoid using any biological origin materials in the ATMP manufacture, yet this is most of the time not possible. With the advancement of scientific understanding and technologies, the use of recombinant proteins in the ATMP manufacture as an alternative solution for biological origin materials has slowly become more common. Nonetheless, materials and processes used to produce the recombinant proteins need to be assessed carefully by ATMP manufacturers.

Using raw materials of biological origin in the processing of ATMP products presents a potential risk of contamination with human or animal pathogens including bacteria, fungi, viruses and Transmissible Spongiform Encephalopathy (TSE)/ Bovine Spongiform Encephalopathy (BSE) agents. For biological raw materials, testing for sterility, residual host cell DNA, endotoxin, mycoplasma, and species-specific adventitious agents are mandatory, and suppliers should provide this information to the ATMP manufacturer. Moreover, the raw material users must understand the source and origin of each raw material and Certificate of Origin (CoO) and TSE/ BSE compliance certification must be provided by the supplier. Whenever necessary, ATMP manufacturers should obtain details on the manufacturing process of the raw materials such as the steps taken in preventing cross-contamination during manufacturing and the methods used in viral inactivation or elimination if applicable. Sufficient information provided by the suppliers allows ATMP manufacturers to perform a proper risk assessment as part of their raw material qualification program.

Managing the lot-to-lot variability of biological origin materials is the biggest challenge as these materials are derived from biological sources and it is well known that the variability of biological materials is very high. The suppliers should provide biological activity data to enable ATMP manufacturers to assess the consistency of performance of the raw material in their manufacturing process. ATMP manufacturers should also be aware of the methodology used by the raw material manufacturer in testing biological activity to determine whether the assay

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is relevant for their intended use of raw material. Furthermore, it is the responsibility of the ATMP manufacturer to study the impact of the lot-to-lot variability of biological raw materials on the final product and if required, at least three batches should be assessed [3,9]. In some cases, it might be incumbent for ATMP manufacturers to collaborate with the supplier to improve the raw material quality and to minimise the lot-to-lot variability. Additionally, the shelf-life of biological origin materials need to be determined by stability studies. The shelf-life data can be provided by suppliers or generated in-house by ATMP manufacturers.

It is also fundamental to assess the risks of the introduction of biological impurities into the final products when materials of a biological origin are used in the ATMP manufacture. Nevertheless, there may be a lack of robust and sensitive analytical methods or the testing regime for biological-derived materials may be highly complicated. These issues may lead to difficulties in assessing the impact of the biological raw material in the final product or to quantitate the amount of residual raw material, if any, remaining in the final product [6]. Consequently, the benefits of using biological raw material need to be weighed against the risks it poses to the final product quality and safety.

Since ATMP products cannot be subject to terminal sterilization, it is crucially important to control the risks of the introduction of contamination through raw materials. According to EP General Chapter 5.2.12, a risk assessment must be performed, taking into account the biological origin and traceability of the raw materials, the steps in production processes, and the ability to remove the raw materials from the final product [8]. In addition, it is indispensable for ATMP manufacturers to understand the critical quality attributes of the biological raw materials and qualify alternative sources at the earliest development phase of their medicinal products. This is because, if there is a need to change the sources or suppliers of biological raw materials, it can be expensive and time-consuming as comparability studies evaluating the impact of the change on final product quality attributes are usually required. More importantly, there is an urgent need for improved guidance documents to govern the manufacturing of biological-derived raw materials.

Confusion in raw material grades & labels

Presently, there is no standardization of compliance claims in the material grades, for example research-grade, clinical-grade and GMP-grade, used by the suppliers. ATMP manufacturer is also not obliged to use a particular grade of raw materials in their manufacturing processes. Therefore, ATMP manufacturers often source raw materials labelled as "GMP-grade" to use in their manufacturing processes. It is paramount to note that GMP is a quality system rather than a grade. GMP is a quality system, defined as the 'sum of all aspects of a system that implements a quality policy and ensures that quality objectives are met' [3]. On the contrary, a grade is a quality standard, which is a 'specification consisting of a list of tests, references to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the test described' [3,10]. Raw material suppliers frequently market their products as 'GMP-grade' to claim the highest quality of their materials, but it actually means that the materials have been manufactured under a robust quality management system, for example ISO 9001, by following a particular GMP guideline. Importantly, raw material users should be aware that there is no GMP guidance specifically for raw material manufacture, and that raw materials manufacturer is usually not audited and licensed by competent regulatory authorities for their compliance with regulatory requirements. On the other hand, pharmaceutical-grade raw materials are manufactured by manufacturing facilities certified as GMP compliant by competent regulatory agencies. Thus, the use of pharmaceutical-grade raw materials

in ATMP manufacture might reduce the burden of qualification. However, additional testing either performed by suppliers or ATMP manufacturers themselves, might still be required, depending on the intended use of the raw materials in specific ATMP manufacturing processes. Another advantage of using pharmaceutical-grade raw materials is that comparability studies are usually not legally required when ATMP manufacturers decided to source the same raw material from alternative suppliers.

On top of that, there is a lack of governance and consistency surrounding the labelling of raw materials. Standard terms for raw material quality have not been established and there is a variety of terminology. For instance, 'chemically-defined', 'xeno-free', 'biological-free', 'TSE/BSE-free' and 'animal component-free' are often used by suppliers for marketing purposes.

Overall, any claims of the raw material by suppliers should be appropriately evaluated by the ATMP manufacturer. ATMP manufacturers can request documented evidence of quality management system certification such as an ISO certificate or GMP certificate issued by the competent regulatory authority. Moreover, an on-site audit of the manufacturing sites of raw materials can also be carried out to confirm that the manufacturing is conducted under strictly controlled processes by following GMP principles. Requirements for in-house raw material qualification should then be determined depending on the quality documentation and certification provided by suppliers as well as the information gathered during the audit.

Suppliers & supply chains management

Apart from choosing highly characterized raw materials manufactured by well-known suppliers with strong evidence of GMP manufacture, a close partnership with suppliers is also required to make the qualification process much more efficient. Building a good relationship with suppliers enables effective collaboration and communication that in turn can significantly reduce the burden of the ATMP manufacturers in qualifying the raw materials. ATMP manufacturers may put in place a Quality Technical Agreement with their critical suppliers to document the terms of the relationship and the expectation of both parties.

To evaluate the identity, quantity, purity and safety of the raw material, detailed quality documentation including Certificate of Analysis (CoA), Certificate of Compliance (CoC), CoO, Safety Data Sheet (SDS) and TSE/BSE compliance certificate should be provided by suppliers. In some situations, ATMP manufacturers might request access to the supplier's Regulatory Support File (RSF) or Drug Master File (DMF) to acquire more comprehensive quality data for the raw material. As RSF or DMF is a confidential document, a confidential disclosure agreement (CDA) will usually be signed between the supplier and the ATMP manufacturer. Documentation on the stability of the raw materials should also be requested by ATMP manufacturer from the supplier to determine the maximum shelf life and storage conditions of the raw materials. When selecting the raw material and supplier, the ATMP manufacturer can obtain data from suppliers regarding their ability to supply a quality raw material consistently during all production cycle including commercialization. The requirements of batch-to-batch consistency of critical raw materials are outlined in EP General Chapter 5.2.12 [8] and ISO Technical Standard-20399 [9]. Furthermore, suppliers should permit on-site audits of their manufacturing facilities for ATMP manufacturers to assess the appropriateness of the system and process used to manufacture their raw materials. Periodic audits or assessments should also take place to ensure ongoing maintenance of the quality standards of the raw materials. Moreover, notifications of any changes on the raw materials manufacturing or specifications should

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be sent in a timely manner by the supplier to the ATMP manufacturer before the change is implemented so that an impact assessment can be performed by the ATMP manufacturer [6,7,11].

Additionally, a robust supply chain should be initiated from the early development stage of a medicinal product to ensure the continuity of critical raw materials supply throughout the ATMP life cycle. ATMP manufacturers should make sure their suppliers are able to scale manufacturing of raw materials to meet the higher demands when the medicinal product reaches the clinical or commercial stage. A secure and reliable supply chain is vital, notably during the COVID-19 pandemic, where a shortage or lead time delay for many raw materials are seen. It is also beneficial to have reliable suppliers that are willing to work with the developer to resolve their supply issues. Ideally, ATMP manufacturers should avoid using single-sourced materials and consider qualifying a secondary supplier for critical raw materials. This is because comparability and validation studies are usually required to support an amendment to the clinical trial authorization (CTA) or variation to marketing authorization (MA) when there is a change in the manufacturing process due to the unavailability of critical raw materials.

In the management of suppliers for critical raw materials, supplier audits, either on-site or virtual, are normally needed to assure raw materials are manufactured under a well-designed and qualified system. Quality and supply agreements between the raw material supplier and ATMP manufacturer is also critical so that potential supply issues and any changes in the raw material production or specification can be communicated to the ATMP manufacturer promptly [6,7,11]. In general, suppliers and supply chains should be properly managed to make sure raw materials are supplied consistently and continuously throughout the ATMP life cycle without any major quality issues or disruptions.

CONCLUSION & RECOMMENDATIONS

Raw materials are key components in the manufacturing processes of ATMP products. When submitting applications of CTA (EU and Canada) or Investigational New Drug, IND (US) and MA to regulatory authorities, raw materials must demonstrate suitability for their intended uses in the manufacturing process by appropriate qualification and risk assessment processes. Nevertheless, numerous challenges still exist in the qualification and management of raw materials for use in the ATMP manufacture. Lack of standardized regulation for raw materials use and no legal requirements for raw material manufacture contribute to the difficulties for both ATMP and raw material manufacturers in understanding the regulatory requirements. In addition, confusion on raw material grades and labels as well as challenges in risk management of biological-derived materials and supply chain further complicate the control of raw material in the ATMP field. Hence, ATMP manufacturers should create a robust framework to select and qualify raw materials used in the manufacturing processes as well as to manage and mitigate associated risks.

An effective raw material qualification process is required to collect data in order to evaluate the source, identity, purity, biological safety and overall suitability of a specific raw material [5]. According to USP General Chapter <1043> and EP General Chapter 5.2.12, a well-established raw material qualification framework should at least include the activities of identification, selection and suitability, characterization, vendor qualification, quality assurance and control, traceability, as well as biological function [5,8]. The level of the raw material qualification program should be defined according to the type of raw material, the type of ATMP product, where and how the raw material is used in the manufacturing process, as well as the amount of residual raw material that remains in the final product.

Given that zero risk may be an unattainable expectation in the use of raw materials in ATMP manufacture, a risk-based approach is typically implemented in the raw material qualification process to manage and control their use [2,5–8,12,13]. The level of quality control and management depends on the criticality of the raw material in the ATMP manufacturing process. The criticality of the raw material should be assessed based on the impact of the raw material on the final product quality and subsequently the patient's safety. A structured risk assessment strategy should involve the steps of knowledge collection about the raw material, general risk assessment, identification and evaluation of the risks of material attributes, and implementation of a plan for risk mitigation accordingly [2]. The risk assessment process for raw materials must be carried out in a timely manner by applying GMP and quality risk management principles [14] and it should be incorporated into the department's quality management system [15]. From our experience during the COVID-19 pandemic and Brexit transition period, it is not only crucial to consider the safety and quality risks of raw material on the final ATMP product, but the risks and the reliability of the supply chain should also not be underestimated.

It is acknowledged that potential changes in raw materials may be very likely throughout the ATMP life cycle. Changes of raw material at the latter stages of product development where more stringent documentation, testing and control are required, can be expensive, time-consuming and labor-intensive [13]. Any changes in raw material need to be carefully evaluated for their impact on the final product quality attributes through comparability and validation studies. Therefore, the raw material qualification process should commence as early as possible during the product development process. Nonetheless, qualified raw materials should be periodically reviewed based on the stage of product development, new knowledge on the raw material and manufacturing process, new scientific understanding, the advancement in raw material testing, as well as the evolution of regulatory requirements [2,6,7,10].

In conclusion, ATMP manufacturers are accountable for ensuring the raw materials are suitable for their intended applications in the manufacturing processes. The raw materials should also not compromise the quality and safety of the final ATMP products. In light of the complexity of raw materials in the ATMP manufacture and the existing challenges in managing raw materials, ATMP manufacturers should design and establish a robust qualification strategy to mitigate overall risks to an acceptable level to comply with regional regulations.

REFERENCES

1. Regulation (EC) No 1394/2007 on the European Parliament and of the council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004.
2. EBE Biomanufacturing WG Concept Paper. Management and control of raw materials used in the manufacture of biological medicinal products and ATMPs. Version 2. December 17, 2018.
3. ICH Q7 Good manufacturing practice guide for active pharmaceutical ingredients. 2000.
4. Directive 2001/83/EC of the European Parliament and of the council of 6 November 2001 on the Community code relating to medicinal products for human use.
5. USP General Chapter <1043> Ancillary materials for cell, gene, and tissue-engineered products.
6. Solomon J, Csontos L, Clarke D *et al.* Current perspectives on the use of ancillary materials for the manufacture of cellular therapies. *Cytotherapy*. 2016;18, 1–12.
7. Ball O and Zylberberg C. Towards a common framework for defining ancillary material quality across the development spectrum. *Cytotherapy.* 2019; 21, 1234–1245.
8. Ph. Eur. General Chapter 5.2.12 Raw materials of biological origin for the production of cell-based and genet therapy medicinal products.
9. ISO/TS 20399 Biotechnology - Ancillary materials present during the production of cellular therapeutic products. 2018.

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10. Bravery CA, Robinson S, and Burger SR. Making the grade: untangling the myths of raw materials used for the manufacture of cell-and gene-based medicinal products. Cell Gene *Ther. Insights.* 2018; 4, 207–225.

11. Leistler B. How to standardize quality requirements of raw materials *Cell Gene Ther. Insights*. 2020; 6, 1555–1560.

12. Lebrun A-S and Brenner C. Master ATMP processes by controlling raw and starting material. *Cell Gene Ther. Insights*. 2021; 7, 265–271.

13. Caraballo J. The role of raw materials in the manufacturing of cell and gene therapy medicinal products: practices and challenges. *Cell Gene Ther. Insights*. 2020; 6, 229–233.

14. ICH Q9 Quality risk management. 2005.

15. ICH Q10 Pharmaceutical quality system. 2008

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

EXPERT INSIGHT

Banking on the future of regenerative medicine with cGMP-compliant iPSC lines

Melissa K Carpenter

The ability to generate cell therapies from pluripotent stem cells is changing the way we think about diseases and how to treat them. Regenerative medicines derived from pluripotent stem cells have the potential to treat a large number of diseases, many of which currently lack efficacious therapies, by identifying the deficient or non-functional cell type involved in the disease and generating the corresponding healthy cell type from pluripotent stem cells. However, like other cell and gene therapy products, pluripotent stem cell-based regenerative medicines face development challenges that must be solved before we can deliver on their promise to patients.

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To date, two types of pluripotent stem cells (PSCs) have been used to develop cell therapies: human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs), both of which can give rise to multiple different cell types. hESCs are derived from discarded 5–7-day human embryos generated during the *in vitro* fertilization (IVF) process. iPSCs are generated by 'reprogramming' a mature cell – typically a skin or blood cell collected from a healthy donor – with genes and other reagents to 'induce' the mature cell to take on a less differentiated, more pluripotent state. For discovering that mature cells can be reprogrammed to become pluripotent, Shinya Yamanaka and Sir John B

Gurdon were jointly awarded the 2012 Nobel Prize in Physiology or Medicine.

Under the right culture conditions, PSCs can proliferate indefinitely and can be directed to differentiate into any type of mature cell. Therefore, PSC lines can be used as the starting material for many different cell therapies to treat a variety of diseases.

Since 2010, about 70 PSC-derived therapies have been in clinical testing [1] Most of these trials use research-grade hESC lines as starting materials and generate the hESC-derived final products in accordance with manufacturing regulations. Although the derivation process for a small number of hESC lines does comply with manufacturing regulations, the IVF process which generates the embryos does not. Further, the embryo donation and IVF processes are not completely compliant with tissue-gathering or donor eligibility regulations, [2,3] and achieving that compliance retroactively is one of the biggest regulatory challenges that hESC-derived products face.

iPSC-derived cell therapies are quickly catching up: the first therapy entered clinical testing in 2013, [4,5] and now there are about 23 iPSC-derived products in clinical studies in at least five other countries, including the US, China, the UK, and Australia [1]. The cell lines in the Japanese and Australian trials were generated in compliance with Pharmaceuticals and Medical Devices Agency (PMDA) and Therapeutic Good Administration (TGA) regulations, respectively.

Interest in developing PSC-derived cell therapies is growing and many groups want to take their products into clinical trials. But there are risks in developing regenerative medicines from research-grade hESC and iPSC lines because the cells have incomplete histories and are not fully compliant with regulations. As PSC-based therapeutics move toward commercialization, strict compliance with manufacturing regulations is becoming more important.

iPSCs have the advantage of avoiding the ethical complications and regulatory compliance concerns associated with hESC-derived therapies. But a key problem facing the field is the paucity of current Good Manufacturing Practice (cGMP)-compliant iPSC lines available for use as starting materials, which creates manufacturing hurdles in developing an iPSC-derived therapy.

ElevateBio is taking steps to solve these problems by creating clinical-grade iPSC lines that can be used in developing and commercializing regenerative medicine products. The approach aims to accelerate the delivery of these powerful therapies to the patients who need them.

GENERATING CLINICAL GRADE IPSCS: A BALANCING ACT

For these purposes, we define 'clinical-grade iPSC lines' as those that have been generated in accordance with local regulations governing tissue-gathering and manufacturing regulations – which in the US are FDA-issued Good Tissue Practices (GTP) [6] and GMP [7], respectively.

Compliance with US GTP requires a donor's tissue or cell sample to be accompanied by the donor's full medical and social history and a blood test demonstrating the donor is free from pathogens. These rules were introduced in the 1990s and are designed to protect the recipient of the donor organ, tissue graft or cell-based therapy from communicable diseases, such as HIV infection [6,8].

Donor cells should be reprogrammed into iPSCs in accordance with GMP guidelines by using the highest-grade reagents possible and GMP-compliant processes, including documentation of the cell line as it was generated, expanded, banked, tested and characterized with appropriate quality oversight.

Unfortunately, cell lines developed in research labs are not compliant with GMP or GTP regulations and many have incomplete histories.

Despite the drawbacks of available iPSCs, most innovators start with research-grade lines for a practical reason: Generation of a clinical-grade, fully GTP- and GMP-compliant

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iPSC line can take 12–24 months. The investment of time and money required upfront, just to create starting material for preclinical studies, can be prohibitive for an academic researcher or company that wants to explore a regenerative medicine concept. It is more feasible for innovators to begin exploring and developing the concept with a research-grade iPSC line and consider the need for clinical-grade material later.

Recognizing the high barriers imposed by GTP and GMP, FDA has allowed therapies derived from research-grade hESCs to enter the clinic; as with other cell therapy products, the Agency has phase-appropriate GMP standards that do not require full GMP compliance in the early stages of clinical testing [9]. The iPSC-derived therapies with approved Investigational New Drug Applications (INDs) have been generated using research-grade and clinical-grade iPSCs.

Although a clinical-grade iPSC line is the preferred starting material, obtaining such a cell line from a contract manufacturing organization (CMO) means putting product development on a lengthy 12- to 24-month pause. At the end of that period, the CMO will provide exactly that: one cell line. Yet researchers know that all iPSC lines are not identical; each cell line can have different differentiation capability [10], for reasons that are not yet fully understood. Furthermore, differing culture conditions can produce clonal variations between the resulting iPSC lines [11]. If the CMO's clinical-grade iPSC cell line doesn't work with the systems and processes originally used to develop the therapeutic product from research-grade cells, the innovator will have to spend even more time and money to modify those systems and processes before moving forward. This may also be the case for developers that, instead of deriving their own cell line, plan to use a cell line from the available repositories of clinical-grade PSC lines: the selected cell line may not be suitable for the developer's manufacturing process.

In addition to the hurdles created by the lack of clinical-grade iPSC lines, iPSC-derived products also face regulatory uncertainties and potential pitfalls. Chief among them is the uncertainty of whether FDA will approve a Biologics License Application (BLA) for a product based on research-grade PSC lines. On the one hand, one can argue that if sponsors demonstrate control of the whole development process with a research-grade cell line to produce a safe and efficacious product, they should be able to commercialize the product – obviating the need for a clinical-grade starting material. On the other hand, no PSC-derived product has gotten as far as a BLA submission, so it is difficult to know what FDA will or won't accept. It's also possible that iPSC-derived products will be held to a higher standard than those derived from hESCs.

The costs in time and money associated with generating clinical-grade iPSC lines, coupled with the regulatory ambiguities, leave many regenerative medicine innovators wondering when – or even whether – to invest in producing those cell lines.

REMOVING THE OBSTACLES

ElevateBio's goal is to minimize the impact of all of the aforementioned development hurdles and regulatory ambiguities by creating clinical-grade iPSC lines generated from multiple donors in a completely controlled environment and with complete histories of their production, characterization, and testing. The iPSC lines will be GTP- and GMP-compliant. Furthermore, additional testing procedures will be added to the process, as needed, to comply with any future changes in the regulations. In short, the intent is for these iPSC lines to become the industry gold standard.

Naturally, the development of these kinds of cell lines requires a significant upfront investment in the platform technologies, needed infrastructure, and talent – and it is a risky venture. It is not easy to create, reprogram and culture iPSCs; there is an art to doing all of that in a controlled, reproducible manner. It requires special skill and expertise in a team

that communicates effectively and trusts one another, because scientists in this field have a special dedication to their work. Apart from a number of groups in Japan, very few have achieved all of this at the manufacturing scale yet.

This is far from an easy endeavor; that's precisely why a lot of people in the US haven't created, or even tried to create a repository of clinical-grade iPSC lines. The government of Japan has appropriated funding to create an iPSC bank that will cover the entire Japanese population with human leukocyte antigen (HLA) matching, using cell lines derived from about 50 donors [12,13]. This is feasible because Japan's population is genetically homogeneous; the US population is far more heterogeneous, making the challenges greater. With the right expertise, the right team and the right business model in place, there is the potential for this approach to de-risk the creation of a clinical-based cell bank in the US.

The science of taking a mature cell, reprogramming it into an iPSC, and then turning it into a liver cell or retinal cell is fascinating. But as amazing as iPSCs are, they aren't as amazing as the therapies that can be made from them – therapies that can ultimately change the lives of thousands of patients in need. ElevateBio is committed to the future of regenerative medicine and our iPSC lines is our first step towards that goal.

REFERENCES

1. Approximate count based on Yamanaka S. *Cell Stem Cell*. 2020; 27(4):523–531. doi: 10.1016/j.stem.2020.09.014; and a search of https://clinicaltrials.gov/
2. Human Cells, Tissues, and Cellular and Tissue-Based Products, 21 CFR Part 1271. *US Food and Drug Administration.* (Oct 01 2021) Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271 (Accessed Jan 2022).
3. Human Cells, Tissues, and Cellular and Tissue-Based Products, 21 CFR 1271, Subpart C, 69 FR 29830. *US Food and Drug Administration.* (Oct 01 2021) Available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271&showFR=1&subpartNode=21:%208.0.1.5.57.3. Accessed January 11, 2022
4. Cyranoski D. Stem cells cruise to clinic. *Nature* 2013; 494, 413.
5. Mandai M, Watanabe A, Kurimoto *et al.* Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *N. Engl. J. Med.* 2017; 376,1038–1046.
6. Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). *FDA.* Dec 2011. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/current-good-tissue-practice-cgtp-and-additional-requirements-manufacturers-human-cells-tissues-and. (Accessed Jan 2022).
7. Current Good Manufacturing Practice (CGMP) Regulations. *FDA*. 2020; https://www.fda.gov/drugs/pharmaceutical-quality-resources/current-good-manufacturing-practice-cgmp-regulations. (Accessed Jan 2022).
8. Couture LA and Carpenter MK. 2005 Donor Eligibility Requirements: Unintended Consequences for Stem Cell Development. *Stem Cells Trans. Med.* 2015; 4, 1097–1100.
9. Guidance for Industry: cGMP for Phase 1 Investigational Drugs. US Food and Drug Administration. July 2008. (Accessed Jan 2022)
10. Rowe RG and Daley GQ. Induced pluripotent stem cells in disease modelling and drug discovery. *Nat. Rev. Genet.* 2019; 20, 377–388. q.v. references 71, 72, 137, 138 , 146 and 147.
11. Mills JA, Wang K, Paluru P *et al.* Clonal genetic and hematopoietic heterogeneity among human-induced pluripotent stem cell lines. *Blood* 2013; 122, 2047–2051.
12. Umekage M, Sato Y, Takasu N. Overview: an iPS cell stock at CiRA. *Inflamm. Regen*. 2019; 39:17.
13. Huang C-Y, Liu C-L, Ting C-Y *et al.* Human iPSC banking: barriers and opportunities. *J. Biomed. Sci*. 2019; 26, 87.

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RAW & STARTING MATERIALS

SPOTLIGHT

Pandemic-related supply chain disruptions in cell therapy require rapid qualification for single-sourced materials

John Duguid & Paul Friedman
Vericel Corporation, Cambridge, USA

VIEWPOINT

"...failure of cell therapy product manufacturers to deliver life-saving treatments can have severe consequences for the patients. As a result, cell therapy companies have more flexibility to hold inventory and spend considerable resources on risk mitigation in this area."

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Cell therapy products seek to treat a diverse range of injuries and diseases from disciplines as varied as regenerative medicine, oncology, and immunology. These treatments have commonalities in their starting materials, which are human tissues, and their drug products, which contain living cells originating from those tissues. They may be autologous, treating a single patient with a product derived from their own cells, or allogeneic, treating many patients from products derived from a donor cell bank. There are a wide variety of raw materials used to manufacture these products including singleuse sterile plastics, well-characterized media and buffers, novel growth factors, cytokines, enzymes, and animal-derived materials subject to biological variability. Supply chain disruptions for any of these materials has the potential to interrupt product supply at a cell therapy manufacturing facility or result in an emergency qualification exercise to find a suitable replacement with a potential regulatory submission before using it.

The cell therapy industry faces supply chain issues that traditional pharmaceutical companies do not generally face. Often only one supplier, the 'sole source', may manufacture specialized materials, which presents difficulties when there are supplier constraints or concerns. To add to the challenge, it may not be feasible to qualify and maintain multiple sources when the process requires small quantities and there is little purchasing leverage to entice one supplier, let alone multiple suppliers, to enter into quality agreements to maintain compliance with cGMP regulations. Finally, an equivalent grade of material may not be readily available. Raw material manufacturers would ideally follow cGMP, so sourcing finished dosage forms already approved by FDA or active pharmaceutical ingredients (APIs) is ideal. That is not always possible, and in many cases, it is necessary to use compendial (USP/EP) grade, ACS grade, cell culture grade, or even research grade materials with an increasing responsibility on the cell therapy product manufacturer to ensure their quality. USP <1043> 'Ancillary Materials for Cell, Gene, and Tissue-Engineered Products' provides an effective risk-based strategy for characterization and qualification of these critical raw materials'.

Cell therapy companies are highly regulated by FDA and require oversight by an effective cGMP quality management system. This impacts the entire supply chain due to the rigorous inspection, testing, and documentation required to change an item's status from 'quarantined' to 'released' for use in the process. The lead times associated with testing and release can be considerable and must be part of the overall strategy. In addition, a comprehensive material and supplier qualification program is essential. Elements of this program include material selection and qualification, initial supplier approval, and ongoing material and supplier surveillance via incoming material testing, supplier audits, and periodic verification of supplier test results provided on a certificate of analysis. Typically, material qualification includes evaluation of at least three lots of a new material for conformance with established material specifications followed by assessment of equivalence to current materials in the manufacturing process via a process validation study. A risk-based approach is useful for prioritizing additional sources of materials for qualification. Things to consider include stability of supply, historical supplier relationship, availability of additional sources, complexity of qualification activities, and safety stock. In the case of sole-sourced materials, the only option to reduce business risk is to maintain adequate safety stock.

Pandemic-related supply chain disruptions require an emergency response to

material qualification instead of a thoughtful risk-based approach. These disruptions can affect even the most stable sources of supply. During the past two years, supplier plants were shut down by COVID-19 infections or the inability of operators to report to work due to illness and child or elder care concerns. In addition, the Defense Production Act required suppliers to prioritize shipments of materials to vaccine producers; the cell therapy industry and vaccine industry use many common materials. The following suggested strategies will help address these challenges.

- Establish adequate safety stock of released materials on site to support demand leadtimes, overcome production lot failures, and include a hedge for disruptions. In the face of COVID-19 challenges, traditional lean and just-in-time strategies did not work and led to irreversible damage at many companies.
- Adjust safety stock based on the source of supply, holding more inventory for sole sourcedmaterials.
- Prepare for vendors to have gaps in communication due to their mitigation strategies or lack of supply chain understanding. Issuing purchase orders with several delivery dates will provide regular feedback about supplier delivery performance.
- Plan for supplier manufacturing schedules to extend past expected due dates. Issuing purchase orders further into the future or issuing blanket purchase orders will mitigate this risk, although it may cause longer-term fiscal liability, which needs to be balanced against the supply chain risk.
- Accelerate logistics to perform concurrent internal quality testing by shipping materials prior to completion of testing and release at the manufacturer. Quality organizations are generally reluctant about this approach, but it may be necessary to provide the fastest time from raw material manufacture to final release, ultimately preventing stock out.
- Pay a higher cost for third party logistics and testing labs to expedite their activities. For example, it is faster and costs more to use air transport to ensure delivery of a raw material than to use ground transport, which costs less but takes longer.

The inventory profile for a cell therapy company is different than for companies in other industries because there is a patient waiting for timely delivery of each therapeutic product. Failure of typical commercial product manufacturers to deliver may result in minor inconvenience to customers, but failure of cell therapy product manufacturers to deliver life-saving treatments can have severe consequences for the patients. As a result, cell therapy companies have more flexibility to hold inventory and spend considerable resources on risk mitigation in this area. Following the suggested strategy recommendations will create the most resilient supply chain during unplanned pandemic events and other unexpected times of adversity.

BIOGRAPHIES

JOHN DUGUID is Senior Director, Research & Development at Vericel Corporation in Cambridge, MA. As a Principal Scientist, Mr Duguid was responsible for developing, validating, and transferring molecular biology assays for rapid microbiology and cell differentiation applications, managing complex projects to implement process changes, and using statistical process control tools to implement process analytical technology for cell therapy products. He has been with CTRM since 1995. Before taking on his current role in R&D, Mr Duguid managed quality control cell therapy operations at Genzyme for over 10 years. He also represented QC during 10 FDA inspections and numerous audits from international regulatory authorities. Mr Duguid received his Bachelor of Science degree in chemistry in 1986 from the University of Michigan in Ann Arbor, MI and taught analytical chemistry in 2000 at Northeastern University in Boston, MA.

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CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

Troubleshooting quality assurance for AAV raw & starting materials

David McCall, Editor, Cell and Gene Therapy Insights **speaks to Biswarup DasGupta**, Quality Professional.

BISWARUP DASGUPTA (Bis) is a Quality and Compliance leader with experience in QA, QC including contamination control over 20 years in the Biotech Industry. Recently, Bis worked as QA Director for Sarepta's Gene Therapy program, since July 2019. Before Sarepta, Bis assumed different roles in Quality organization with increasing responsibilities at Sanofi, GSK Biologics, and Altana Pharma. In these roles, he has developed effective contamination control programs, led successful CD verification, implemented phase-appropriate GMPs, provided strategic leadership, and built a culture of quality and continuous improvement. Bis has a master's in Microbiology and BS in Chemistry, Biology, and Microbiology. Bis has co-authored TR13-2 and currently co-leading the new TR on Contamination Control Strategy. Bis is also a member of the current revision of TR-56.

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Q What are you working on right now?

BD: Currently, I am transitioning to a new job. At my last job, I worked within the Quality Function in gene therapy for rare disease.

Q What are the main current challenges regarding raw and starting materials for AAV (adeno-associated virus) manufacture?

BD: The critical starting materials for AAV are in many cases the plasmids and mammalian cell banks.

For plasmids, the first challenge is the number of qualified vendors who can produce GMP quality grade plasmids. Secondly, due to the COVID-19 situation, the supply chain is always challenging and when you do not have a back-up supplier, you are always worried about stock out situation. My advice to people would be to have at least one back-up vendor to avoid this situation.

Mammalian cell banks are another critical raw material that are used in Gene Therapy (GT) manufacturing. You need to think about where you are manufacturing your master and working cell banks and qualifying it accordingly in adherence to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (ICH Q5D) [1.2].

Finally, the rate of consumption may also be a problem. If the drug substance yield is low, then you are consuming critical raw materials much more frequently. Creating that number of cell banks and qualifying them on time prior to use is always going to be a challenge.

Q What are the key differences between regulatory expectations for plasmid quality, qualification, and testing requirements in early development versus late-phase development versus commercial?

BD: For gene therapy products, there is limited regulatory guidance in place for plasmids in US, although there is some guidance that we leverage in the US [3–6], but EU [7,8] provides more details information in that regards. Regulatory Guidance on the European side is a little more in-depth, for qualifying and testing plasmids. Right now, there are no clear regulatory instructions or expectations, but we can expect more guidelines soon. There are industries who use plasmids as a drug product which do not undergo further manufacturing thus safety and quality attributes needs to be much more stringent than the ones go through further manufacturing. Due to the relatively recent advancement of the gene therapy field many vendors are unwilling to share information regarding their process,

"For plasmids... due to the COVID-19 situation, the supply chain is always challenging and when you do not have a back-up supplier, you are always worried about stock out situation. My advice to people would be to have at least one back-up vendor to avoid this situation."

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materials, and test methods due to IP concerns. This makes it very challenging to address yield and product quality issues with vendors who are unwilling to be transparent with the sponsor company. This issue will likely subside overtime as gene therapy technology becomes more common place. In the meantime, go over contracts with a fine toothcomb and insist that information, within reason, be shared with the sponsor/partner company before committing to the contract. Consequently, in the coming years, we can expect that there will be a much greater degree of regulatory oversight and guidance.

In terms of early versus late-phase development, the expectation in the gene therapy world is having phase-appropriate GMPs. You need to define the quality for what you need for intended purpose. For late-phase development, you obviously need more robustly characterized and suitable plasmids for your manufacturing process.

Q What is your corresponding advice for gene therapy developers?

BD: It may be very difficult to characterize your product in the early stages, as you may not have enough data. You should build a library with whatever you have, and you must keep that data generating machine going. You need to test whatever lots you can, whether pilot scale or laboratory scale or manufacturing scale, so that you have enough data that you can leverage for late your later stage specification development. In gene therapy, people sometimes go directly from Phase 1 to Phase 3, skipping Phase 2. Ultimately, the burden is on the manufacturer to ensure the product is safe for the patient. Safety at every phase is key.

The proper classification (starting material or intermediate) and required level of GMP scrutiny for plasmids used for AAV gene therapy products remains uncertain. In the future it is expected that regulatory agencies will provide more clarity; however, manufactures should be prepared to justify their control strategy for plasmid production and release based on how they designate plasmids in their regulatory submissions.

In the early phase, you should select the parameters you want to check and characterize so you are comfortable that you have the right raw material or plasmid with which to proceed. Through your product testing, you need to gather data to ensure whatever you assumed in the raw material stage is still correct in the drug product.

In the late phase, specification becomes more comprehensive and detailed. To start with, manufacturer can leverage the suggested testing for Plasmid stated in *FDA Guidance for Industry, Considerations for Plasmid*

"Due to the relatively recent advancement of the gene therapy field many vendors are unwilling to share information regarding their process, materials, and test methods due to IP concerns. This makes it very challenging to address yield and product quality issues with vendors who are unwilling to be transparent with the sponsor company."

DNA Vaccines for Infectious Disease Indications [1]. *November 2007*. For cell bank, ICH (Q5D) has comprehensive guidelines. The manufacturer should further characterize the cell banks and plasmids based on their sources and intend usage. My advice is that during the late phase of development, you need to have a very good understanding of your plasmid and cell bank.

Q Tell us about the key considerations regarding one's choice of plasmid backbone

BD: The plasmid backbone contains features such as the origin of replication and antibiotic resistance gene that are crucial to the overall success of the gene therapy drug product. One of the important decisions a manufacturer must make is the type of antibiotic resistance gene used for bacterial selection during the plasmid manufacturing process. The FDA has recommended that manufacturers should avoid beta-lactam antibiotics such as ampicillin for the production of therapeutic products for humans whenever possible. If the use of a beta-lactam resistant gene is unavoidable the manufacturer should be prepared to justify the use of that antibiotic in the plasmid manufacturing process.

The backbone you choose for plasmid depends on your application. It depends entirely on your characterization and the product that you are trying to manufacture. Whether you choose **AMP** plasmid or **KAN** plasmid, you need to defend your choice to ensure patient safety and product stability.

In my past, I did not see any stability-related issues caused by the backbone. We have used different backbones in the early phase and in the late phase, and with over two years of stability data, we have yet to see anything that is concerning related to stability. Our quality of plasmids so far has been very stable.

Q What are the main considerations for QA in this area?

BD: A lot goes into developing the Specifications and it must start at a very early phase. You must work very closely with your development team to understand and characterize your critical starting materials like plasmids and cell banks. Once you lock down the specification, it becomes difficult to change without a significant rationale to regulatory bodies.

Data is what is going to drive this specification and ultimately, that is always what defines QA's position. If the data makes the specification, we in QA are happy; if it does not, we need to understand why and investigate on the cause.

"One of the important decisions a manufacturer must make is the type of antibiotic resistance gene used for bacterial selection during the plasmid manufacturing process."

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Q What are the major considerations stemming from the latest guidance on qualification of AAV raw materials, especially those with significant lot-to-lot variability such as media formulations?

BD: There can be issues with media given that there are certain levels of lot-to-lot variation. The challenges in some cases are with the vendors. Vendor management is a key part to receive quality materials. A strong Quality Agreement (QA) with the vendor is always helpful to ensure consistent Quality product. If you do not understand your media or raw materials vendor's Quality Management system and do not require them to notify you regarding changes that are planning to make, thus assess the impact of changes to your product, then you may never understand why you suddenly start seeing change in Quality of your product. A minor change in raw material may cause significant impact.

Taking serum as an example, differing factors can affect its quality, such as whether it comes from the US versus Australia, the age of the cow, and what the cows are eating. These are all very important things to keep in mind. In some cases that when a vendor makes changes, such as changing the cow's feed, it can ultimately impact the serum.

In those cases, it is very important to have an effective Quality Agreement so that the vendor notifies you for all changes, and in turn, you can assess the change through your change control system and understand the impact on your product. In some cases, those impacts may not be evident immediately, but, if you think the change is significant, then you need to start trending your data over time to see if you need to take additional actions. Some companies do a small-scale study in their PD lab to see if there are any immediate impact from the change on the product. This is a good practice, if you have capabilities.

Q How do these quality considerations play into the decision of whether or not to bring plasmid production in-house?

BD: From my view, there are both pros and cons in both in-house and outsourced plasmid manufacturing. It is never easy to bring in -house manufacturing if you do not already have the infrastructure. A small start-up company may not have enough financing or the technology in place to bring plasmid manufacturing in-house. And if you do bring it in-house, there is a process change to factor to understand comparability if you have already used plasmids from other vendor for manufacturing purpose. An important consideration to make is whether you have the capability or resources to do that technology transfer and manufacture comparable Quality and Safe product.

That said, anything that you can have within your own control becomes easier to manage. However, you must build in infrastructure including phase appropriate Quality Management systems around this. In addition, you are also exposing yourselves to regulatory inspections if you are manufacturing in-house.

People need to think about what is best for their business and their sustainability. In the current environment, we are seeing high demand for plasmid that impacts almost every gene therapy product around the world.

Q What are your thoughts on standardization initiatives relating to AAV raw and starting materials?

BD: As an industry, we have this great opportunity of being at such an early stage for GT products. Many things are confidential and involve proprietary information, but I think that if there are any mechanisms through which we can share our knowledge in a common platform and have an open discussion with regulators, then changes of harmonization is high. There are some forums out there that are trying to do this by publishing white papers or seeking feedback from different manufacturers. We should consider sharing knowledge as appropriate. This approach opens discussion with the regulators to ensure harmonization. As a result, I am sure there will be some new guidelines or regulations coming out soon.

Q How would you characterize the current state of harmonization in the field across different regulatory jurisdictions – for example, between the US and Europe?

BD: In the industry, it is always good to have one pathway to follow. There are some challenges when you try to bring multiple vendors in under one umbrella. From an industry perspective, if the regulators come together to provide a harmonized guidance for plasmids used in the GT manufacturing, clarifying the phase appropriate requirements, also makes vendor management easy and consistent.

REFERENCES:

1. FDA Chemistry, Manufacturing, Controls Information for Human Gene Therapy Investigational New Drug Applications (IND), January 2020.
2. ICH Q5D: Derivation and Characterisation of cell substrates used for production of biotechnological/biological products.
3. FDA Guidance for Industry, Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. November 2007.
4. United States Pharmacopeia (USP), Gene Therapy Products, USP <1047>, November 2020.
5. FDA Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications.
6. FDA Guidance for Industry, Recommendations for Microbial Vectors used for Gene Therapy. September 2016.
7. European Medicines Agency, 24FEB2021, EMA/246400/2021, Inspection, Human Medicines, Pharmacovigilance and Committees Division, Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin. used to transfer genetic material for the manufacturing of ATMPs.
8. European Medicines Agency, 22MAR2018, EMA/CAT/80183/2014, Committee for Advanced Therapies (CAT), Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products.

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS

SPOTLIGHT

INTERVIEW

David McCall, Commissioning Editor, *Cell & Gene Therapy Insights*, talks to Dennis Royal, Associate Director, Supply Chain, Nkarta, Inc

Adapting materials supply chain management for a post-pandemic world

DENNIS ROYAL is an Associate Director at Nkarta Therapeutics, where he leads the Supply Chain functions for Nkarta's clinical stage programs. He has been in the industry for over 20 years and has been involved in small molecules, polymer and cell therapy.

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Q What are you working on right now?

DR: As the Associate Director for a clinical stage cell therapy company, the main objective of our team is the

continuous supply of raw materials to ensure manufacturing runs are not interrupted as a result of not being able to provide the required materials. With that said, continuous interaction with vendors has been a vital function for me over the past couple years, because of what the COVID-19 pandemic has done to supply chain as a whole.

Q What have been the chief challenges and issues that the COVID-19 pandemic has presented to you in your role, and how have you adapted your risk management strategy as a result?

DR: As I mentioned, being aware of the challenges vendors are dealing with that relate to raw materials we procure from them for our needs has been a significant task over the past couple years now. Keeping the lines of communication open with vendors that have high risk items has been crucial. It has not only been open but also constant communication, as lead times for any given item can change rapidly. Therefore, it is crucial to ensure you have the most up to date information from those vendors.

The mind set has changed from minimizing storage costs and not wanting to spend excessively on materials to maximizing internal raw material inventory. In the past, the idea was always to carry what you need for your short-term outlook and replenish as you use up those materials, ensuring you always had a sufficient buffer to the next delivery. This was a result of not wanting to spend excessively on materials and potential additional storage costs. What this pandemic has done to the supply chain has completely removed these concerns about having too much in stock, as lead times for items have significantly increased and there is a high level of uncertainty as to when things will go back to normal. Today, our thought process is based on how much we can order to get us out as far as possible but without having to worry about materials expiring – that is the approach we have implemented. Again, this does not pertain to all items, but once an item hits that bump where the lead time is drastically changed, then it is placed in this bucket of high-risk items that needs additional oversight.

Q Finally, what are your chief goals and priorities for your role over the coming 12–24 months?

DR: Over the next year or two, the main priority is the same as it is for any clinical stage company: ensure we get the product to the patient. With the ever-changing dynamics of supply chain, especially around materials, it will be crucial to continue to pay close attention to what items could potentially be impacted by global shortages. We will then need to work with our vendors to plan our long-term demand

"...continuous interaction with vendors has been a vital function for me over the past couple years, because of what the COVID-19 pandemic has done to supply chain as a whole."

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needs, which will in turn allow the vendors to then forecast out needs from within their own manufacturing environment.

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CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

INNOVATOR INSIGHT

Current technological trends & advancements in vector purification

Elisa Manzotti speaks to Ying Cai, Nathalie Clement, Chantelle Gaskin, Matt Roach & Ashish Saksule

YING CAI is the Sr. Director of Process Development at Ultragenyx Pharmaceutical. She heads AAV downstream process development and formulation development functions, also a CMC lead of AAV clinical programs. Prior to joining Ultragenyx, Ying worked at Sanofi, Biogen, Merck, and a few CDMOs. Ying has over 20 years' experience in the development, validation, manufacturing and commercialization of different modalities including AAV, plasmid DNA, oligonucleotides, antibodies, antibody conjugates, and fusion proteins. Ying holds a Ph.D. in Chemical Engineering from the University of Arkansas at Fayetteville and a B.S. in Biochemical Engineering from Zhejiang University in China.

NATHALIE CLEMENT has more than 25 years of experience in the field of Gene Therapy, with a strong expertise in viral vectors, specifically adeno-associated vectors, in the academic and industry settings. Her research focus has strongly been focused on optimizing processes to support large-scale production of high quality rAAV stocks and their implementation into the GMP settings. During her thesis work at the University Libre of Brussels, Belgium, she developed new recombinant viruses derived from the parvovirus Minute Virus of Mice (MVM) for cancer-selective gene therapy treatments. She then joined Dr. Michael Linden's laboratory at Mount Sinai School of Medicine, New York, where she developed novel recombinant AAV vectors and directed the AAV Vector Core. She next joined the Powell Gene Therapy Center in 2008 as the Associate Director to supervise AAV production and

testing at research, preclinical and clinical grades. She led the Process and Development Group and the Quality Control group responsible for the production and release of all AAV pre-clinical and clinical lots. During her time at UF she oversaw manufacturing, release and stability campaigns of more than 7 AAV INDs from start to finish, including CMC preparations and interactions with FDA. More recently, she spent several months at Resilience, Alachua, Florida, as the Director of Process and Development of the Viral Vaccines and Gene Therapy franchises. IN that role she oversaw viral vaccine and AAV production scales up to 200L in suspension format and in the icellis 500 platform for adherent platforms of a variety of viruses and AAV vectors. Currently Nathalie is taking a break before starting a new adventure in 2022.

CHANTELLE GASKIN is a Field Applications Scientist, specializing in protein and viral vector purification and downstream process development. She held leadership positions at Applied Genetic Technology Corporation and Brammer Bio, prior to joining the Thermo Fisher Scientific Bioproduction Division in 2020. With over 10 years of experience in gene therapy, Chantelle has accumulated comprehensive knowledge of standard industry practices and regulatory standards, applying this knowledge to advance development of therapies for a variety of indications including ocular, CNS and systemic disease.Chantelle holds a Master's degree in Chemistry from University of Florida and a Bachelor's in Chemistry from Smith College.

MATT ROACH leads the AAV Process Development group at Precision BioSciences, which is focused on designing and implementing new strategies for the production and purification of adeno-associated virus. Matt completed his Bachelor's degree in Biological Sciences at North Carolina State University and his Master's degree in Microbiology and Cell Science at the University of Florida. Prior to Precision, Matt spent time at Pfizer working on the purification of AAV and the Biomanufacturing Training and Education Center training industry professionals on downstream bioprocessing operations.

ASHISH SAKSULE is the Cell and Gene Therapy process development lead and technical expert on bioprocessing platforms for viral vectors (Lentivirus and Adeno-associated virus vector) and non-viral vectors with more than 7 years of experience. Ashish has graduate degree in Chemical Engineering from Michigan Tech University, and Biotechnology graduate degree from Harvard University. His experience spans research & drug development, clinical stage and CRO/CMO settings. Ashish is currently working at Takeda within Global Gene Therapy and have previously worked at MilliporeSigma and Miltenyi Biotec.

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Q Can you sum up the key current technological trends and advancements in AAV vector downstream processing?

YC: There are three key trends regarding AAV gene therapy. First, we want the enrichment for full AAV particles to be as high as possible. This is not only done by removing empty capsids, but also partially filled AAV, which is quite challenging. Secondly, there is a rising regulatory bar for the control of adventitious agents including viral clearance and inactivation. The third trend is manufacturing cost reduction from the clinical phase to commercial. Manufacturing cost consideration is becoming more important. We have seen high cost per dose, especially for AAV and cell therapies. Moving forward, we not only need to improve product quality, but we also need to reduce manufacturing cost per dose. Our ultimate goal is to make these drugs affordable to all patients.

MR: Somewhat unsurprisingly, we are all still working towards improved recovery and purity. There have been innovative revelations on the separation of empty and full capsids that have added to this potential solution. There has been a move to continue reducing the number of purification steps whilst also maintaining sufficient purity, especially around the harvest and capture purification steps.

NC: One substantial advancement over the past five years is the CaptureSelect™ column, or affinity capture column, specifically for AAV8 and 9. Having worked on AAV9 for more than 10 years, this was a huge change in the field. We have seen it widely implemented in downstream processes in the industry.

Another new trend is the enrichment in full capsids. There has also been an effort to develop new reagents to better remove DNA and RNA residuals. Instead of or in addition to benzonase, there are current efforts to remove some DNA species that may be more resistant.

AS: A newer trend I have seen is regarding novel variants and new serotypes. Generation and screening of libraries for AAV variants has emerged as a powerful method for identifying novel capsids. Novel capsids are emerging with numerous advancements in the construct design, and we have multiple synthetic capsid variants that can outperform their natural counterparts. These include new liver-tropic serotypes such as AAV-DJ or AAV-DJ/8, muscle-tropic AAV9MYO, or even the newer AAV7m8.

For downstream processing of this novel capsid, we are still using traditional methods, which were developed for the proteins and monoclonal antibody (mAB) space. There is a key technological need to focus on the newer novel serotypes. There are tools that are being developed specifically for AAV such as CaptureSelect™ AAVX. There are also new key players emerging who can provide custom AAV serotype-specific affinity ligands, as well as newer formats of chromatography media such as monolith or membrane adsorbers formats, which can deliver higher performance as compared to traditional resin formats.

The separation of empty and full creates a mandate for chromatography suppliers to explore new surface chemistries and methods with the goal of achieving adequate separation for all the serotypes. Until then, many of us are still relying on traditional methods such as ultracentrifugation.

Lastly, the application of fast and high-resolution analytical tools is important. Confirmation of all the results with the techniques that we work with for weeks to months is not a problem. Relying on them for day-to-day guidance, especially within process development where decisions need to be made on the spot, is a burden. That is where high-resolution and quick analytical technologies will be necessary.

CG: From the vendor side of things, I personally am looking at the column-free systems on the horizon. One example is essentially a liquid-liquid phase separation approach, based on a hydrophobic affinity reagent binding to the target molecule in the crude harvest phase. This is combined with tangential flow filtration to produce purified material. Another example is a single-use flow-path system using a chromatography resin in a recirculation flow path. The different process buffers are connected and are allowed to circulate in the flow-path along with the crude material. If they are applied at the correct time, then the purified material is eluted in a separate vessel.

Q How are current solutions helping to address the challenge of empty/full capsid separation?

MR: This is an exciting topic that has made significant progress in the last few years. Companies are moving towards designing platforms for AAV. It has become more apparent just how different various AAV capsids are from each other. Additionally, you need to account for the differences in production systems, heterogeneity of viral proteins, and heterogeneity of packaging, which can be a challenge.

The good news is that many groups are tackling this. We have seen an increase in the number of resin and column manufacturers providing specific solutions to empty/full capsid separation. Four years ago, vendors had no specific solutions, only general recommendations and examples of model proteins, like BSA being separated with an anion-exchange resin. We have seen a large increase in the number of vendors approaching us personally with initial methods that have been tested for AAV. It is pretty promising.

There is still a large space to be explored regarding additives. We are seeing that start to develop, and it is promising that people are willing to share that information.

CG: From a regulatory perspective, people are finding they want to get ahead of the bar being raised, as there are not many regulatory guidelines yet. Like Matt said, it is very interesting how people are willing to share methods. A handful of papers and posters published in 2021 have tackled the subject. I have seen the use of divalent salts and other additives to modulate the retention times between the two species, so that you could get baseline separation and even proceed to step gradients in some cases. We have made some advancements, but it is still challenging.

NC: I would like to emphasize the challenge of separating full and empty capsids. The innate nature of these capsids is that the isoelectric point (pI) is so close and requires a specific method. This is why chromatography methods have been slow in becoming efficient,

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though we have seen progress here. Successful separation may end up being very product and platform specific. We will be able to develop standard protocols, but we need to keep in mind that each product will be different. The percentage of empty in a harvest is affected by the AAV construct itself: the length of the genome and the sequence of the genome. It is also affected by the type of helper being used, such as a *rep/cap* helper or adenovirus.

AS: We still need more technological advancements in platforms that can be applied for multiple serotypes. Right now, it is time-consuming, and we need to develop a process individually for each serotype. If we are working with 10 different targets, a lot of hours and resources are spent developing a process for the individual serotypes.

There are technical difficulties and challenges existing specifically around elution. For example, the close similarity of elution conditions leaves the separation vulnerable. The variation in temperature, buffer formulation or lot-to-lot differences among the buffers, the chromatography media, or the AAV feed material itself, can add a lot of challenges. Even small variations can compromise the separation and recovery of AAV.

Empty capsids are reported to have some beneficial effects, under certain conditions, based on their immunological similarity. Empty capsids can act as an effective decoy to reduce the neutralization of AAV vectors by pre-existing antibodies, thus increasing the target tissue transduction following systemic administration. We need to find out exactly how much percent empty and full AAV are beneficial, and whether we should focus on removing all the empty particles. We must balance both sides of the separation, and this will be useful for systemic administration.

YC: Empty/full separation is based on small differences in the pI. Recently, in the October issue of *Cell and Gene Therapy Insights*, my team published a paper using capillary isoelectric focusing to explore this. We demonstrate that measured pH is different from calculated pI; there is also a heterogenous species of different charge profile ranking between 6.2 to 7.0, which is very different from the theoretical pI being reported: 5.9 to 6.3.

This is caused by several factors including the capsid post translational modifications (PTM), not just the length and sequence of the genome. Certain PTMs can shift the charge profile drastically. This is also highlighted during this forced degradation study, where the shifting of the charge profile is visible. The heterogeneity of charge profiles are observed in different products, as well as the same product of different origin (clone, serotype, or different upstream conditions).

Right now, it is more of an art than a science, as we do not understand all the root causes contributing to this charge profile heterogeneity. Mixed mode chromatography will become interesting to apply to this field. We are also exploring gradient separation, and how can we apply it to the industry, but the challenge from the GMP environment is whether it is possible for it to become single use.

Q If you were starting a new process development initiative today, would you recommend utilizing ultracentrifugation or would you bank on chromatography techniques or other new technologies to enrich full capsids?

MR: We have been through this at Precision, and our choice was to go with chromatography. We have devoted effort on the process development side, which has been no small feat. As you get to the later stages of a clinical trial, you think more about comparability. When you are transitioning from phase I/II to phase III, you are going to modify your initial production process. If this is a chromatography method, which it more than likely is for commercial production, it is better to start early. You are likely going to get a worst-case scenario for your percent full at the beginning, but it is ideal to build it early and then improve upon it. That being said, you must hit certain metrics. I would not recommend doing that if you are 10 to 20% full, for example. As you approach higher than 40% full, you are probably in a good state to switch over to chromatography.

Q What are the chief implications of residual testing, for example in terms of cost and time, and what are the keys to optimizing this aspect?

NC: Measuring residuals, whether DNA, protein, or product or process derived, has become a very hot topic over the past few years. In my opinion, there are two reasons. The technology has advanced tremendously, so all the testing has become more sensitive and more accurate, for example ddPCR, next generation sequencing, and RNA sequencing. In parallel to the technology improvements in the assay itself, the clinical doses have dramatically increased, mostly because of the type of indication treated. With higher doses in the clinic, there comes a higher burden of residuals, and therefore a need to better determine the amount and the type of residuals. We have seen toxicity in humans during the course of several trials, furthering the importance of measuring residuals.

One of the keys to success in residual testing is to determine the type of residuals you are going to face in your own platform with your own product. Separate the ones that are very common to every single product and platform like host cell DNA and host cell protein. CMOs have invested in the field, and they are going to be able to offer assays that have been, to some extent at least, validated and standardized.

Spend more time looking at what is going to be specific to your product. If you are using a specific helper virus, for example baculovirus or HSV, you are going to require different types of assays. Keep in mind that developing specific assays for your product could be more time consuming and therefore more expensive, so take this task on early. You also need a well-defined clinical dosing regimen early on. It is sometimes difficult to think about the clinic if you are just about to start screening candidates. However, the importance of your residuals will be impacted by the dose you are going to choose, the route of administration, and also the localization of your administration. Being in the eye, the liver, or being systemic will present very different impacts.

There is still a significant need for improving the technologies, mostly for accuracy and consistency across various products. It is becoming more important to know that the techniques developed are validated across multiple Investigational New Drugs (INDs), so you can have a

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basis for comparability between a product that may show some toxicity or immune reaction in patients and another product that would not. Moving the field towards sharing and standardizing more is critical for everyone, especially in terms of residuals.

AS: There are a lot of guidelines available for residual testing. For example, with cell-substrate DNA, we want to have less than 10 nanograms per dosage with a median DNA size of 200 bp or lower. From a process development point of view, these guidelines need to be addressed by establishing process optimization strategies when the residual host cell DNA is present as a nuclease-sensitive process-related impurity. One of the challenges is that there is residual nuclease-resistant host-cell DNA that has been packaged within the AAV capsid.

For process optimization, based on the close similarity with the desired vector product, it is difficult to eliminate the AAV package host cell DNA impurities by regular vector purification methods. The separation of AAV particles based on density or by gradient ultracentrifugation can remove the AAV package nucleic acid impurity, as they can differ significantly in length from the vector genome based on different densities of the respective particles. In addition to chromatography, gradient ultracentrifugation has been shown to improve this to four to five-fold. This again can represent a scalability challenge when we move into large clinical programs.

CG: One of the more critical aspects of assay development is getting hold of representative reference standards, which the downstream process development team is usually responsible for. It can pose a bit of a problem because before a process is finalized, you must start assay development; it must be developed concurrently with the process development. This means the process that you use to make any reference standards for assay development might not be your final process.

It can be helpful for a sponsor company to establish your formulation buffer early on during the process development. This allows you to use a standard platform column chromatography, or even an affinity chromatography step, then buffer exchange your material into your final formulation buffer, to serve as a surrogate for your reference standard in the interim.

YC: We should always push for improving methods and the manufacturing process. We are always being asked for the residual specifications from a safety perspective as early as possible, but we are reluctant to set the specifications based on very early data. It is equally important to demonstrate impurity clearance as early as possible, by designing scale-down studies to analyze impurities that could introduce certain safety concerns with higher doses. Overall, the keys are demonstrating testing, improving methods, and also demonstrating the process capability for downstream operations as early as possible.

MR: To echo what other panelists have said, an interesting way to approach this is to prioritize testing for final material studies, supply and animal studies, initial animal studies, then compare to confirmation runs as the process improves as a check. These tests, especially if they are outsourced, can get quite expensive. Designing specific Design of Experiments (DOEs) carefully around key steps like the harvest process and in various buffer conditions for possible chromatography steps is important.

Q Is there any trend of companies being more open with sharing critical quality attributes (CQAs) and residuals information, to better understand how products are affecting patients?

CG: In industry, there is a sense of keeping CQAs and other material information close to the chest. However, there is a very slow-moving trend towards being more open with data on reduction of host cell protein and other types of residuals.

NC: Being able to see data, especially on residuals, would be critical to the field. I do agree with Chantelle that there is a trend there, but we are far from being where we should be. I hope that the FDA will push towards sharing this information, because this is exactly how we are going to understand the role of residuals and their toxicity, if any, in a human body.

Turning to adventitious agent inactivation, removal, and viral clearance – what is the current state of the art?

YC: Currently, we inactivate and remove adventitious agents through more traditional approaches. For example, inactivation is typically done through heat, detergent, or lower pH. The removal process typically uses different chromatography modes, including affinity-based modes to find the protein, and different anion exchange steps during separation.

With AAV, we need to be careful when selecting viral filters. Viral clearance is dependent on the manufacturing platform being used. In the early clinical stages, it is possible to get away with not executing viral clearance, especially if you have a low-risk manufacturing process. This is a small part of the control strategy, and you can still test your raw materials, cell bank, starting material, or seed bank. If we have a high-risk process using adenovirus or helper virus, then removal needs to be demonstrated with a viral clearance study, as well as inactivation. If you do not have a key inactivation step, then it can be difficult to add during a later clinical phase.

Due to the rising regulatory bar, I recommend thinking about what the risks of your process are. Also, justify the choice of your model virus. For example, AAV is relatively small, so consider the smallest model virus you want to use. We are not currently using very small viruses; we are not trying to use MNV yet, although we have tried SV40, which has been quite successful.

MR: Having worked on later stage projects, this should be dealt with earlier rather than later. If you do not have something like detergent or an inactivation step built in, it can be quite disruptive to the process to add later. Otherwise, the general steps that people are going through – affinity purification and anion exchange – will help in providing the appropriate log removal values. There may be slight modifications, like low pH holds, that can be added to achieve sufficient viral clearance. It is important to rely on the quality organization within companies as well and have robust raw and starting material qualification.

NC: Focusing on testing your raw material and your cell banks early on is critical. Viral clearance, as Ying said, is not required for phase I or II, so it may not prevent you moving to the clinic, but it is still something to consider. An issue I faced myself is when you are using a virus as a helper, like HSV in my case, testing the raw material, your HSV stock, is a challenge

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in itself because you may get false positives. This makes the development of your adventitious assay a little more complicated.

CG: I have seen the introduction of older technologies like viral reduction filters specifically designed for the removal of larger viruses. This has been adopted in some processes and has worked really well with high recovery.

Q Chantelle, regarding a previous answer: did you observe high aggregation levels in your process intermediates, and what did you include to reduce or remove aggregation? Could you find a good purification solution?

CG: There are certainly some serotypes that have more of a tendency to aggregate, such as AAV2. Some other novel capsids might have some aggregation problems depending on the buffer background. I have worked with a few processes where aggregation was alleviated by adding different excipients throughout the process. Intermediates can suffer aggregation because sometimes you need to include longer hold times between unit operations.

If you have an unstable intermediate product, I recommend looking into either non-ionic detergents or potentially different amino acids in a small-scale screening study. Including a stability study early on during process development allows you to get an idea of what your stability really is.

Q What issues can a lack of serotype-specific technologies present to process development, and what solutions are available?

AS: Unfortunately, due to current lack of serotype specific technology, the approaches that we are using are still traditional methods such as cesium chloride gradient or iodixanol gradient ultracentrifugation combined with filtration technologies. One benefit of this is that we can distinguish our serotypes based on the physical characteristics versus chemical characteristics.

It is easy to develop a process based on the physical characteristics of AAV, because regardless of the serotypes and the capsid differences, we still see similar physical characteristics. Due to this, we can utilize many filtration-based technologies, making process development easier. We can adapt the process based on the physical characteristics of the viruses. On the other hand, if we consider chemical properties, we see multiple differences. As an industry, we need to continuously work on bringing new technologies that can address multiple serotypes and novel variants.

YC: For AAV we have some choices for accommodating different serotypes. We need more publications regarding the fundamental mechanism, regarding which part of the serotype the peptides are binding to associated with the resin. There is continual work to do around developing the technology and working closely with resin manufacturers and vendors.

This includes possible work on the isolation of certain peptides or antibodies which have more specificity to the serotype the company is using.

NC: If you are lucky enough that your serotype or capsid variant works well on any of the current tools, specifically the affinity resins that are available, such as AVB or CaptureSelect™ resins, there is no problem with using the same method for each serotype you have. The exception is the possibility for cross contamination that needs to be assessed once you are in the clinical environment.

MR: We have taken the approach where we have a whole platform, then we test a given serotype as it comes through. We may have to modify a portion of the platform, but the rest will ideally stay intact. However, something like empty/full separation may have to be modified significantly. The various serotypes we have tested to date fall in a few buckets depending on their homology. They may need slightly different buffer conditions, or slightly different load conditions. These options for manufacturing make things easier when novel serotypes come through.

CG: Putting the time in to design a high-throughput screening experiment usually gets overlooked. Often, people want to brute force through small-scale experiments using 1 or 5 ml columns. Static mode small-scale screening tools can be useful in this case, to give good data early on in your process run. I would encourage people not to shy away from doing something like that.

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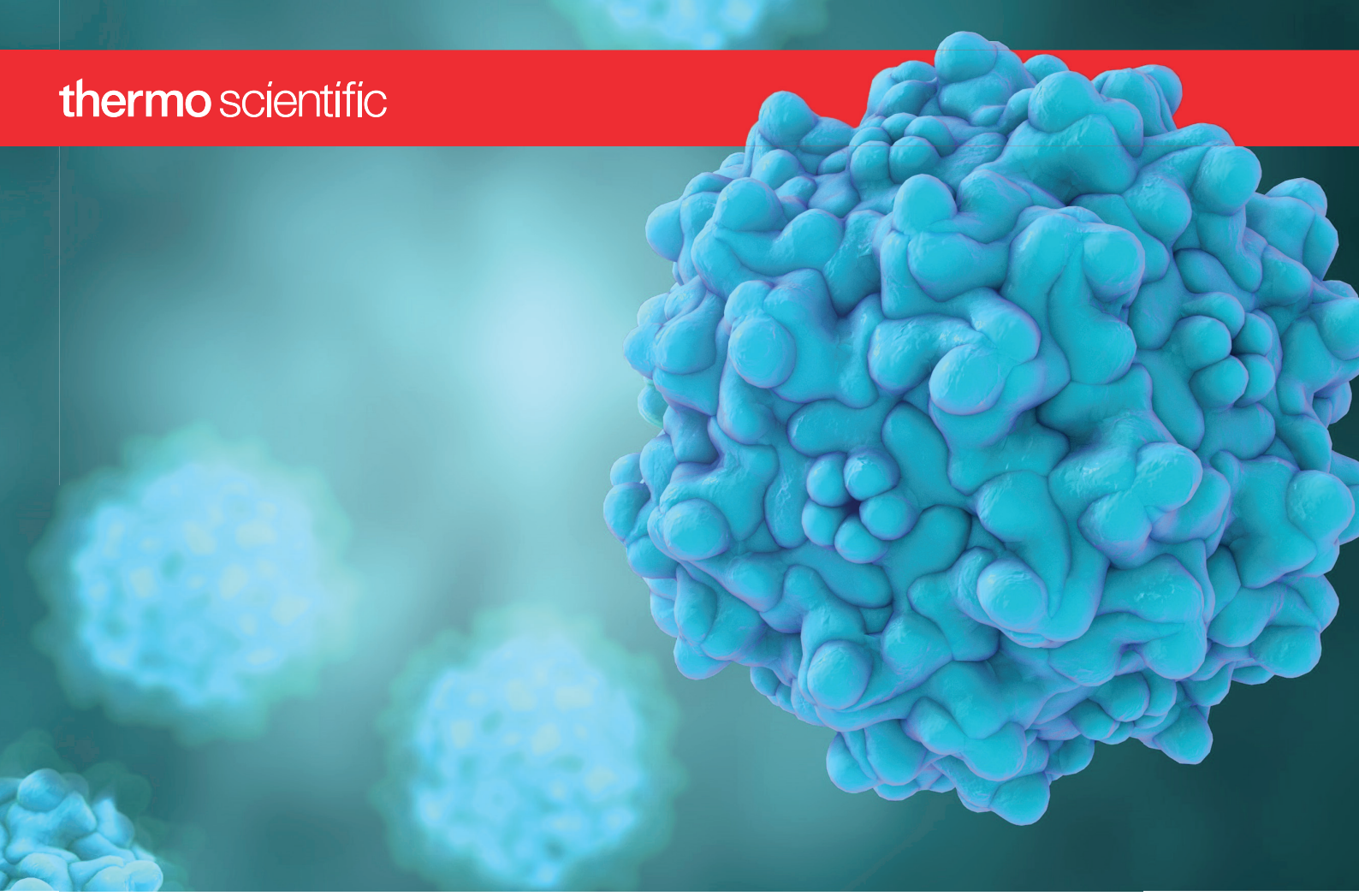

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CELL & GENE THERAPY INSIGHTS

PODCAST INTERVIEW

Key factors to consider for successful cell therapy manufacturing: a case study



Dave Humphries, Content Marketing Manager, Thermo Fisher Scientific, **speaks to Valentina Becherucci,** QC Scientist, Children's Hospital Meyer, **Øystein Åmellem,** Director of Cell Therapy, Thermo Fisher Scientific, **and Xavier de Mollerat du Jeu,** Senior Director R&D Cell Therapy, Thermo Fisher Scientific.

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DH: Today, we'll be discussing the key factors to consider for successful cell therapy manufacture. Valentina, can you tell us a little bit more about what you do at the Meyer Children's Hospital?

VB: I work for a small cell factory located in Florence, Italy, at the Meyer Children's Hospital. Our cell therapy is represented by allogeneic bone marrow derived mesenchymal stromal cells (MSCs), and we have two approved clinical protocols. One is for the treatment of steroid-resistant graft versus host disease (GvHD) in pediatric patients. GvHD is a pathological condition that can occur after an allogeneic transplant system. The second clinical protocol is a multi-center Phase 2 study in collaboration with several Italian cell factories, for the treatment of older patients with COVID-19-related pneumonia.

In short, the production process is completely open, as we work under a class A cabinet, with a class B background. The production process starts with the isolation of MSCs from 10ml of bone marrow, and thanks to their ability to grow on plastic surfaces, we use plastic flasks of different sizes for the culture. As you know, MSCs are only 0.001% of bone marrow white blood cells, so they must be isolated and cultured to reach therapeutic doses.

Our production process takes about four weeks of cell culture, with media changes two times per week. Our cell culture medium is composed of the gene element supplemented with a 5% human platelet-lysate, produced completely internally. After one week of culture, the cells reach about 80% confluence on flasks, and they are decanted, counted, and treated again until we reach the therapeutic dose (1 million cells per kg for pediatric patients, and 3 to 4 million cells per kg for older patients). After about three or four weeks of culture and at the last passage, the cells are counted, frozen, and stored into the nitrogen enclosure until they are injected into the patient. It is important to understand that for both protocols we produce one batch for one patient, and for a media of two batches produced in one month. As I said before, we are a small cell factory.

XMJ: Are those autologous therapies?

VB: It is allogenic for both protocols. It is one donor, one batch, one patient.

XMJ: How do you find your donors?

VB: Our healthy donors give bone marrow for transplantation to the international bone marrow bank.

XMJ: Do you do any selection before?

VB: We count the white blood cells.

ØÅ: In the field of MSCs, it is also normal to use a source from umbilical cord blood or adipose derived materials as well as bone marrow. Is there any specific reason you have selected the cells from bone marrow rather than from other sources?

VB: It depends on the type of therapy. In our hospital, we work in the onco-hematology department, so we have practiced bone marrow transplantation for other malignancies. The cells are the same; with MSCs, the potency is the same whether they are derived from bone marrow or from adipose tissue or cord blood. In our case, we used bone marrow-derived because it was easier for us to source.

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ØÅ: That makes sense. When you have a four-week manufacturing time, that means that the cells are undergoing several passages. Do you have criteria for how many passages you run in your manufacturing process, in order to not lose the cell's characteristics? Do you count the number of passages so that you get to the desired end point of your drug?

VB: The data of all culture comes out after process validation. The goal is to reach the therapeutic dosage. The culture can be shorter – you can stop it at three weeks and not four weeks. It cannot be more than four weeks because, according to the literature, if you culture for more than four or five weeks, you can get some unwanted effects on cells. For example, you can get genetic variation that is not good for the patient. The four weeks comes from our process validation, where we produced five batches of MSCs, and in these batches we saw that the variability was low in terms of the number of cells after four weeks of culture. We also checked other parameters of MSCs, for example the antigen expression of specific markers that must be positive or negative according to International Society of Cell Therapy.

XMJ: Valentina, in this four-week process, how do you ensure you maintain sterility? Do you do weekly/daily QC monitoring on your process?

VB: In our process, we perform initial sterility before starting the culture directly on the bone marrow. Then, we perform an in-process control of sterility after two weeks of culture, and at the end of the culture, before freezing. In our process, cells will be frozen after four weeks of culture and then stored in liquid nitrogen until you get the patient. In this case, the sterility is performed both on cells and on the cell culture media, on the supernatant.

Q **DH:** What are the QC or analytical tests you implement in your process to ensure the safety and quality of the product?

VB: According to the regulatory specification, the testing methods must be validated, and mandatory regular testing includes testing of the sterility, endotoxin, mycoplasma, and karyotype, and in our case, we also perform cell identification with flow cytometry. All these tests are performed as in-process control at different steps of the process, and also for the lot-release at the end of the process.

ØÅ: Valentina – as you are using flasks, you operate in Class A cell culture conditions. Have you tested bags, or a more closed system that you could operate in a hood?

VB: We have tested different kinds of flasks with more surface for culture. However, we do not use bags. Bags are only used in the final step for freezing and storage in liquid nitrogen. We only use open system and flasks.

XMJ: You mentioned it is a Phase 2 process. As you move to Phase 3 and commercial, you will need to scale this process. How are you thinking about doing this?

VB: This is a good question. The goal is to reach therapeutic doses. One way to get good results and to scale up the process for the final production in Phase 3 and commercialization could be, for example, the use of a bioreactor. We are not planning to change the process, because the process is validated and works well like this. We could increase the number of rooms for the production, or we could introduce a closed system.

Q **DH:** Xavier and Øystein, you are working very hard to solve some of the challenges Valentina mentions. What things have you identified? What things are we working on at Thermo Fisher Scientific to address the challenges in Valentina's process?

XMJ: What we hear from Valentina is very common in cell therapy. Cell therapy has incredible potential to cure cancer and other disease, such as long COVID-19. A lot of the process is taught at the bench in R&D. The main question is how to take this to the commercial or industrial phase and scale-up.

Typically, they are either donor- or patient-specific, so you cannot just scale-up the volume and have 1000 doses. It is a scale-out model, where you have many different donors. You not only need to find a way to scale-out, but you also need to find a way to constantly monitor the culture because you are dealing with a moving target. Each donor behaves a little differently. We heard from Valentina, it can go from two weeks to four weeks, so you have to find ways to adapt to this.

At Thermo Fisher, we are trying to develop the tools to allow you to do this. For example, having a closed- system, meaning a platform that can be in bags outside the hoods, so you can do it in different classes of rooms to reduce cost. This will increase your ability to put multiple instruments in the same room and treat many patients in the same room. The other way is the idea of in-line analytics: being able to constantly monitor your process so you can adapt and change the culture based on how the donor behaves.

The goal is to enable commercialization and industrialization at scale, and we think through technology, you can achieve that. We are very connected to a network of collaborators and researchers, like Valentina, to really understand each different process. We want to learn more about those process, and apply what we know about technology, working together in partnership and collaboration so the drugs become available to all.

ØÅ: One of the clear trends in the cell therapy industry is that everything is changing all of the time. We see scale-up, we see scale-down, we see scale-out. It is a very dynamic environment depending on the drug platform of choice. That gives the industry quite a lot of challenges to solve, because there is no (or very limited) standardization. Everyone wants to see more standardization, but as long as everything changes all the time, that is a difficult game.

The important thing for us is to work closely with the customer. Manufacturing and developing technologies that will work in a GMP environment takes quite a long time. Working closely with customers allows us to find a better way to improve the system and make it more

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efficient, more tailored, and more potent. To solve some of the biggest challenges in this industry and target difficult application areas, for example solid tumors, we require more tailor-made technologies and systems. Scalability is a key word for us, in terms of providing systems but also the technologies that can be scaled, either up, down, or out.

XMJ: Not only do we want to make and provide good products that people need, but we have also created a network of labs, some based in US, some based in Singapore, to work in collaboration with our customers. We are going beyond just the product and are now thinking about solutions. I agree with Øystein about the importance of collaborations. Once we better understand what a researcher wants to do in a process, then we can apply different tools and work together in a partnership to help them scale.

Q **DH:** Valentina, can you detail your experience with Ilaria Scarfone implementing the MycoSEQ analytics that are critical to the process?

VB: I agree with Xavier in that the collaboration between the final user – in our case our cell factory – and Thermo Fisher specialists was very important in our experience. At the beginning of our process, we had problems with the validation of the mycoplasma testing. We were using the MycoSEQ, and we had some problems because we did not have any amplification of our cells or our medium. We were unsure of the problem with this inhibition, as we did not get any amplification during the PCR reaction. We started a collaboration with Thermo Fisher specialists in Italy, and thanks to this collaboration we solved the problem. It was due to an inhibition caused by the presence of the heparin in our culture medium. After many trials, it was with these Thermo Fisher specialists that we finally solved the problem, and we can now use and validate the MycoSEQ in our process.

Collaboration is very, very important. It is also useful for the development of new QC testing, for example sterility testing. The future of the sterility testing is dependent on the molecular way, instead of classic culture testing.

XMJ: That is a great point and a great example of why this is so critical. When we developed those products, we had standard assays. We used typical media and we tested. Once we put the product on the market, if it does not work, we cannot just say sorry – we need to follow up. Then we found the heparin in the media, and that allows us to better understand our products, and design better.

The second point Valentina made, which is very important to us, is getting feedback so we can develop the right products. It takes a long time, up to four or five years to make a product, so you have to choose it right. If you do not have constant feedback, then you may spend five years in development to launch the same product as someone else, and it will not help anybody. A constant back and forth helps to better design. In cell therapy, where things move so fast, we need to always be able to adapt if needed. Ultimately, it is about providing the right products that allows standardization, scale-up, commercialization, and make those drugs available to everybody. That is the goal.

Q DH: The MycoSEQ product is an interesting example because it has been around for a while and is used in many processes. As Xavier said, cell therapy is moving so fast, and suddenly there are requirements to test for mycoplasma in sample matrixes that contain heparin, which is critical to cell processes. Solving that problem, alongside our customers, is critical to meet regulatory requirements and bring these therapies safely to the clinic. Are there any other features we are working on now in Thermo Fisher, or from your side Valentina, that you think will be critical in the future?

VB: The most important innovation for the future in cell therapy field is the sterile processing. Manufacturers of synthetic products must address the requirements for sterile processing, especially for autologous settings. We use allogenic cells, but there are many cell therapy products used in autologous settings. They have short shelf lives, so sterility testing is critical for these kinds of products. Good sterility testing must be as fast as possible, so it is important to think about molecular assays for sterility testing. The European Pharmacopeia is going to approve rapid sterility testing with PCR assay. I think this will be the future of sterility testing.

ØÅ: Valentina, you mentioned that you free the cells after manufacturing. Have you met any challenges regarding thawing and stability of the product after freezing?

VB: After testing the stability of the product, we found that our product is stable for ten years after freezing. We tested during the process validation, and after freezing the cells they were stored for liquid nitrogen, six months, one year, and two years. To test the cells, they were thawed, then cultured again, then we evaluated the ability and adherence to plastic surfaces. We found the ability of overall 90% after thawing after six months, one year, and two years, meaning cells were stable.

XMJ: Valentia, we hear what you said about rapid sterility a lot. As you mentioned, it can take too long, and people want to release the drugs. We see processes getting shorter, but it is important to still wait for the sterility before you inject your patients. We are working on rapid sterility tests like you mentioned, using molecular tests. Just as importantly, you want to ensure you work with the regulatory body to make sure the tests are being validated and approved for this kind of use.

Even to do a QC analysis, people can use up to 50% of the total cells. They make the cell product and 50% of it is used just for QC. We are trying to make smaller miniaturizations, e.g., multiplex assays, to reduce the need for those cells. This way, you save the products instead of using everything for QC. QC takes a lot of labor and people to run those assays, so we believe that automation would help the field, especially as you scale.

Right now, for a single product you need to run many assays, which means as you scale-out, you need to multiply those assays, and therefore there is an incredible demand for labor. We think automation could be the way to scale-out.

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VB: I agree. You have to eliminate the operator dependence, even for mycoplasma testing. As you know, the European Pharmacopeia allows three methods for mycoplasma sterility. One is the culture media, but this is operator dependent. If you use PCR or MycoSEQ, you eliminate this variability, and this standardizes the whole process.

ØÅ: Valentina, in your network of academic partners, have you discussed transferring the manual, open process to an automated, closed, bioreactor-type process?

VB: The reality here in Italy is that we are academics in hospital cell factories. We are a small group, not an industrial or commercial group. Considering scalability, we have discussed the use of closed systems. On the market, there are some examples of closed systems, such as bioreactors, which could be advantageous for our production, for example to reduce the personnel that operate inside clean rooms. As you know, you have to understand how to work inside a clean room and pay attention to how you move and the products that you bring inside. There are some issues in dealing with the presence of people inside the production room.

ØÅ: The MSC space is a perfect example of the need for people coming together with different abilities: the industry, academia, and all to progress the field. It seems obvious that it is hard for an academic environment to take on a huge industrialization and transfer to an automated, closed system. It speaks to the importance for more collaboration across the industry to advance this field.

VB: One of the issues in cell therapy production is control of the variables in the process. In our process, even if validated, but controlling variability, you are still using human-derived products, starting from the cells. In our case, even the culture media is derived from humans. One of the goals in future cell therapies is to develop tests to control this variability and to standardize both QC testing and the production itself.

XMJ: Regarding your last point Valentina, we should have better chemically-defined media to avoid variability. You already have variability with your donors, so we should not add variability with the media of the products. This highlights **Øystein's** point that it is a concerted effort between academia, industry, and tool providers like us to work together and provide the best technology to be able to manufacture those drugs.

This is a case where we know the potential of this therapy. Now, we need to figure out how to make them at scale. Usually, it is the opposite: you make them, and you hope they are going to work. The difference here is that we already know the results of those drugs, we just have a huge challenge to be able to manufacture them and it will take a concerted effort between the different industry areas.

BIOGRAPHIES

Valentina Becherucci

QC Scientist, Children's Hospital Meyer

Valentina Becherucci is a QC Scientist at Children's Hospital Meyer. Valentina has a master's degree in medical and pharmaceutical biotechnologies, and a PhD in biochemistry and clinical pathology from the University of Florence, Italy. She has worked since 2010 as a senior scientist in the field of advanced therapy medicinal products (ATMPs), with a special focus on drafting and execution of analytical validation protocols, in compliance with international requirements. During her 10 years as medical and pharmaceutical biotechnologist, Valentina has gained experience in process development, technology transfer, process validation, manufacturing, and biological characterization of cell-based products.

Øystein Åmellem

Director of Cell Therapy, Thermo Fisher Scientific

Øystein Åmellem is the Director of Cell Therapy at Thermo Fisher Scientific For more than 20 years, Øystein has held a variety of leadership positions in R&D, Product Management and Business at Thermo Fisher Scientific. He was responsible for development and commercialization of several products and services, including many for the cell therapy market. He received by PhD from the University of Oslo in the field of molecular cell biology. During his academic career, he focused on the study of physiological & molecular mechanisms of tumor cell growth and was involved in investigating the method of actions for a novel group of anti-cancer compounds developed by Norsk Hydro.

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

The EuLV® System, an inducible stable producer cell line for lentiviral vector production

Bofu Xue, Nathan Yang, Amon Liu, Bright Liu, Jessie Liu & Aaron Lin

Lentiviral vectors (LVV) are widely used in gene and cell therapy. A stable cell line based production technology is crucial to the gene and cell therapy industry. This article introduces a stable producer cell line for LVV and its performance. In Phase 1, the packaging cell line, with lentiviral packaging genes gag/pol, rev and VSV-G is developed. In Phase 2, the producer cell line, with GOI stably inserted is developed. In Phase 3, all upstream and downstream processes based on stable producer cell line are developed.

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BACKGROUND

There are two methods for producing lentivirus, one of which is transient transfection, using different transfection reagents that can form complexes with DNA, allowing cells to take them up through endocytosis. This method is widely used clinically, but due to its low titre and difficulty in scaling up, it cannot meet the current industrial needs.

Another method of lentivirus production is using stable producer cell lines. Due to the low efficiency of stable gene insertion, the reported methods for constructing stable producer cell lines usually require introducing a large number of resistance genes for simultaneous or step-by-step screening. As a result of insufficient regulation and expression optimization of each lentiviral packaging gene, the final stable cell line may have high leaky expression during the construction of producer cell lines, resulting in unstable producer cell lines. These are unacceptable for pharmaceutical industry.

EuLV® SYSTEM

The EuLV® system produces lentiviral vectors using a stable producer cell line that enables high-density cell culture and inducible lentiviral production in chemically defined media with low uninduced leakage of producer cells to manageable levels (Supplementary Data 1).

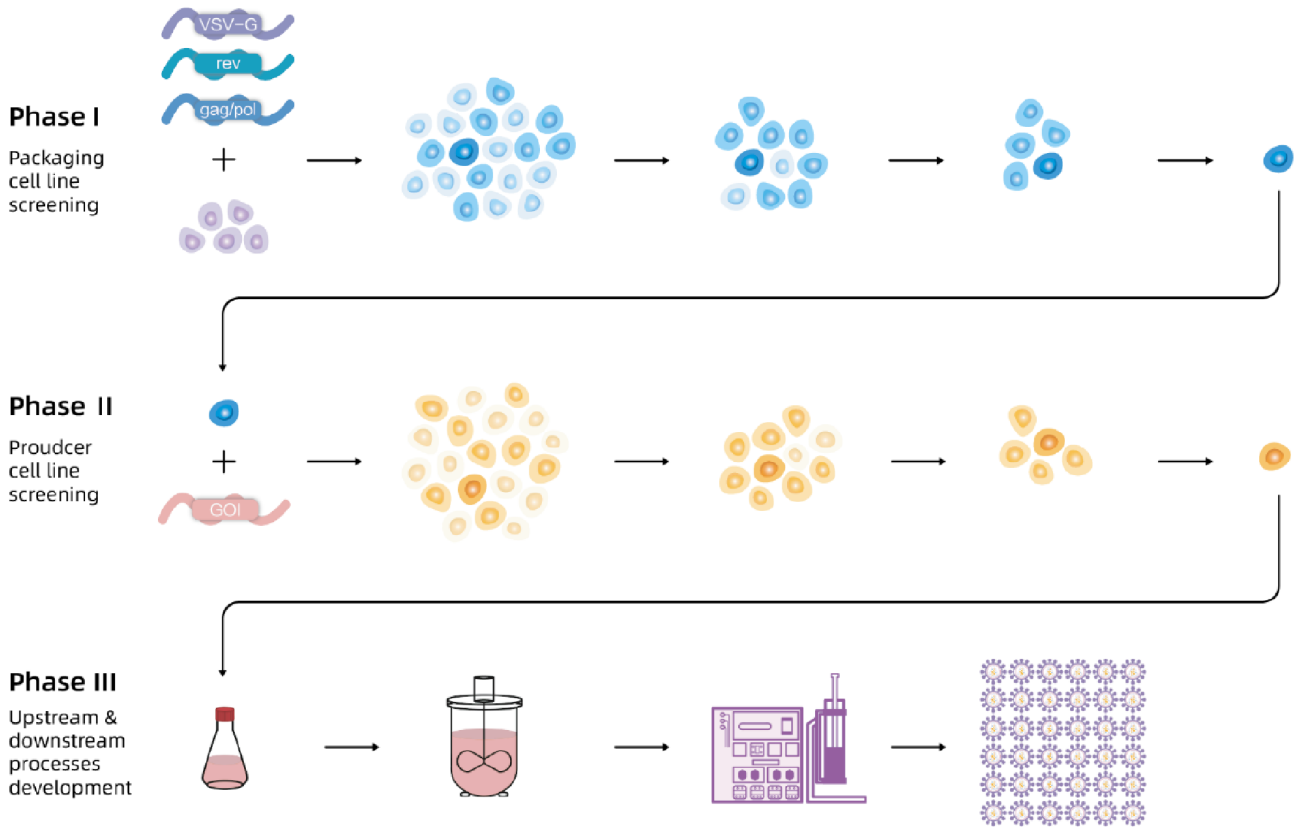
The flow chart of EuLV® system to construct the producer cell line is shown in Figure 1. First, VSV-G, gag/pol, and rev were integrated into 293T cells, and the optimal packaging cell line was obtained through monoclonal and titre screening; then, the viral genome transcription cassette carrying target nucleic acid fragment was integrated into the packaging cells, after monoclonal and titre screening, the optimal producer cell line was obtained. Finally, production and purification processes were developed based on producer cell lines to obtain high-titre and high-quality lentiviral vectors.

GENERATION OF PACKING CELL LINE & PRODUCER CELL LINE

VSV-G, gag/pol, and rev were stably inserted into 293T cells to obtain packaging cell populations, which were screened for monoclonal cells using EuBioX (Supplementary Data 2), best 10 high yielding cells were chosen by transient transfection using hPGK-luciferase- IRES-EGFP plasmids (Figure 2). Then hPGK-luciferase- IRES-EGFP was stably integrated into the chosen packing cell to obtain a population of producer cells, and the same procedure was performed to get high-yield producer cells. The virus titre during the screening process is shown in Figure 3.

FIGURE 1

In Phase 1, the packaging cell line with lentiviral packaging genes gag/pol, rev and VSV-G is developed.



In Phase 2, the producer cell line is developed with GOI stably inserted. In Phase 3, all upstream and downstream processes based on stable producer cell lines are developed.

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FIGURE 2

The hPGK-luciferase-IRES-EGFP (plasmid 19BF081) expression cassette is the Gene of Interest (GOI) , which is used for packaging cell line screening (single-plasmid transient transfection) and evaluation as well as producer cell line construction.

Luciferase assay is used to quantify the transduction titer (TU (RLU) /mL) of the produced lentivirus.

Cell culture

Freestyle 293 and other 6 commercially available CDM medium was tested for lentivirus production and CDM#2 was chosen (Supplementary Data 3). The producer cells are grown in suspension in shake flasks with an agitation of 170 rpm using 2.6cm orbital shakers, 8% CO^2 at 37 °C. Cells were passaged to 0.5×10^6/mL when the cell density reached 4×10^6-6×10^6/mL.

Medium, feed & inducer

In many lentivirus production processes, the fresh medium needs to be replaced before induction or lentivirus production. These steps hinder the scale-up of the process. In EuLV®system, media, inducers, and feeds were screened and optimized, and a feed batch method for lentiviral production was developed (Supplementary Data 4).

LV production in tube & flask

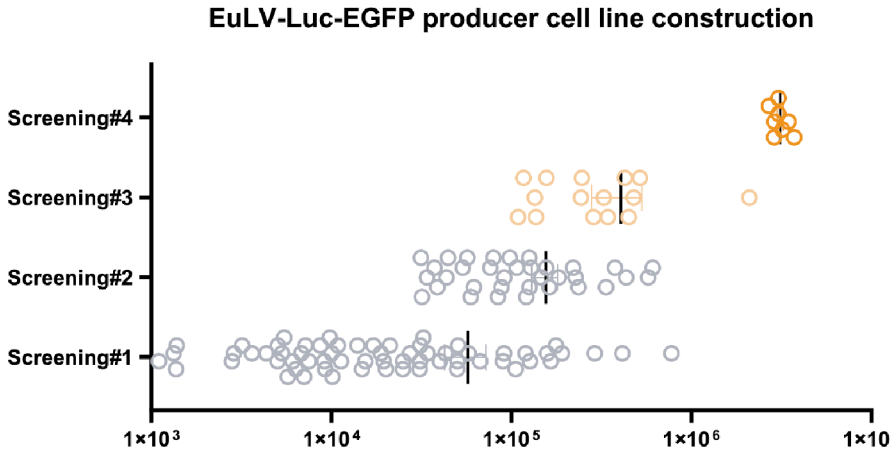
The producer cells were inoculated into 50mL tubes or shake flasks at a density of 0.5×10^6/mL, and cultured for 5 days until the cell density reached about 1.2×10^7/mL, then the inducer and feed were added to start the lentivirus production, feed was added again after 24 hours, and the supernatant was harvested after another 24 hours. The supernatant titre results in Figure 4.

LV production in WAVE bioreactor

Producer cells were seeded into WAVE bioreactors at 0.5×10^6/mL in a total reaction

FIGURE 3

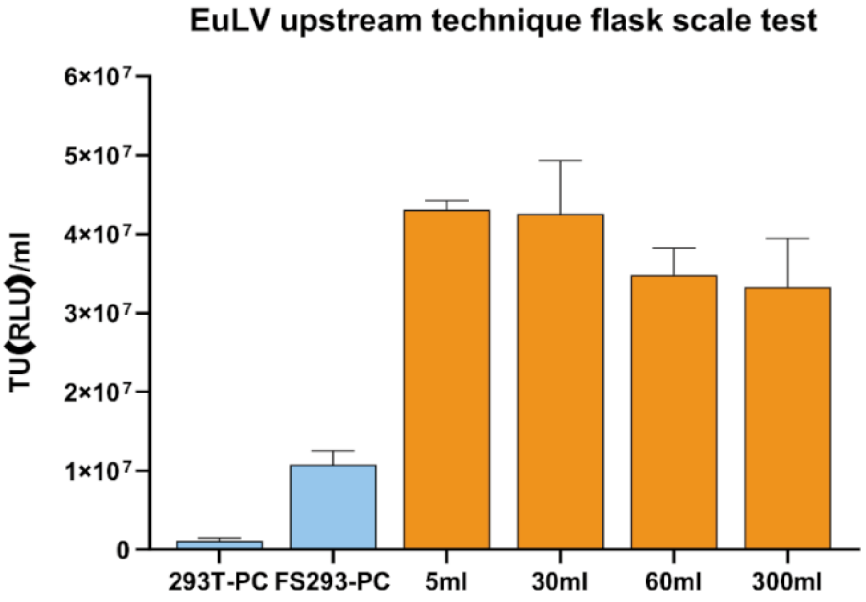
The viral titer from each clone in the process of producer cell line screening.



The x-axis shows the transduction titer measured from luciferase assay, and four rounds of screening are performed. The average titer increases nearly 100 folds after the fourth screening step. One of the best producer clones is selected to develop the lentiviral production process.

FIGURE 4

Viral titer produced in this process can increase 4 folds compared to the condition in FS293 medium (induced at 4×10⁶ cell/mL with 100% medium re-placement) , and this process is stable when tested in the shaking flask scale.



volume of 1L. The reaction conditions were 37°C, 8% CO^2, the stirring speed was 20 rpm, the angle was 10°, and the ventilation rate was 0.1 L/min. For the 1L reaction system, cells were directly inoculated into a 1L reaction system and induced to produce lentivirus when the induction density was reached (Figure 5). For the 25L reaction system, cells were first inoculated into a 1L reaction system, cultured for 3 days, then expanded to a 5L system, cultured for 3 days, and finally expanded into a 25L system, as shown in Figure 6. The cells were cultured in the final system for 5 days, and the cell density reached about 1×10^7/mL. The inducer and feed were added, and the culture was continued for 24 hours. The feed was added for the second time, and the supernatant was harvested after 24 hours.

PURIFICATION

The purification steps are shown in Table 1. First, Clarification by centrifugation or depth filtration and microfiltration to remove impurities such as cell debris (step 1). Then, the purified lentivirus was obtained by ion-exchange chromatography (step 2), ultrafiltration concentration washing (step 3), gel filtration chromatography (step 4), and sterile filtration (step 5). See Supplementary Data 5 for the lentiviral purity profile.

ANTI-CD19 CAR-IE PRODUCER CELL LINE

We also constructed the EuLV® antiCD19 CAR-IRES-EGFP producer cell line (Figure 7). Cell screening and process optimization are similar to hPGK-luciferase-IRES-EGFP. Results are shown in Figures 8 & 9.

For this batch of experiment, we get 8.13E10 TU per litre in the culture medium and 2.91E10 TU per litre after purification. From the result of HPLC analysis, the viral particle purity is 84.5%. The manufacturing process is still under optimization (Table 2).

OTHER GOIS

In order to test the versatility of the EuLV® system, the other four GOIs were also used

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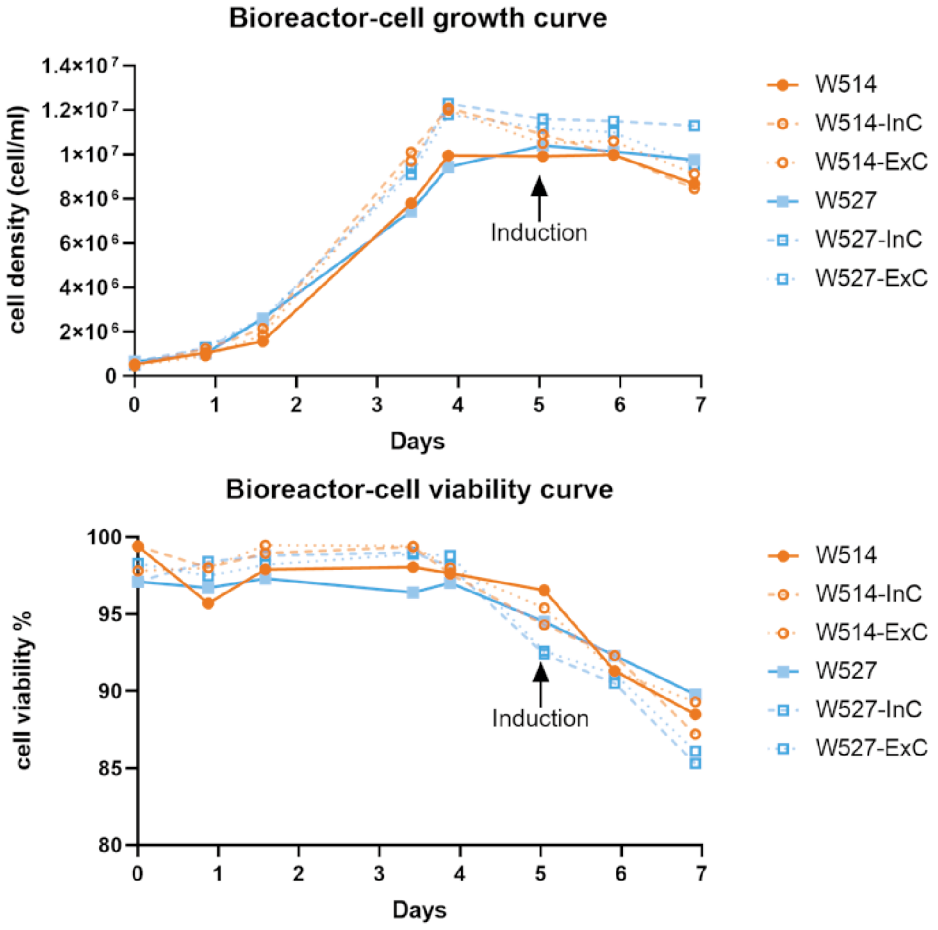
to construct producer cell lines through packaging cells, and the lentiviral yield was tested under adherent conditions. The structures of each GOI are shown in Figure 10, and the detection results of lentiviral infection titres are shown in Figure 11.

DISCUSSION

Developing stable lentiviral vector-producing cell lines is time-consuming and labour-intensive complex systems engineering that takes a year or more to fully develop and characterize cell line platforms. Due to the complexity of the work, much published work ultimately failed to meet the needs of the industry due to issues such as titre, cell line stability, and culture adaptation. However, as compensation, once a stable virus producer cell line is successfully developed, the technology has irreplaceable advantages in the field of clinical and industrial applications, the stable producer cell line is more efficient than transient transfection

FIGURE 5

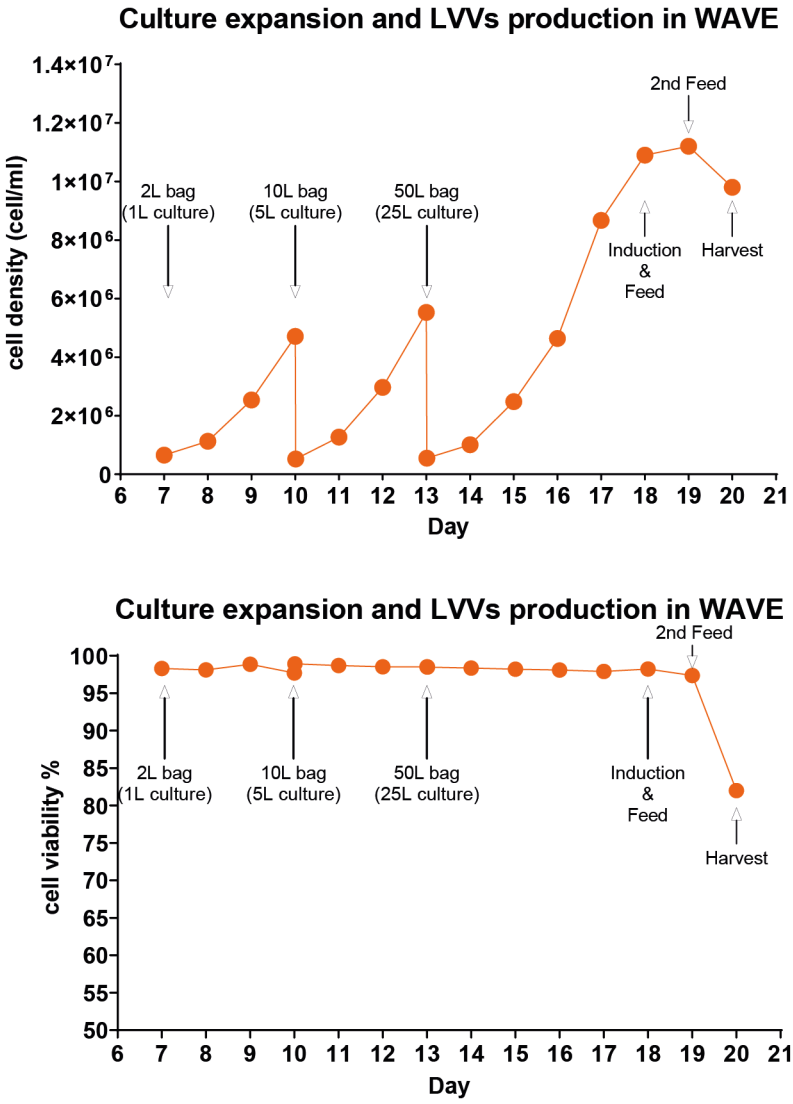
Results of two batches of 1L-scale lentiviral production using EuLV® stable producer cell line in a WAVE20/50 bioreactor, and summarize the cell density and viability data during the 7-day culture and virus production period.



W514 (orange) and W527 (blue) are independent batches. InC stands for internal control, a 20 mL sample collected after cells are filled into the culture bag; ExC stands for external control, a 20 mL sample prepared from the same batch of seed. Both InC and ExC follow the same procedure as the WAVE bioreactor in a CO_2 shaker for culture and virus production. On day 0, cells are seeded at a density of 0.5×10^6 cell/mL and continue to expand until day 5, when the inducer is added. 48 hours later, on day 7, the virus in the culture medium is harvested and purified in the downstream process. The cell viability on day 7 remains above 85%, which is obviously beneficial to the downstream process.

FIGURE 6

Cell density and viability data when the producer cells were amplified and induced for LVV production in the WAVE bioreactor.



Calculated as day 0 from the recovery of cells, on days 10 and 13, when the cell density reached around 5 million per mL, the cells were transferred to the next size of wave bag, and on day 18, inducer was added for the virus production. Same as the 1L data, cell viability is controlled over 80% upon harvest.
And for the virus titer, we detected 3.8×10^{8} TU/mL in the culture medium, 9.5×10^{12} TU/mL before harvest, and 3.4×10^{12} after purification.

production in terms of process reliability, scale-up capability, production cost, and virus product safety. First, the stable producer cell line production process is more stable and can provide a fully characterized production platform to produce safer viral vectors with low batch-to-batch variability; second, the process is easier to scale up, and the titre without drops rapidly problem when the culture volume increases; in addition, without the addition of raw materials such as DNA plasmids and transfection reagents, the company does not need to establish an additional GMP production line for producing plasmids; finally, it has the higher unit yield and simpler production process quality control. When expanding the production scale, the production process of stable production cell line will further highlight its advantages

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TABLE 1

Key data in the main purification steps from two 1L-batch of virus production.

Process flow	Purity %	TU titer 1E8TU(RLU)/ml	Viral activity 1E6TU/ng p24	Inducer residue
Medium	1.55 ± 0.09	5.31 ± 1.73	1.92 ± 0.84	N/A
Step 2	73.61 ± 4.95	3.26 ± 1.03	1.89 ± 0.89	N/A
Step 3	97.86 ± 0.76	28.60 ± 5.94	2.21 ± 1.05	N/A
Step 5	97.94 ± 1.07	11.31 ± 4.24	2.02 ± 1.03	Not detected

The purity is measured by HPLC-SEC column. The transduction titer (TU titer) is measured by luciferase assay. The recovery rate based on physical titer (recovery-VP) is calculated based on the integration of peak area in the HPLC-SEC column analysis. The recovery rate based on transduction titer (recovery-TU) is calculated based on the luciferase assay. The virus activity is calculated by dividing the TU titer by the physical titer measured by ELSIA-based P24 protein quantitation method. The result is illustrated as transduction unit per ng of p24 protein.

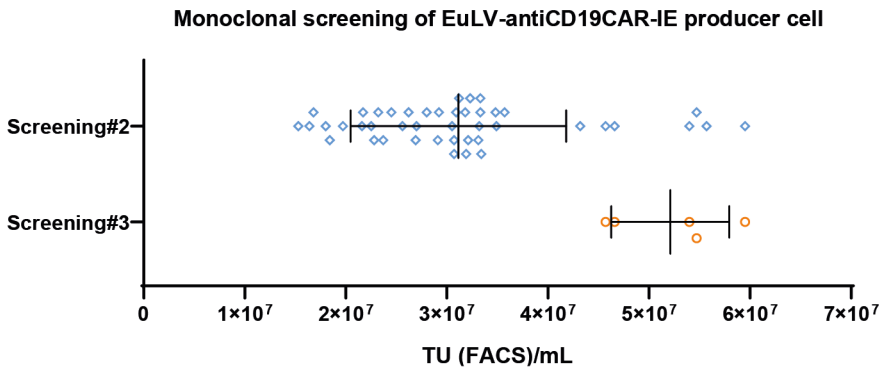
FIGURE 7

The map of the GOI sequence has the typical anti CD19 CAR [1] sequence with additional IRES EGFP sequence.

It is almost twice the length of the original anti CD19 CAR sequence.

FIGURE 8

The monoclonal screening result.



The x-axis represents the viral titer measured by FACS, representing the 2nd and 3rd screening results. Each dot represents a transduction titer result from one single clone. After 3 rounds of screening, the best clone can reach 5×10^7 TU/mL measured by FACS.

TABLE 2

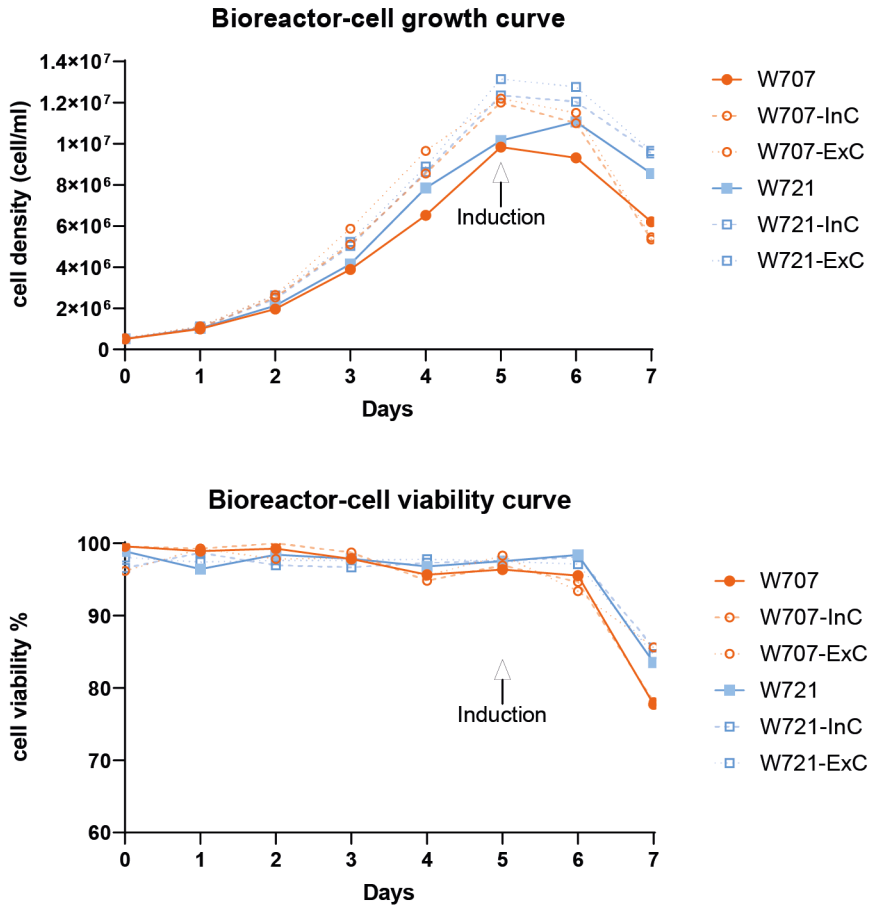
First, the lentivirus is removed by centrifugation or depth filtration, and then by 0.45-micron filtration to further remove impurities such as cell debris (step 1) and benzonase digestion (step 2).

Process flow	Purity %	TU titer 1E07Tu(RLU)/ml	Recovery (TU based %)	Inducer residue
Medium	N/A	8.13	100	N/A
Step 1	0.16	7.74	80.14	N/A
Step 3	1.38	5.94	76.16	N/A
Step 4	34.4	7.61	49.85	N/A
Step 6	84.5	78.6	35.78	Not detected

Then, the purified lentivirus was obtained by ion-exchange chromatography (step 3), gel filtration chromatography (step 4), ultrafiltration concentration washing (step 5), and sterile filtration (step 6).

FIGURE 9

Cell density and cell viability data in 1L WAVE bioreactor.



Similar to flasks, they are induced on day 5 and harvested after 48 hours.

FIGURE 10

Structures of 4 GOI, A:anti-BCMA vector [2], B:anti-HIV vector [3], C:Thalassemia Therapy vector [4], D:Temophilia Therapy vector [5].

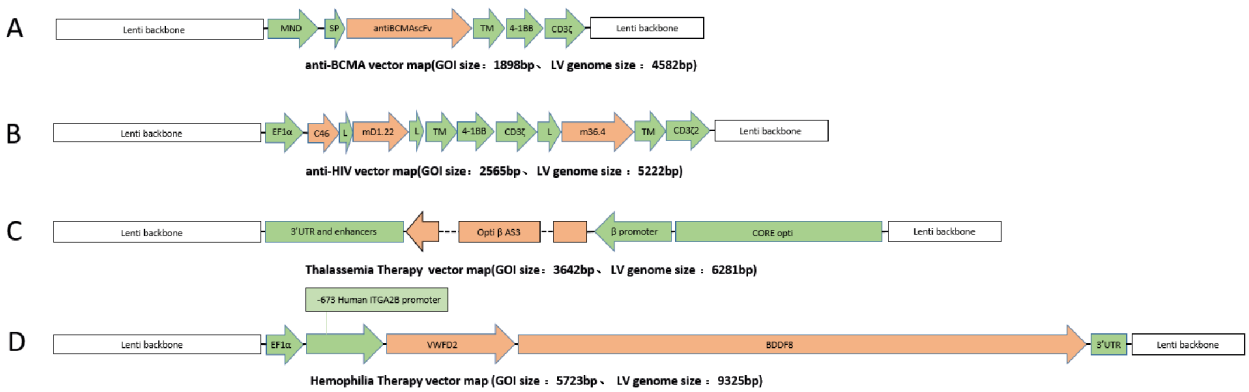


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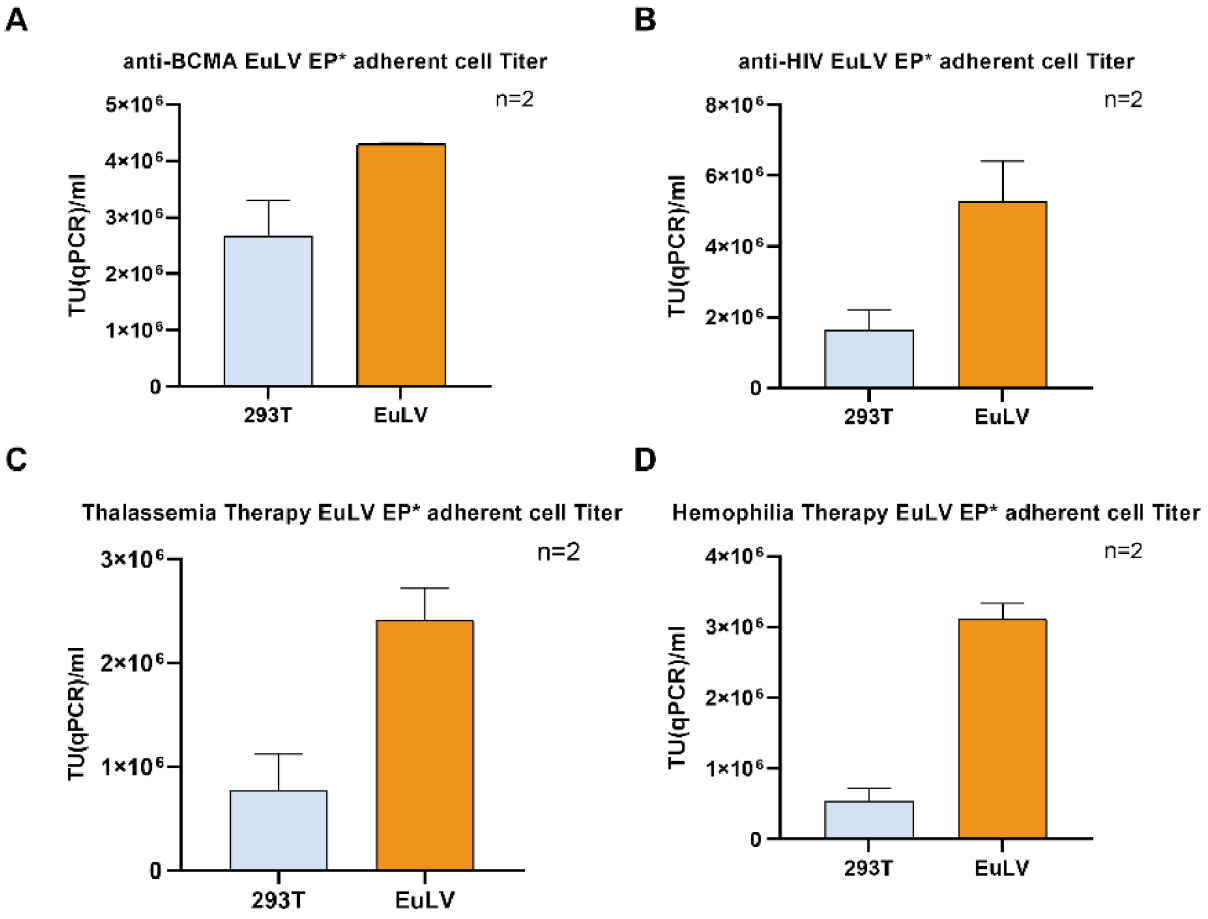
FIGURE 11

During this study, the transduction titer of the enriched pool (EP*) of adherent EuLV® producer cells (EuLV®) is measured against the transient transfection method viral yield (293T).



The viral titer is higher than the transient method. Since only EP* adherent cells are used for comparison, the viral yield will be improved over 10-fold after cell single clone screening and suspension adaption.

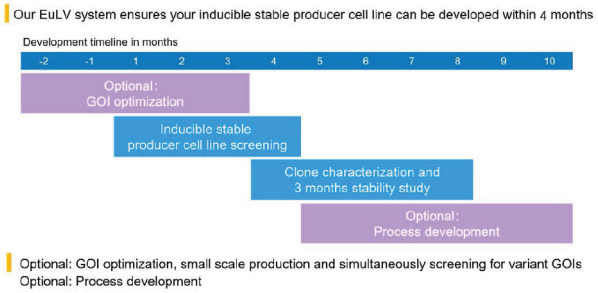
in R&D, production, management, operation and maintenance, and cost. These advantages are beneficial to the promotion of technology and drug industrialization in the field of causative therapy and cell therapy.

STABLE PRODUCER CELL LINE FOR LENTIVIRAL VECTOR PRODUCTION

EurekaBio provides CRO services of the EuLV® system. Customers only need to provide gene sequences or plasmids, and EurekaBio will deliver the corresponding monoclonal cell line within 4 months (Figure 12). Other optional services are also provided, including GOI optimization, clone identification, stability research, upstream and downstream process development.

FIGURE 12

EuLV CRO project development timeline.



REFERENCES

1. Brogdon J, June C H, Loew A, *et al.* Treatment of cancer using humanized anti-CD19 chimeric antigen receptor: U.S. Patent 10,221,245[P]. 2019; 3–5.
2. Friedman KM, Garrett TE, Evans J W *et al.* (2018). Effective Targeting of Multiple B-Cell Maturation Antigen–Expressing Hematological Malignances by Anti-B-Cell Maturation Antigen Chimeric Antigen Receptor T Cells. *Hum. Gen. Ther.* 2018; 29; 585–601.
3. Anthony-Gonda K, Bardhi A, Ray A *et al.* Multispecific anti-HIV duoCAR-T cells display broad in vitro antiviral activity and potent in vivo elimination of HIV-infected cells in a humanized mouse model. *Sci. Trans. Med.* 2019; 11.
4. Kohn D B, Morgan R A, Hollis R P. Optimized lentiviral vector for stem cell gene therapy of hemoglobinopathies: U.S. Patent Application 16/466,970[P]. 2020; 4–9
5. Du LM, Nurden P, Nurden AT *et al.* Platelet-targeted gene therapy with human factor VIII establishes haemostasis in dogs with hemophilia A. *Nat Commun.* 2013; 4, 2773

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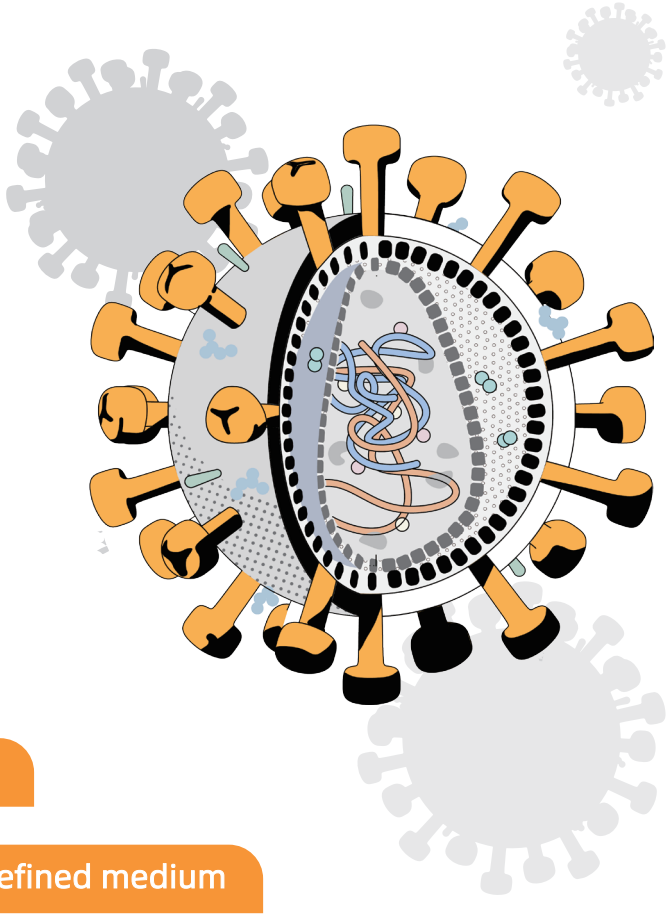
EurekaBio

EuLV® System

Inducible Lentiviral Vector Producer Cell Line



- No plasmids & transient transfection
- Suspension culture in the chemically defined medium
- High degree of batch-to-batch consistency

Production process reduced

by **50%**

Increase production efficiency

by **100 times**

Total cost reduced

by **80%**

Eureka Biotechnology Co., Limited

Tel: +86-755-86562586
E-mail: enquiry@eurekabio.com
Add: 8F, City Garden Cyberport Building, No.1079 Nanhai Street, Nanshan District, Shenzhen, China 518067

CELL & GENE THERAPY INSIGHTS

EXPERT ROUNDTABLE ARTICLE

Strategies for scaling up and out in gene therapy manufacturing: addressing AAV's growing pains

Elisa Manzotti, Founder, Cell and Gene Therapy Insights, speaks to **Chris Reardon, Phillip Vermilion** and **John Yoshi Shyu**

CHRIS REARDON is an Associate Scientist on the vector production team at Dyno Therapeutics. Chris and his team lead the scale-up and automation of the manufacturing platform for high-throughput production of AAV variant libraries.

PHILLIP VERMILION oversees GMP Vector Manufacturing at Andelyn Biosciences. He leads a team of diverse staff in the manufacture of clinical AAV vectors of many serotypes for clients around the globe.

JOHN YOSHI SHYU manages field and internal technical application scientists at Corning Life Sciences. In his role, Yoshi supports product development and application in the fields of cell and regenerative therapy and viral vector production.

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Q First, can you frame for us the state-of-the-art platforms, as well as the challenges remaining in scaling AAV vector manufacturing and boosting yield to enable continued growth in the space?

JYS: For me as a scientist, one of the most exciting things is how far viral vectors have come in the last decade. They have gone from use in disease models to treating life-threatening disease.

As to the challenges, one we definitely encounter in this field is how to keep up with demand. Demand is going to go higher and higher. More disease models or disease treatments employing viral vectors will become available, and manufacturers like Corning need to come up with solutions that allow researchers, biotechs, and pharma companies to produce more on a smaller scale, be able to produce smarter, and be able to produce better and have better controls.

CR: I'll echo this. It is very exciting to see the field growing and coming to the clinical space after so many years of development and R&D, and now to be asking the question of how we manufacture a therapy at scale.

Personally, I am really looking forward to, and have enjoyed watching, the growth of the adherent systems at scale as opposed to a suspension system for production. This is leaving the R&D or academic lab and moving into the clinically relevant scale. In addition, we are seeing many of the downstream processes mature, which is addressing a lot of challenges that are still faced in scaling. Pairing those upstream and downstream processes and having these new systems come to market is exciting to watch.

PV: From our perspective here at Andelyn Biosciences, it is exciting to see clients that have started their early-phase production with us, move to later-phase and commercial production. Internally, as we progress to later-phase production, the challenges are going to be scaling, showing the equivalency of the platforms we are working on now with all of those raw materials, and getting those to a scale where they can perform equivalently in that later phase at a much greater capacity.

Q How is the increasing availability of 'right-sized', purpose-built bioprocessing technology impacting scale up and out approaches for gene therapy?

CR: Having the right-sized equipment is extremely beneficial. The biggest

"It is very exciting to see the [AAV vector manufacturing] field growing and coming to the clinical space after so many years of development and R&D, and now to be asking the question of how we manufacture a therapy at scale."

– Chris Reardon

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thing it allows us to do as researchers or manufacturers is to let the science dictate the manufacturing, instead of letting the equipment dictate the manufacturing. We can choose a process that fits what we are making and the goals we want to reach, instead of saying we have Goal A or Goal B that are ten times separated in volume and production with no middle ground. Now we can pick a goal and let the equipment follow us.

Specifically, for clinical applications, it is beneficial to be able to produce the amount you need, especially if you are researching an orphan disease or rare disease. You can produce the exact amount you need; you might not be continuously doing run after run for years. You might just want two runs and having the right-sized equipment will cut your costs, increase your speed, and simplify your process. Even with single-use systems.

It's the same for downstream equipment – being able to run in one process to the correct size and not use an excessively large process or multiple runs of an excessively small process is very beneficial to the product and allows us to keep to the science.

PV: From our perspective, right-sized technology is going to get you up and running much more quickly. You don't have a whole lot of that development earlier on to be able to fit it into your scope, or your capacity needs. If it is right-sized and appropriate for your purpose, it is going to make that development time period much shorter.

JYS: I definitely can relate to what both Chris and Phil said in regard to the right size. I don't know if there is a perfect right size, because you have to balance a lot of different things. What is the right instrument to use? What is the right equipment to use? How fast do you want to get there?

Phil mentioned having to develop the process. If you have the wrong size to fit the disease model you are trying to study, to address the customer that you are trying to acquire, not having the right infrastructure or right size becomes detrimental. It becomes a hurdle to providing that solution.

Right size is something that comes up often: am I buying the right-sized technology to enable me to address exactly what is being asked for by the company, the CMO, or the biotech or pharma?

Q How have customization and/or creativity helped you achieve your vector bioprocessing goals?

PV: For us, growing out of research and the early-phase clinical production arena, the creativity and customization is taking something that may have been intended for a different purpose and making it fit our production scheme.

We have had to custom-build a lot of tube sets, single-use disposable flow paths, to be able to take components and fit them in our upstream or downstream processes and get them to do what we need. Early on that just didn't exist, or it wasn't readily available. Whereas

now, it is becoming much more so. Now our scale is increasing, and we are getting into that area where the purpose-built materials or equipment are in line with the scale that we are expecting to operate at.

We have had to do a lot of in-house development and modifying equipment and materials to fit that need and be able to adjust "on the fly," based on markets and resource availability. I am sure everyone out there knows that in the last two years, resources in the biomedical arena have been stretched very thinly.

Resources that we have been accustomed to being off-the-shelf are now something we have had to adapt to building a custom replacement for. Staying nimble and creative is keeping us going, and keeping the whole industry moving forward. It is about being able to use your imagination and develop a process to get your goals met.

JYS: Coming from a manufacturer's perspective, Corning has created equipment to enable customers to use their imagination. I have seen one specific product be used a hundred different times, in a hundred different ways. This goes back to the notion that customization is very relevant to the specific customer and their specific process.

Creating solutions that allow customization makes your production faster and better, enabling you to achieve your targets in a faster, smarter way. Customization is part of the vocabulary whenever you are working with viral vectors and is going to help to enable the next breakthrough in disease treatment.

CR: I think customization of any process is incredibly valuable. The standard consumables that come with most bioreactors are great starting points. They are usually very well thought out. But everyone's process is just a little different. You may take a sample differently or at a different time point. You may have some different needs, maybe you run a closed system or you run perfusion or continual feed, and being able to add that one extra line or take out a spare line you are not using is really valuable. It increases the quality of the process. It reduces the risk and number of failure points, and it is incredibly valuable to be able to have that.

There are a lot of suppliers offering services that are not off-the-shelf, but continually made custom loops. You tell them exactly what you want – the lengths, the fittings, and the connectors – and you can order 100 of those sterile bagged and delivered to your facility, which is really exciting.

We ran some process development experiments on some adherent bioreactors where we kept media in cold storage, perfused continually through a warm set of tubing straight into the bioreactor, perfused and then continually harvested on the other side. That customization of being able to splice into and out of the standard consumable set was really valuable, and the consumable set was designed to be able to work with it how we wanted to.

Q Turning to facility design, how can you safeguard against both over- and under-sizing vector manufacturing facilities from a strategic standpoint?

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PV: It is all going to start with planning. You have got to have an idea of what your target is going to be and lay out the goals to get to it. Frame that facility around what you expect to be able to create as an output and be able to still be economically viable to justify it.

A lot of your goals along the way are appropriately assessing the risk that is going to be involved, and then scaling up your process. Be able to measure your process controls to ensure that you have got control over your procedures. Ensure that your finished product can meet the quality and output yield that you are expecting to be able to get out of it.

"Having seen so many of our customers go into this field of viral vector manufacturing, one thing that comes to mind very clearly is that by the time the facility is being designed, it is very important to understand what the demand is, and what the output you need to make or generate is."

– John Yoshi Shyu

JYS: Having seen so many of our customers go into this field of viral vector manufacturing, one thing that comes to mind very clearly is that by the time the facility is being designed, it is very important to understand what the demand is, and what the output you need to make or generate is.

I have noticed that by the time someone is ready to manufacture at that level, they are already under the size they need. Maybe this is a little naïve, but my recommendation would be to look at what you currently need as the deliverable size, and double that output. Because by the time you reach that level, you are already out of the size that you need. This is something I see newcomers do year after year, and then they are feeling that pinch several years into a new facility.

It may be a little bit scary for the company, but as I mentioned earlier, the viral vector field is just going to continue growing for quite some time. By the time you make that investment and have the potential to manufacture that specific size, your facility might already be out of the size that you need.

CR: I would agree with Yoshi on this question of scale, and by the time things are made they are not enough. Your facility not being big as you need it by the time it is done being constructed is a very real concern, and has been seen in a number of examples. The field is just so popular.

My take on it is there are some early questions to ask – do you design a facility to have individual production suites, or an open facility that is flexible and can work together with multiple processes in parallel? Something that would enable that is consumables and having true closed-loop production, raw product to final product, so you can have multiple productions in the same facility without cross-contamination. And then if you have a large product coming through, you can consider having two major lines run together, going to the same

downstream loop, instead of having two separate suites where you have to run two products in parallel.

So there are certainly questions for a facility designer to ask about what kind of product they are going to make. Are they making treatments for orphan diseases, are they running a single large product and designing around that?

To echo what Yoshi said, you need to make it bigger than you think.

Q With more and more vector manufacturing facilities with over 2,000L capacity coming online, what do you see as the key challenges and solutions at large production scales?

JYS: Challenges are always going to exist with any type of manufacturing process. What I have personally noticed is that, as we are getting bigger and bigger on the equipment, we have to consider how to manage so much liquid. Chris mentioned moving media from cold storage into warm storage, and continuous perfusion. That is okay on a small scale, but if you go into a 2,000-liter scale, the management of that liquid, both upstream and downstream, is so critical. What can the industry and manufacturers create to address these large volumes in their manufacturing process?

PV: The logistics of the liquid handling is an interesting problem; not just the feed source, but when it comes to the waste handling you are going to have a lot of volume to deal with. Circling back to what we spoke about previously, this plays a role in your planning of the new facility.

The logistics of your liquid handling, and ensuring your process is controlled, are big challenges. If you can't ensure you have got control of your process, you may not have an effective product at the end. Your process at that scale is not necessarily what it was at 50, 200, or 500 liters. That is where keeping control of your process as you are developing and scaling up to that level of output is absolutely critical to ensure your end product is what you expect it to be.

"The logistics of your liquid handling, and ensuring your process is controlled, are big challenges. If you can't ensure you have got control of your process, you may not have an effective product at the end."

– Phillip Vermilion

CR: That liquid volume is phenomenal at these scales. It is truly a unique challenge to face. Consider that when many of these processes are invented, they are going on in an R&D lab where a scientist warms up a half-liter of media or has to dispose of a half-liter of waste. That is inconsequential to deal with.

As Yoshi said, when you get to these large scales, even just pumping liquid from one

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tank to another, or mixing it uniformly, becomes incredibly difficult. There is a phenomenal amount of collaboration with engineering and other process specialists just to figure out how to handle your liquid. Also, the amount of time it takes to do a single step might be very challenging scientifically. If transfection takes 4–10 hours, you have got a very difficult chemistry question there, whereas before it might have taken a scientist in the R&D lab 30 seconds. There are some real issues with the physical volumes at these large scales.

Q What are the comparative advantages and challenges of adherent platforms for scaling up AAV production to industrial commercial scale?

CR: For decades, suspension culture has had the upper hand. It is very easy to just get a bigger bucket, so to speak, and make more in a suspension bioreactor.

The technology for adherent systems is relatively new, and there hasn't been a lot of competition in the market until recently. I am very excited to see Corning and others putting out some really good adherent equipment aimed at large-scale manufacturing and clinically relevant scales of manufacturing. To me, making AAV in an adherent system is scientifically better. You get a little bit more yield per cell, and you get a little more yield per reagent.

However, mechanistically it is very difficult to control that process. I know a process engineer would certainly prefer a suspension process. There is give and take here, and it is exciting to watch the field tackle these new adherent systems.

There are some other issues, such as the seed train. In a suspension system, the seed train is rather straightforward. You can put cells in a reactor, you can underfill your reactor, and then as the density increases you can begin to fill up your reactor over a couple of days, or a couple of weeks if need be. All in one vessel.

With adherent culture, it is a little more difficult. If you start in a 15cm petri dish, you can't just stretch that to be a 15m dish. You need to physically remove the cells and place them somewhere new. There is a lot more mechanical intervention and hands-on time; it is a whole different beast.

It is important to ask yourself what the goal of your process is when you are deciding on which one to do. If you have a single product and you just need it made, there is no reason suspension can't be wonderful. If you are thinking about producing a product consistently and constantly, I think adherent would be the way to go, if the new technologies work well for you. We are just getting to the beginning of evaluating those new technologies and seeing where they fit in the field.

JYS: This question comes up often with customers. Should we stay in adherent, should we switch to suspension? Is suspension going to be better in manufacturing? Is suspension going to be better on the biology?

I would go back to the lifecycle of the project. If your lifecycle is to make one single product, and you know exactly what that deliverable is, potentially suspension will be much

easier. For example, you know exactly the size that you need, so you buy a 1000L bioreactor and you keep producing that single product in that same process for about 5 years.

But if your production needs change, if your deliverable changes, potentially that 1,000L bioreactor becomes too big or too small. If you stay with adherent, and in my opinion, you can actually be more modular in the adherent process, your translation between a smaller size and a bigger size is more direct.

But when you take the process from a 250L bioreactor to a 2,000L bioreactor, all the parameters that you developed for the design of the 250L bioreactor do not apply. There is a large optimization phase that you might not necessarily encounter with adherent.

Going back to the advantages and disadvantages, one of the most important things is to understand the lifecycle of your manufacturing process. Do you want to produce it in a shorter period of time? Do you have a long process? Do you have a long-term production? That will guide you on the most appropriate platform.

PV: My opinion is a bit biased. Adherent is trusty and it is fairly predictable, as far as cells can be predictable. It is a robust platform, but at some point you are going to reach a maximum threshold where you just cannot expand more in footprint or output capacity.

Bioreactor suspension-based platforms are going to give you a lot more versatility to be able to expand your output capacity, weighing in that difference in output that each cell will be able to produce, that historically science has been able to perfect in an adherent platform.

The choice is your trusty go-to, versus the prospect of much greater expansion capacity.

JYS: In simple terms, when you are working with adherent platforms, you are dealing with biology and chemistry. If you go into suspension, physics gets involved.

Whenever I am trying to explain why you would want to move from one technology to the other, or consider different things, it is important to note that you are involving different types of sciences. You may already understand the biology and chemistry; how to grow your cells, how to transfect your cells. But now you are growing in an environment where physics is a key component to maintain the viability of the cells, to be able to modify your cells in suspension. It takes on a whole different avenue.

Q Cell culture has often been overlooked as a key process to improve when tackling scalability. Is the field coming up with better and smarter transfection reagents, and do you feel this is the right approach to take?

JYS: Cell culture has been around for quite some time, and often we are pushing ourselves to produce more things. But a lot of things that we are asking the cells to produce don't naturally occur. We have to insert genes of interest and modify the cell lines.

In the last decade or so, manufacturers like Corning have produced products from CellSTACK® to HYPERStack® vessels, and now going into bioreactors such as the Ascent™ FBR

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System. Often, we are looking at how to contain the cells, how to put more cells in a smaller footprint. Sometimes we forget that you still need to modify that cell line; you still need to ask the cell to produce something.

We are very excited to see companies try to improve the infection reagent in order to make cell uptake of the genetic information much faster. It is definitely an area of growth and an area that will enable easier manufacturing processes if we can improve the transfection reagents.

"There is also a lot of space on the equipment to design a novel way to apply the reagent, and the two of them can work together. You can use the best reagent and the best process to get the best transfection. Instead of using a reagent that maybe isn't as good, but is more durable, to deal with the equipment limitations."

– Chris Reardon

CR: The nuances of applying the genes to the cells, the basic transfection of how to tell the cells what to do, change with every system. Even if it is the same system and you are scaling it, the nuances are going to change. If it is suspension, how do you get that volume mixed in in time? If it takes an extra hour to add that reagent, is the reagent still good?

It is exciting to see transfection reagents that have longer windows of function; that are robust through more sheer force or more stirring.

For adherent systems, when you are just working in the plates you can do rounds of transfection and set up your transfections with a lot of labor. When you get into an adherent bioreactor, some of these questions resurface. You have to ask yourself how you are going to apply your transfection reagent if your pump speed is set at a certain rate, consider the size of pump, the size tube diameter, and the maximum flow rate so you don't blast the cells off the adherent surface. How you apply the reagents is a huge question, and a lot of the onus ends up on the reagent manufacturer to manufacture a robust reagent.

There is also a lot of space on the equipment to design a novel way to apply the reagent, and the two of them can work together. You can use the best reagent and the best process to get the best transfection. Instead of using a reagent that maybe isn't as good, but is more durable, to deal with the equipment limitations.

There is a lot of room for ingenuity there, and I have seen a lot of very fun, smart ideas come out of various companies to address this.

PV: The platform that we have employed is pretty robust. It is relatively predictable. As far as being able to change and move into a different type of transfection reagent, there is going to be a tremendous burden of process development and justification of the cost.

When we know what we know and what we are comfortable with, switching to something new that may have better promise is going to be a challenge. We are going to have to do a lot of work to show that it is worth the change. Because not only are we changing reagents and suppliers potentially, but then also you are impacting your process in some manner that you are going to have to understand.

The promise of change and improvement is always tempting. But clinging to what you know and what you can predict is always going to be the safest course. There is always another possible improvement out there, you have just got to put the work in to justify the change.

CR: I like to talk a lot about "the ideal". A new process could be great, but it can take years to define. That is a significant burden, especially when we are talking about a clinically relevant therapy. Do you want to delay two years to treat a patient, because you want to change your transfection?

Phil makes a really good point. Having something robust, knowing how it works, and sticking with it has a lot of value. Knowing when not to change is a really important skill.

Q How can the rapidly expanding gene therapy manufacturing field address the increasingly acute shortage of adequately trained and experienced personnel?

CR: This is difficult. The field is exploding and new facilities are popping up everywhere. Every major academic and research institute is getting into gene therapy. That is a lot of new staffing demand, so where will all of these people come from?

I see a few really important steps that any employer can take to increase their staffing. One of them is to offer true entry-level positions. Don't be afraid of the person who has a bachelor's with no experience. Just because they don't have any experience doesn't mean they aren't an incredible scientist or engineer, it just means they haven't had an opportunity to demonstrate it yet. As an employer, you can train them. You can offer them something and help them grow.

By providing a clear career path and training you are going to tell an employee that they are valuable, and that you want them around for the next 10 years. If you hire someone and don't train them, they are going to leave in 2 years. Then you are going to lose employees on top of not being able to hire.

Another point is continuing education along with training. Helping employees to continue to take classes to expand their field can help them understand if they are in the right field. It might seem counterintuitive, but employees that know they are in the right field are going to do higher-quality work. They are going to find a lot more passion and excitement in it. You get a much bigger return per person with that high-quality investment in them. The ones that didn't want to be in that field will go somewhere else, but you will have had them for a very good time.

Don't be afraid to reach out to universities and tell them when you are interviewing and that you want students who graduate with these skills. Can you help you develop a curriculum or sponsor a curriculum? Maybe those students will go somewhere else, but you will be helping both the field and yourself.

Finally, be upfront about pay. A lot of employers hesitate to talk about pay until the last second possible; they keep it secret. But when folks are looking for jobs they are going to be

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asking themselves if this job is worth the effort they are going to put in, and that is the basic transaction – effort and expertise for money. If the employer doesn't want to talk about what they are willing to trade, it becomes a difficult negotiation. It is really important to acknowledge that that is the deal – it is money for work. Not everyone is going to be passionate about making 10,000 liters of PBS, but someone has to do it.

Q Phil, has staff retention or recruitment been a challenge for you over the last couple of years?

PV: A little bit, yes. We are at a point where we are expanding quite rapidly, so this is a subject near and dear to my heart. Recruiting, retaining, and training staff is always a challenge.

Right now, when we are going through "the great resignation," there are a lot of talented people out there and they know what they are worth. Being able to attract them and compensate them for the experience that they have, which can be integral to your success, is about giving them a goal to work towards; a path of progression. This could be either within the particular focus they got hired for, or they may actually have other interests within your organization. Spending time in your group for a couple of years may allow them to succeed in another area much more successfully.

As an example, I have had a number of people working in operations who, after a couple of years, have moved to quality assurance. That is a huge benefit to the organization because they can do their job much more effectively in quality assurance knowing the intricacies of our processes.

One of the other big challenges right now, as Chris said, is that the industry is exploding. It is exciting, and often it's a little scary too. If you are not able to reach beyond where you are at to find those talented people, you are going to be limited. Utilize all of the resources at your disposal – the internet, job fairs, conferences, and so on. If you can't make those connections you are not going to have those people available to you.

JYS: Chris and Phil addressed training, investing in, and promoting personnel.

One of the challenges we have seen is people that are graduating with degrees that might not necessarily have all the training they need. What I see lacking in academic training is bioprocessing

"Recruiting, retaining, and training staff is always a challenge... there are a lot of talented people out there and they know what they are worth. Being able to attract them and compensate them for the experience that they have, which can be integral to your success, is about giving them a goal to work towards; a path of progression."

– Phillip Vermilion

engineering – training scientists to think you are not just grabbing a pipette, you are not just using a tip, you are not just running an assay, you are actually running a biological production. Programs and opportunities are needed to educate new students that come into a university that they need to get trained on these things. I have seen some universities start bioprocessing projects, with specific programs tailored to training scientists to be in manufacturing, and I think that's very important.

Looking at hiring, I definitely agree with both Chris and Phil that trying to get people into the company, and then retaining that talent, is a challenge in this current market.

Q What would be your parting words of advice in terms of AAV vector scale-up/scale-out planning from an early stage of development?

JYS: Often we focus on understanding the process and understanding the biology, but I believe the industry sometimes forgets that manufacturers can help. At Corning we have personnel and talent that can help customers improve their processes. We are not necessarily just providing a product – we can guide you in some of the science, and how to use this product to truly complement your process and potentially produce more than what was expected.

Don't be shy of reaching out to your suppliers to ask them how their product can make your system work better.

PV: We have been discussing process procedures, materials, and equipment. But in the end, you have got to remember that the end-user is a patient that needs this therapy.

In order to be able to scale up to expand your clinical trial, you have got to be able to control your process. Ensure the end goal is a process in control, and the same product you started with. If you can get to the end and make a massive amount of vector, but it is not of sufficient quality, you are going back to the drawing board.

When we first bring people in and train them, the first thing we tell them is to always keep the patient in mind. Everything we are doing day-to-day is going to treat a human being. It really helps to keep the end goal in perspective.

CR: If you are designing a new production, or if you are scaling up and scaling out a therapy, don't be afraid of the cost or the regulatory path. They are daunting, they can take time, money, and expertise, but it is worth confronting these things in order to have the best process and the best science.

At the end of the day, if the product that you make isn't good, it doesn't matter how much money you have saved, or how many regulatory loopholes you have managed to sneak past. If you don't have a good product you have nothing. So don't be afraid of the very difficult hurdles of price and regulation. Confront them, work with them, work with producers, manufacturers, suppliers, and regulators, and get the best product out for the patient.

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AUTHORSHIP & CONFLICT OF INTEREST

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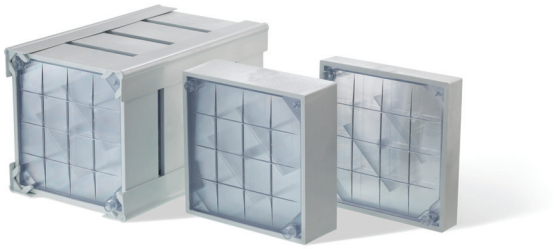

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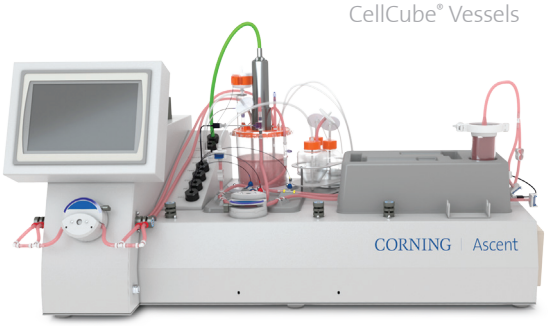

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INNOVATOR INSIGHT

Accelerating AAV capsid analysis using a new multi-capillary electrophoresis platform

Susan Darling

Adeno-associated viral (AAV) vectors, while offering numerous advantages over other viruses (non-pathogenic, low immunogenicity, and can readily enter a variety of cell types), are highly complex molecules that present significant manufacturing challenges. There are a large number of serotypes to choose from, and the need to implement transfection processes that afford high yields of capsids containing the gene of interest and purification hurdles to overcome. From an analytical perspective, samples are getting more complex, more numerous, and require more complex analytical methods that involve complex method set ups, but results are needed in less time. Despite these challenges, developers of gene therapies must be able to understand the molecular liabilities of AAV vectors as soon as possible in the development process in order to ensure the manufacturability of robust, stable molecules prior to clinical trials. Existing approaches to detect and characterize product changes during drug development are part of the problem because they take too long. High-throughput analytical techniques that can overcome these complexities are becoming essential. A new system designed to enable parallel processing of eight samples simultaneously using two well-established capillary electrophoresis (CE) techniques combined with two different detection methods is filling the gap. The SCIEX BioPhase 8800 system accelerates analysis and dramatically shortens new therapy development timelines while providing the sensitive, high-resolution data expected in the biopharma industry for bioprocessing to R&D to QA/QC.

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IMPORTANCE OF AAV PURITY & GENOME INTEGRITY

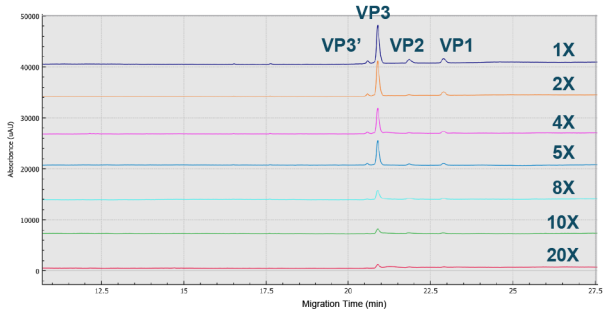
AAV is a small virus with a protein shell, or capsid, comprising three viral protein monomers (VP1, VP2, VP3) that surround a single-stranded DNA. The viral proteins have molecular weights of approximately 87, 73, and 61 kDa, respectively, totaling 60 monomers arranged in icosahedral symmetry in a ratio of 1:1:10, with an estimated size of 3.9 MDa. The DNA is approximately 4.8 kilobases in size.

To produce recombinant AAV (rAAV) vectors, host cells (typically HEK293) are transfected with three plasmids, one of which contains the entire rAAV genome and two helper plasmids that contain special Rep and Cap genes that enable the host cells to make virions. The *Rep* gene encodes four proteins (*Rep78, Rep68, Rep52* and *Rep40*) with overlapping sequences that are required for gene regulation and replication of the AAV. The *Cap* gene encodes the three capsid proteins and a non-structural protein named AAP (assembly-activating protein). The capsid viral proteins participate in the assembly of both the capsid and genome and determine the efficacy of the gene therapy product [1].

The genome of an AAV vector for gene therapy is usually composed of two inverted terminal repeats (ITR), a promoter, a transgene and a poly-A tail. AAV genome integrity analysis is a critical quality test for AAVs because it provides insights into transgene integrity and ensures product safety and efficacy [2]. It is essential that AAV capsids be expressed correctly with respect to size, peptide sequence and post-translational modifications (PTMs). Minimizing the production of capsids that do not contain the vector genome (empty) or contain truncated versions or contaminant genetic material (partial) is equally important. The purity of the capsids is also a critical quality attribute with respect to host-cell protein (HCP) and other contaminants, as they can contribute to immunogenicity and off-target effects [3].

FIGURE 1

Best peak intensity of all three AAV8 capsid proteins were obtained using a 1x dilution.



Sample buffer (SDS-MW kit, 100 mM Tris-HCl pH 9.0, 1% SDS) optimization with dilutions from 1×–20× using CE-SDS-UV on the BioPhase 8800 system.

THE VALUE OF CE FOR AAV CAPSID ANALYSIS

While traditional mAb-based protein therapeutics have been highly optimized for production and purification, AAVs are significantly more difficult and more expensive to produce. They typically require a multiple transfection system and produce very low titers of functional AAV. Therefore, some of the traditional techniques utilized for protein and nucleic acid analyses, such as agarose gel electrophoresis and PAGE, can be used for AAV analysis, but are quite crude compared to capillary-based methods. Capillary gel electrophoresis provides a rapid, robust and highly sensitive method for both capsid purity and genome integrity analysis, effectively separating proteins with very similar molecular weights as reflected by their migration times.

For purity analysis, CE-SDS (sodium dodecyl sulfate) offers high resolving power and excellent quantitation and reproducibility combined with automated operation and is effective even at the low concentrations of viral proteins found in AAV samples [4,5]. Detection with UV is appropriate for samples with AAV titers greater than 1×10^{13} genome copies per mL (GC/mL) or lower titers but sufficient sample volumes [4,5]. Sample labeling using fluorescent dye and laser induced fluorescence (LIF) detection can also be used to improve sensitivity of the assay.

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For genome integrity analysis, CE-LIF is a rapid, automated biophysical method for genome size analysis of double-stranded DNA (dsDNA), including restriction fragment analysis of its vectors, as well as single-stranded DNA (ssDNA) and RNA and offers higher resolution than HPLC [3].

BIOPHASE 8800 SYSTEM FEATURES

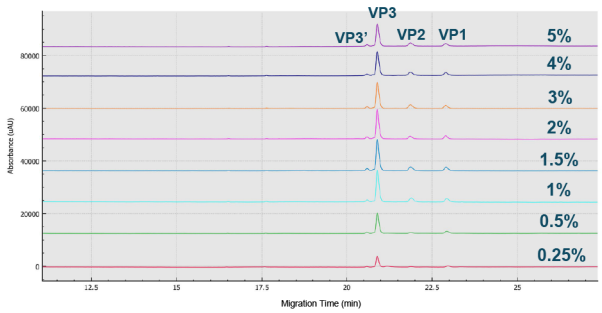
The BioPhase 8800 system leverages a new cartridge that allows parallel processing of eight different CE samples simultaneously, delivering consistent, accurate results so that more samples can be analyzed in less time. Parallel processing can be achieved using either CE-SDS or capillary isoelectric focusing (cIEF) on the same or different samples containing the same or different molecules.

ACCELERATING AAV PURITY ANALYSIS

The three viral proteins in AAV capsids differ only slightly in length and the N-terminus, and each can exist as different variants with a range of PTMs, making these samples highly complex. Furthermore, the relative ratio of VP1:VP2:VP3 can be a factor impacting the potency of AAVs. The AAV protein concentrations in most gene therapies are quite low (~50 ng/mL) compared to traditional protein therapeutics. AAVs are also significantly more difficult and more expensive to produce than protein therapeutics. All of these factors taken together clearly necessitate an assay with significantly greater sensitivity and resolution than what is afforded by either agarose gel electrophoresis or SDS-PAGE. Both the PA 800 Plus and BioPhase 8800 system provide a highly reproducible and sensitive platform for AAV capsid purity analysis. The BioPhase 8800 system also provides the scalability you would expect with an 8-channel system, but also a very unique capability to dramatically accelerate method development.

FIGURE 2

Optimized value obtained was 1–1.5% SDS.



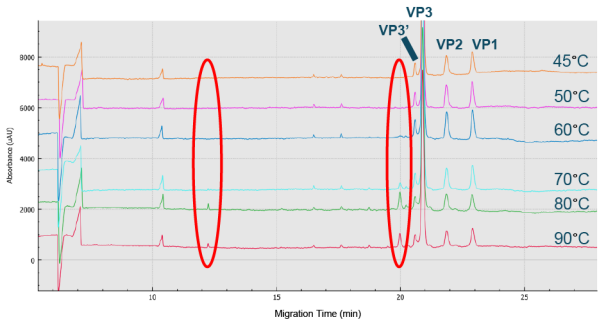
BioPhase 8800 electropherograms from CE-SDS-UV AAV8 sample analysis using various %SDS ranging from 0.25 to 5%.

To demonstrate the utility of the BioPhase 8800 system, a one factor at a time (OFAT) method development study was performed using AAV8 (pAV-CMV-GFP, Vigene Biosciences) samples to determine the optimal sample buffer concentration, %SDS and incubation temperature for analysis of these viral vectors. The samples were analyzed using CE-SDS-UV, and the conditions that provided the maximum peak intensity were selected as the optimum.

The application note 'Acceleration of method optimization for AAV capsid purity analysis using multi-capillary electrophoresis platform' [6] fully describes the preparation, capsid purity analysis and data processing of the AAV8 vector. Briefly, the AAV8 capsids were chemically reduced and then diluted. The samples were separated on a BioPhase BFS Capillary

FIGURE 3

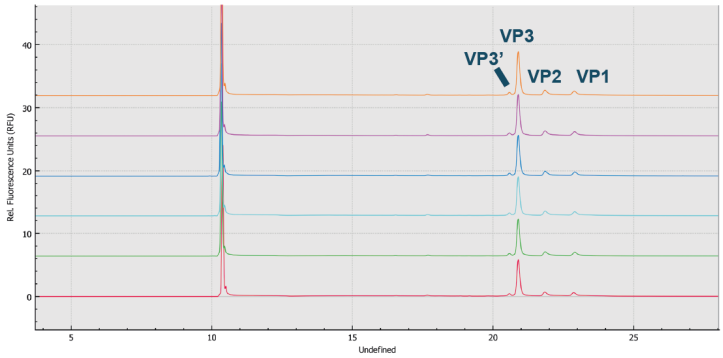
The peak intensity of all 3 capsids was optimized at 50° C.



Electropherograms from CE-SDS-UV AAV8 analysis at temperatures ranging from 45°C to 90°C to determine the optimized incubation temperature.

FIGURE 4

The results were highly reproducible.



	MT		CPA%	
	Ave.	RSD%	Ave.	RSD%
VP3'	21.20	0.62	4.36	1.27
VP3	21.53	0.61	75.49	0.36
VP2	22.50	0.62	10.00	1.46
VP1	23.57	0.63	10.15	1.23

Results (CE-SDS-UV) for six consecutive injections of the AAV8 sample on the BioPhase 8800 System.

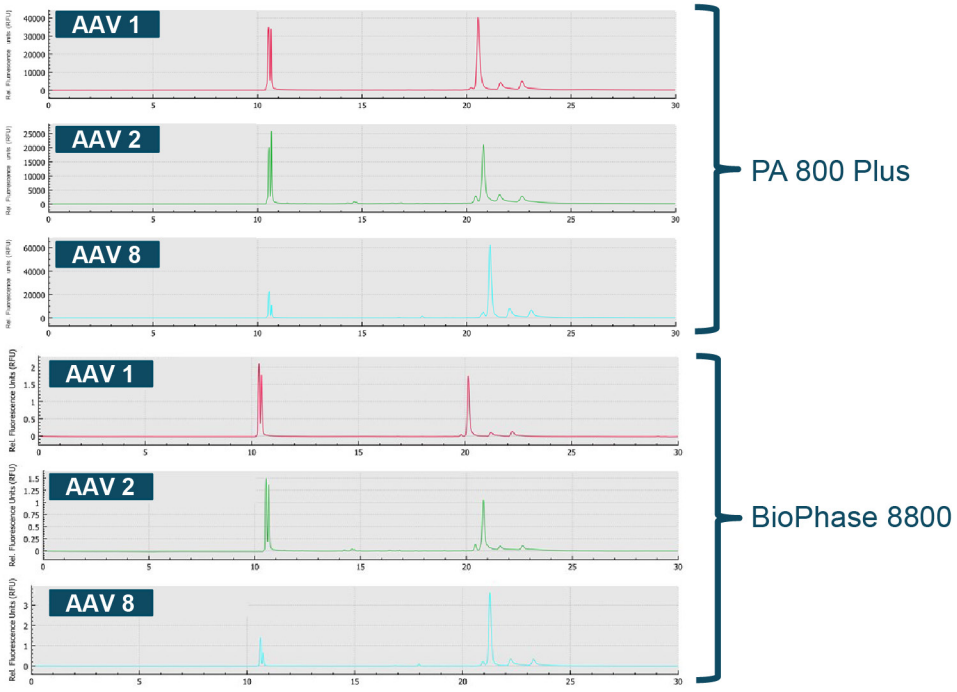
Cartridge. The BioPhase software package was used for data acquisition and processing.

Different sample preparations and buffers were evaluated to achieve optimal sensitivity and resolution of the capsid proteins for the AAV8 serotype on the BioPhase 8800 system using CE-SDS-UV. A buffer dilution of 1× was found to be the best (Figure 1), while the optimal %SDS was found to fall in the range 1–1.5% (Figure 2) and the peak intensity of all three capsids was optimized at 50°C (Figure 3).

Most notably, all of the method optimization for all three of these parameters was completed in 4 hours using the BioPhase 8800 system compared to 48 hours for a single capillary system – 12 fold faster. This increase is due to

FIGURE 5

Excellent correlation in performance across multiple AAV serotypes.



Comparison of CE-SDS-LIF analysis data AAV1, AAV2 and AAV8 on the single capillary PA 800 Plus and the multi-capillary BioPhase 8800 system.

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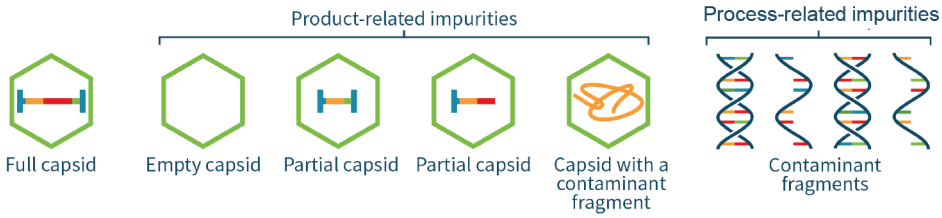
FIGURE 6

Genome impurities impact safety and efficacy.



each of the parallel 8 channels exhibiting highly reproducible performance across channels as well as compared to capillaries in the PA 800 Plus. To demonstrate the repeatability of analyses on the BioPhase 8800 system, results for six consecutive injections of the AAV8 sample were compared. As can be seen in Figure 4, the relative standard deviation (RSD) of the migration time (MT) and corrected peak area (CPA)% values for the VP1, 2, 3 and VP3' (fragment of VP3) peaks were no more than 1% and no more than 1.5%, respectively.

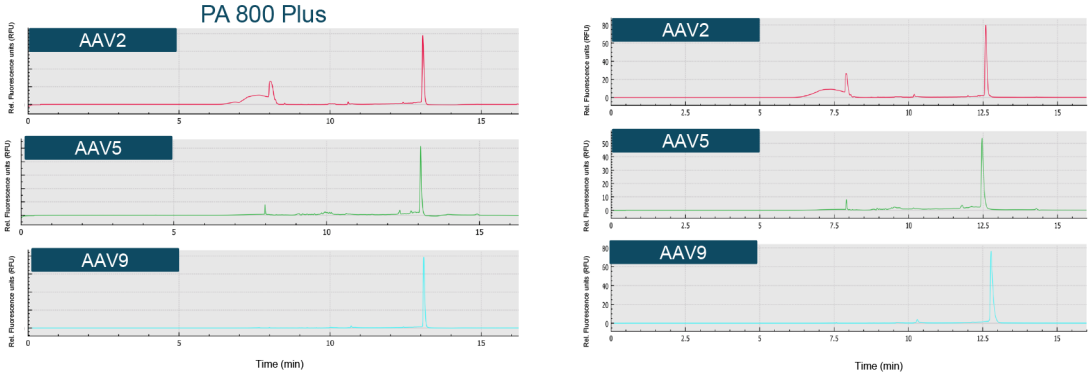
Although this example leverages a one factor at a time (OFAT) design, the BioPhase 8800 has tremendous potential to perform full design-of-experiment studies (DOE). These highly optimized assays can then be run on the BioPhase 8800 or PA 800 Plus. To illustrate this point, three different AAV serotypes (AAV1, AAV2, AAV8) were analyzed by CE-SDS-LIF on both the BioPhase 8800 and the PA 800 Plus [7]. The results demonstrate extraordinary correlation between the results across the two systems (Figure 5).

Dramatically faster method development with the BioPhase 8800 system is also enabled by the software with advanced capabilities and drag-and-drop functionality for easy and confident method and sequence creation. The software also leverages advanced data analysis capabilities to further accelerate method development – even within a fully compliant-ready environment. Also, new validated assay kits simplify operation.

Temperature control provided on both the sample chamber and the factory-built, multi-capillary cartridge of the BioPhase 8800 system ensures maximum reproducibility by preventing degradation of the analyte(s) prior to analysis. A constant temperature in the capillaries also ensures a consistent environment for all samples and every run.

FIGURE 7

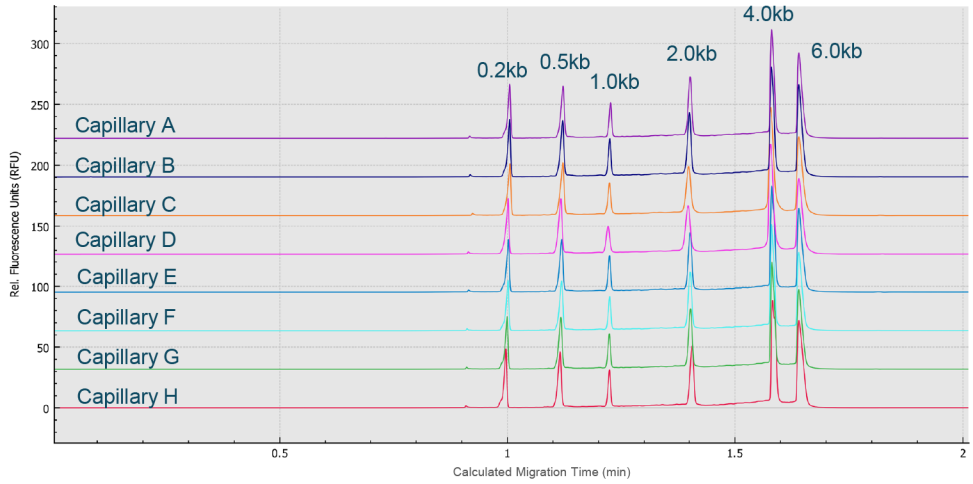
Very good alignment of genome profile across systems.



Comparison of genome integrity analysis results for AAV2, AAV5 and AAV9 on the single-capillary PA 800 Plus and the multi-capillary BioPhase 8800 system using a LIF detector.

FIGURE 8

High resolution separation of all RNA size markers.



Traces A to H represent the separation in the eight individual capillaries of the BioPhase 8800 system.

The capsid proteins of different AAV serotypes can, and frequently do, have different physical properties. Initial method development, or even worse – mid program method development, is often a significant and unpredictable delay to project timelines in an AAV therapeutic program. The unique capabilities of the BioPhase 8800 system provide a solution to avoid this project impact.

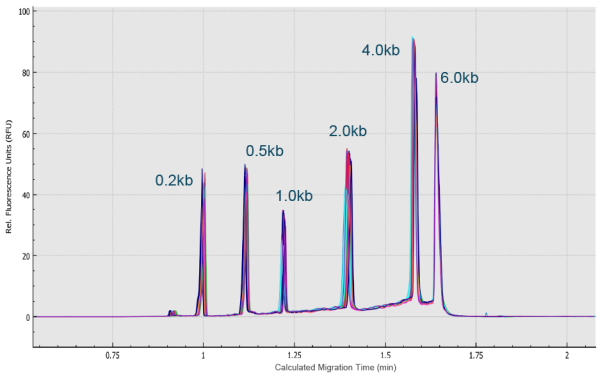
The quality of the transgene inside a viral vector impacts the infectivity, efficacy and safety of the gene therapy product. The genome cassette encapsulated in the AAV capsid could be absent, truncated, or occupied by fragments from the host-cell genome or plasmid. The analysis of AAV genome integrity is therefore of significant importance because it provides insights into transgene integrity and ensures product safety and efficacy (Figure 6).

Currently, AAV genome integrity analysis by CE-LIF is performed one sample at a time using the single-capillary system. Multiplexing the analysis can help decrease the analysis or profiling time. The multi-capillary BioPhase 8800 system has been shown to effectively accelerate the execution of sensitive AAV genome integrity analysis for multiple AAV samples with different serotypes or different genome sizes while retaining the excellent resolution, sensitivity and repeatability obtained when using the single-capillary PA 800 Plus [8].

The application note 'Genome Integrity analysis of adeno-associated viruses (AAV) using multi-capillary gel electrophoreses' [8] fully describes the preparation, genome integrity analysis and data processing for several AAV (AAV2, AAV5, AAV9) serotypes. Briefly, AAV samples were treated to remove non-capsid genetic impurities, purified with the QIAquick PCR kit and then separated on the BioPhase 8800 system with a PVP

FIGURE 9

Excellent inter- and intra-capillary repeatability.



Overlaid traces of 80 injections (ten consecutive injections of eight capillary channels) of RNA size ladder on the multi-capillary electrophoresis system.

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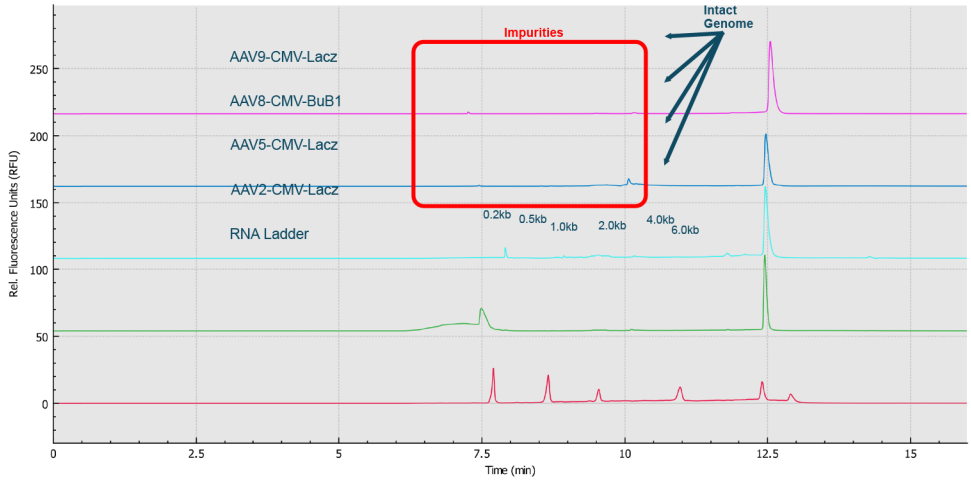
FIGURE 10

Genome Integrity Analysis of AAV samples of different serotypes (Serotype 2, 5, 8 and 9) done in parallel on the BioPhase 8800 system.



Red trace: RNA size standards with sizes marked in dark blue font. Green trace: AAV2-CMV-Lacz sample. Light blue trace: AAV5- CMV- Lacz sample. Dark blue trace: AAV8-CMV-BuB1. Pink trace: AAV9- CMV-Lacz sample.

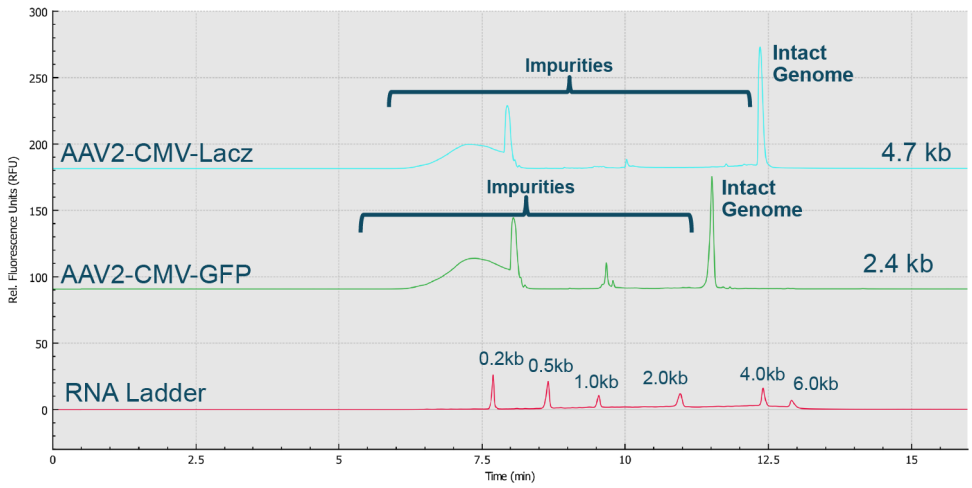
gel-based capillary with LIF detection. Equivalent separation and analyses were performed in a single-capillary mode on the PA 800 Plus.

The genome profile and the migration times of the nucleic acid peaks aligned well between the two systems (Figure 7). Similarly, the % corrected peak areas of the intact genome and the impurities (including truncated genome and other small sized nucleic acid impurities) correlated well.

Next, an RNA ladder sample (RNA 6000 Ladder, Thermo Fisher Scientific) was used to evaluate the reproducibility of the migration time and corrected peak area values for eight analyses simultaneously performed on the eight capillaries of the multi-capillary electrophoresis system. High-resolution separation of all RNA size markers (0.2 kb, 0.5 kb, 1.0 kb, 2.0 kb, 4.0 kb, and 6.0 kb) was obtained (Figure 8). In addition, when ten consecutive

FIGURE 11

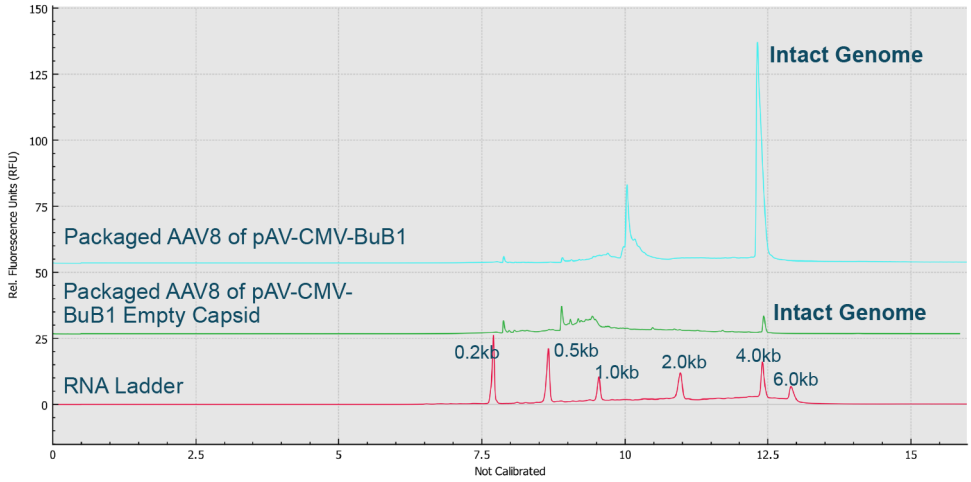
Genome size analysis of AAV2 samples of different genome loads on the BioPhase 8800 system.



Red trace: RNA size standards with sizes marked in dark blue font. Green trace: AAV2-CMV-GFP sample with genome size about 2.4 kb. Blue trace: AAV2-CMV-Lacz sample with genome size about 4.7 kb.

FIGURE 12

Genome Integrity Analysis of enriched full capsids and enriched empty capsids of AAV8-CMV-BuB1 on the BioPhase 8800 system.



Red trace: RNA size standards with sizes marked in dark blue font. Blue trace: Enriched full capsids of AAV8-CMV-BuB1 sample. Green trace: Enriched empty capsids of AAV8-CMV-BuB1 sample.

injections of the RNA ladder sample on the eight capillaries were evaluated (Figure 9), the migration time reproducibility (RSD%) of the 80 analyses for each RNA size marker was less than 1%, while the RSD% for the corrected peak area% was <5% for the RNA markers. Next, simultaneous analysis of AAV vectors with different serotypes and the same serotype with different genome sizes were simultaneously analyzed using the BioPhase 8800 system. In the first case, samples of AAV2, AAV5, AAV8 and AAV9 were analyzed in parallel along with the RNA ladder. The intact genome of AAV was well separated from the partial or truncated genome and other small size impurities for different serotypes of AAV samples (Figure 10). Notably, it took less than 25 min to screen eight samples using the BioPhase 8800 system. In the second case, AAV2 samples encapsulating different genome sizes were analyzed along with the RNA ladder sample (Figure 11). The genome size different can be clearly seen. It is worth noting that the RNA size standards migrate slower in this PVP gel buffer than the single stranded AAV genome of the same size due to the differences in base composition in these nucleic acids, and the differences related to ribose in RNA versus deoxyribose in single stranded DNA.

Finally, the AAV samples with enriched full and empty capsids were analyzed on the BioPhase 8800 system along with the RNA ladder (Figure 12). The small amount of intact genome observed in the enriched empty AAV8-CMV-BuB1 sample indicated the presence of a small amount of full capsids in the enriched empty capsids sample.

CONCLUSION

The ability of the BioPhase 8800 multi-capillary CE system to rapidly analyze AAV capsid purity and genome integrity with the same high resolution and sensitivity well known for CE analyses on established single-capillary PA 800 Plus system was clearly demonstrated. The capability of analyzing eight AAV samples of multiple serotypes and different genome sizes at the same time on the same analytical platform can dramatically accelerate screening and process development of AAV products. The easy transferability of methods from one system to the other was also confirmed, enabling seamless movement of analyses from process development into QA/QC.

Overall, the results of these studies show that by using the SCIEX multi-capillary

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BioPhase 8800 system, drug developers can reduce development timelines by leveraging the efficient generation of sensitive, high-resolution data. With parallel analysis capabilities, biopharmaceutical scientists can quickly develop methods for screening and characterizing AAV vectors for gene therapies, dramatically shortening gene therapy development timelines and accelerating their time to market.

REFERENCES

1. Kewal JK, Blouin V, Brument N, Agbandje-McKenna M, Snyder RO. The Role of the Adeno-Associated Virus Capsid in Gene Transfer. *Drug Deliv. Syst.* 2008; 437: 51–91.
2. Naso MF, Tomkowicz B, Perry WL Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs* 2017; 31, 317–334.
3. Darling S. Facilitating gene therapy development with solutions to four capsid analytical challenges. *Cell Gene Ther. Ins.* 2021; 7, 411–415.
4. Zhang C, Meagher, MM. Sample stacking provides three orders of magnitude sensitivity enhancement in SDS capillary gel electrophoresis of adeno-associated virus capsid proteins. *Anal. Chem.* 2017; 89, 3285–3292.
5. Sensitive AAV capsid protein impurity analysis by CE using easy to label fluorescent Chromeo dye P503. SCIEX. RUO-MKT-02-10600-A.
6. Li T, Mollah S. Acceleration of method optimization for AAV capsid purity analysis using multi-capillary electrophoresis platform. SCIEX Technical Note.
7. Acceleration of method optimization for AAV capsid purity analysis using multi-capillary electrophoresis platform.
8. Li T, Luo J, Mollah S. Genome integrity analysis of adeno-associated viruses (AAV) using multi-capillary gel electrophoresis. SCIEX Technical Note.

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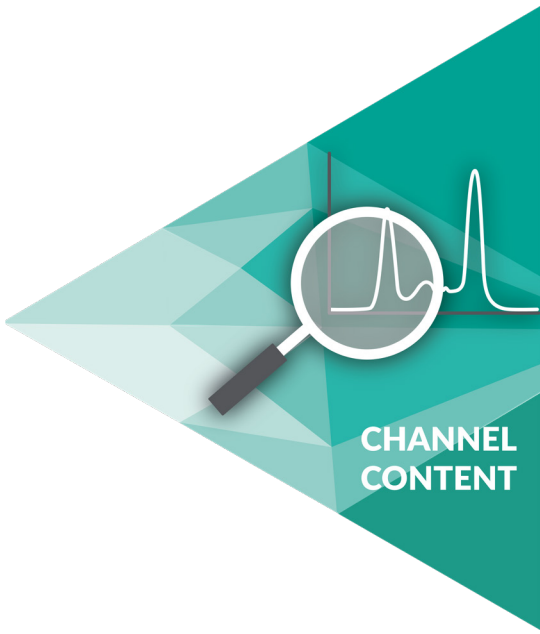

INNOVATOR INSIGHT

Accelerating process development with at-line concentration measurement

Joe Ferraiolo

Currently, the most used methods for adeno-associated viral (AAV) vector quantitative analysis are qPCR or ddPCR and ELISA assays, along with analytical ultracentrifugation (AUC) and transmission electron microscopy (TEM). The time to results when using these methods can range from days to several weeks, and the acceptable tolerance range is high. Rapid, reliable in-process testing offers a significant benefit to AAV downstream process development and can be achieved with Slope Spectroscopy® utilizing Variable Pathlength Technology (VPT). The CTech™ SoloVPE® System, when used at-line in one or more stages of a process, can help to quickly identify process characteristics, provide key insights, and allow for process optimization in minutes. VPT has enabled at-line measurement of concentration by eliminating the need for sample dilution or manipulation and delivering rapid and highly accurate real-time results.

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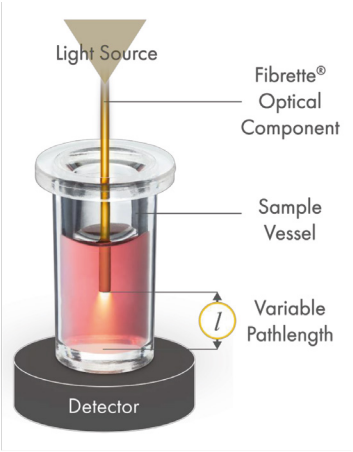
INTRODUCTION TO SLOPE SPECTROSCOPY

Traditional ultraviolet–visible (UV-Vis) spectroscopy measurements typically require a fixed pathlength to be used for analysis. This means the variable is concentration, which introduces dilution error in limited ranges of spectrophotometers. This is the challenge for most industries using UV spectroscopy for concentration determination.

FIGURE 1

Illustration of variable pathlength technology.



Our solution is to make the pathlength variable using VPT within the SoloVPE System, so that the concentration stays fixed (Figure 1). The pathlength is determined from the distance of light from the Fibrette® Optical Component to the bottom of the sample vessel, also known at the Detector (Figure 2). The pathlength ranges from 5 microns up to 15 mm, with 5-micron steps. This gives the SoloVPE the option to scan up to 3,000 different choices of pathlengths to find the best linear regression data. Therefore, without sample dilution, we are able to measure the most highly-concentrated samples without any further sample manipulation thus, avoiding dilution error.

We rely on multiple absorbance values to calculate concentration, plotting a slope regression which shows the change of absorbance over pathlength, shown in Figure 2A. Each data point sitting on the regression line represents an additional pathlength. This is also represented spectrally in Figure 2B, where each spectrum is collected at a different pathlength data point. This allows us to quantify the regression line with a confidence provide by the R^2 value.

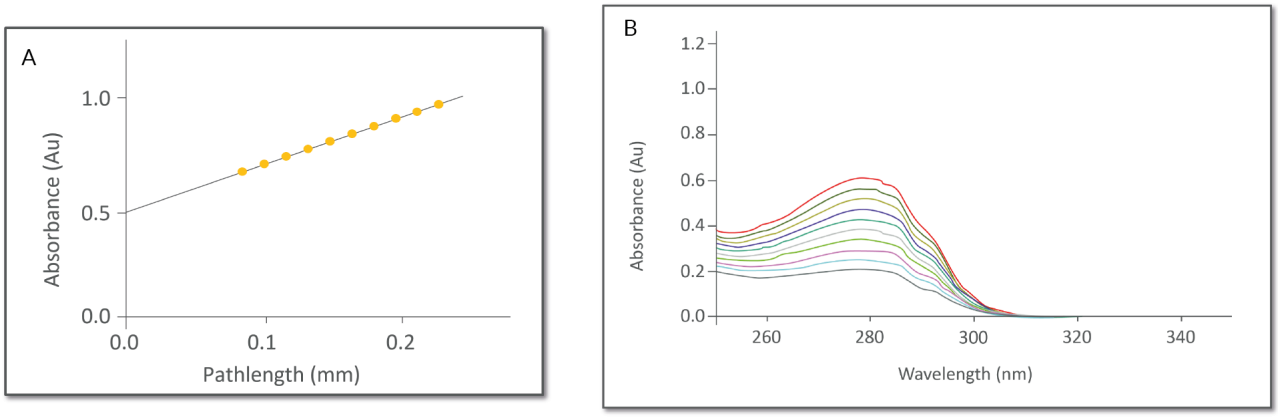
This process utilizes the Beer-Lambert law (Figure 3), in which slope (m) divided by extinction coefficient (ε) will calculate concentration (c). This has allowed us to significantly reduce process steps for simplicity and speed. The system collects absorbance data for up to ten different pathways, to calculate a slope in less than a minute.

HOW THE GENE THERAPY WORKFLOW CAN BE ACCELERATED BY SLOPE SPECTROSCOPY

A typical UV-Vis process is defined by 7 steps, as shown in Figure 4. The full process takes between 30 and 180 minutes depending on the number of samples. The SoloVPE System has been refined to a two-step analysis, measure and report, taking a total of two minutes. The automation of multiple steps saves time and has introduced robustness and simplicity to the optical density

FIGURE 2

a) The SoloVPE System uses variable pathlengths and collects multiple absorbance data points to calculate a linear slope regression. b) Pathlength spectra data set confirms wavelength peak maxima of collected sample.



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measurements. Electronic data signatures and storage simplify the process, so it can be used in GMP environments without user manipulation of sample volume or data at the end of the process.

APPLICATIONS FOR VIRAL VECTOR PRODUCTION

The SoloVPE System can be applied in viral vector production as well as other gene therapy-related applications. Slope spectroscopy is an established method in protein concentration measurement. We believe that the same technology will be beneficial for gene therapy applications.

In the following case studies, our objectives were to demonstrate the Slope Spectroscopy to accurately determine viral titer concentration, using the slope value ratio of DNA/Protein. We wanted to establish the SoloVPE System as a process development accelerator based on immediate at-line analysis that drives real-time decisions.

Case study: determination of plasmid DNA purity in human gene therapy products

Our initial case study was a collaboration with Pfizer to determine plasmid DNA purity in human gene therapy products. We aimed to demonstrate that the SoloVPE System can accurately measure the R value (purity ratio) within plasmids (DNA purity). To demonstrate the R value, we took 25 different levels of insulin and compared the theoretical purity ratio versus the SoloVPE System measurements.

The SoloVPE System and its dedicated software allowed measurements to be taken simultaneously at 260 nm and 280 nm, with results automatically reported. The Slope Spectroscopy technique addresses end user's challenges with sample volume and dilution, and inconsistent wavelength readings using traditional UV-Vis spectroscopy, enabling the SoloVPE System to provide reliable data. As shown in Table 1, the results were comparable between the theoretical value and actual value with differences of less than +/- 2%, and were further validated on purchased material through a third-party vendor, achieving the same results.

FIGURE 3

Beer-Lambert law and associated slope formula.

Beer's law

$$A = \varepsilon l c$$

$$m = A/l$$

$$m = \varepsilon c$$

A = absorbance
ε = extinction coefficient
l = pathlength
c = concentration
m = slope

Case study: antisense oligonucleotides (ASO)

In this collaboration with Ionis Pharmaceuticals, our objectives were to firstly demonstrate the SoloVPE System's universal ability to measure ASOs of different chemical modifications (base modifications, sugar modifications, inter-nucleoside linkage modification, and N-acetylgalactosamine [GaINAc] conjugates), precisely and accurately.

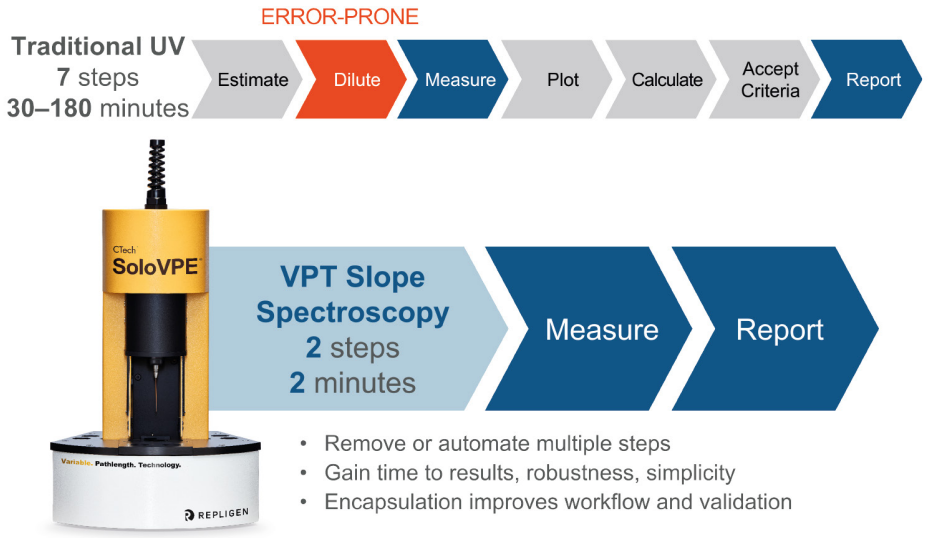
Ionis Pharmaceuticals was interested in leveraging a UV-Vis solution for highly concentrated oligonucleotides. They were able to use our VPT method in their process, and move from an HPLC method to a UV-Vis method, as the SoloVPE System provides quick time to results and does not require dilution. This project was developed, validated, and ultimately transferred to two of Ionis Pharmaceuticals' contract manufacturers.

As shown in Figure 5, SoloVPE System achieved repeatable and accurate results of representative ASOs in different aqueous solutions.

Between the three different manufacturing sites, the differences between the in-house samples versus the transfer method on the

FIGURE 4

The Slope Spectroscopy process in comparison to the traditional UV process.



SoloVPE System were all well within +/- 2%. The validity of the measurements based on the R^2 value of each slope regression provides all parties with the evidence that they can achieve accurate and repeatable results between varying sites and organizations.

TABLE 1

Purity ratio accuracy.

Level	Theoretical purity ratio	Observed purity ratio	% Difference
1	0.6259	0.6273	0.21%
2	0.87087	0.90315	-0.22%
3	1.05311	1.06122	0.74%
4	1.18483	1.17076	-1.14%
5	1.28451	1.28847	0.30%
6	1.42528	1.42358	-0.11%
7	1.51996	1.52481	0.31%
8	1.58798	1.58959	0.10%
9	1.63927	1.65568	0.96%
10	1.67930	1.67489	-0.25%
11	1.71134	1.69203	-1.09%
12	1.73770	1.71515	-1.25%
13	1.75964	1.73533	-1.33%
14	1.77821	1.77064	-0.41%
15	1.79418	1.81956	1.36%
16	1.80804	1.77874	-1.56%
17	1.82026	1.80414	-0.85%
18	1.83094	1.81184	-1.00%
19	1.84046	1.82975	-0.56%
20	1.84902	1.85457	0.29%
21	1.85672	1.84667	-0.52%
22	1.86028	1.88278	1.22%
23	1.86365	1.85282	-0.56%
24	1.86692	1.84941	-0.90%
25	1.87	1.87147	0.08%

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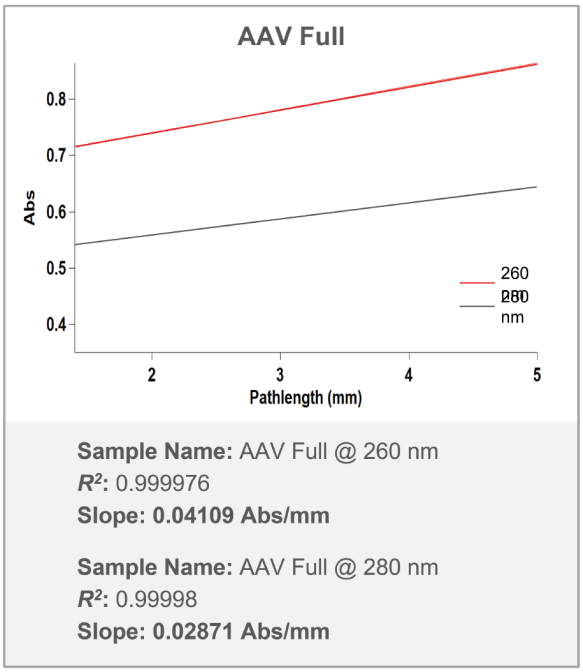
FIGURE 5

AAV sample concentration.



Sample Name: AAV Full @ 260 nm
***R*²:** 0.999976
Slope: 0.04109 Abs/mm

Sample Name: AAV Full @ 280 nm
***R*²:** 0.99998
Slope: 0.02871 Abs/mm

Sample Name: AAV Empty @ 260 nm
***R*²:** 0.999990
Slope: 0.09463 Abs/mm

Sample Name: AAV Empty @ 280 nm
***R*²:** 0.999995
Slope: 0.13454 Abs/mm

Method	% Full	Tolerance (%RSD)	Time to Results
qPCR / ELISA	69%	+/- 20% to 50%	~1 week
SoloVPE System	78%	+/- 2%	5 minutes

Using the SoloVPE System, in-process samples can be measured in less than 2 hours, thus providing a 67% cost/time reduction compared to high-performance liquid chromatography (HPLC). The Slope Spectroscopy method enables significant process improvements resulting in increased turnaround time, reduced time to market, and

FIGURE 6

Onsite SoloVPE System evaluation of AAV8 and AAV9 serotypes.

Sample Name	SoloVPE System			qPCR/ELISA	% Diff.
	DNA (vg/mL)	CAPSID (cp/mL)	% F/E	% F/E	
AAV8 Empty	9.38E+11	2.18E+12	43.0%	15.4%	27.6%
AAV8 Full	1.24E+12	1.40E+12	88.0%	74.8%	13.2%
AAV9 Empty	3.00E+11	4.59E+12	6.5%	7.9%	1.4%
AAV9 Full	7.51E+11	9.81E+11	76.5%	82.3%	5.8%

FIGURE 7

Sample AAV empty/full determination using SoloVPE System versus qPCR/ELISA.

Sample	Expected DNA vg/ml	Expected Capsid cp/ml	DNA vg/ml SoloVPE	Capsid cp/ml SoloVPE	DNA % Diff. log_{10}	Capsid % Diff. log_{10}
RS-AAV2-FL	1.00E+12	3.26E+12	1.82E+11	2.56E+11	-7%	-10%
RS-AAV2-ET	8.55E+11	1.72E+12	6.35E+09	1.27E+12	-22%	-1%
RS-AVV8-FL	1.07E+12	9.81E+11	7.97E+11	1.07E+12	-1%	0%
RS-AAV8-ET	4.27E+11	4.59E+12	1.76E+11	4.40E+12	-3%	0%
RS-AAV9-FL	1.23E+12	1.36E+12	3.86E+11	4.69E+11	-4%	-4%
RS-AAV9-ET	5.78E+11	4.36E+12	1.21E+11	1.76E+12	-6%	-3%

increased throughput. Therefore, the Slope Spectroscopy method was qualified to be the method of choice as the most efficient and universal in-process control (IPC) assay instrument and method for ASO drug product manufacturing.

Case study: AAV viral titer concentration

The current industry standard methods for measurement of AAV empty/full capsid ratio are qPCR /ELISA, ddPCR/ ELISA, AUC, and TEM. Our goal is to find a comparable technology that provides rapid, yet reproducible and reliable data that would enable true in-process, real-time results so that you can accelerate process development steps without long wait times. To achieve this, we needed to demonstrate that Slope Spectroscopy can quantify a change of absorbance from a perceived full capsid to a perceived empty capsid. This was tested within our third case study.

The data presented in Figure 5 confirms that the SoloVPE System can effectively

FIGURE 8

Method equivalency of the SoloVPE system and current methods using sample data from the Bioprocess Technology Institute (BTI) in Singapore.

Average % difference between **qPCR** and SoloVPE	**6.1%**
Average % difference between **ELISA** and SoloVPE	**0.67%**
Average % difference between **ddPCR** and SoloVPE	**5.0%**
Average % difference between **ELISA** and SoloVPE	**0.74%**
Average % difference between **dPCR** and SoloVPE	**3.86%**
Average % difference between **ELISA** and SoloVPE	**0.80%**

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quantify a change in absorbance between empty and full capsids. Figure 5 shows that the SoloVPE System and the qPCR/ELISA method had a difference in results of just 9%. The SoloVPE System achieved these results in just 5 minutes, while it took a processing time of around one week for the qPCR/ELISA method.

Vigene certified reference standards can be used as universal system suitability controls. We aimed to demonstrate the SoloVPE System's ability to use the DNA/Protein ratio slope value to make real-time decisions prior to subsequent analysis as an in-process tool. Our results showed equivalency between the SoloVPE System and the current Vigene qPCR/ELISA method, well within the +/- 40% range. The SoloVPE System demonstrated rapid analytics providing DNA and capsid concentrations measurements in a single test within minutes rather than days using the current qPCR/ELISA method.

Vigene Biosciences provided in-process samples for both AAV8 and AAV9 serotypes, which were compared to the SoloVPE System values versus their expected values (Figure 6). We aligned well to their given processes. The SoloVPE System was able to provide rapid in-process testing results to indicate that the AAV8 Empty standard was out of specification, and further refinement of their process was necessary.

The next step was to purchase traceable standards, to understand how well the Slope Spectroscopy method compares to these certified standards. We looked at empty and full capsids with three different serotypes (Figure 7). When comparing viral titer concentrations, equivalency of both methods was well within the +/- 40% range for each serotype tested, with an average of 7.5% for ddPCR, and 3.7% for ELISA. This confirmed that the SoloVPE System technique is serotype agnostic.

Case study: AAV viral titer concentration

In addition to the Vigene Standardsstandards, we purchased certified material from Biotechnology Institute of Singapore (BTI) in three different batches, at five different concentrations. The samples were tested using three different methods: qPCR and ELISA, ddPCR and ELISA, and dPCR (digital PCR) and ELISA (Figure 8). The three batches showed highly consistent data from batch to batch, and test to test. Compared against all three methods, the SoloVPE System showed equivalent results.

SUMMARY

The purpose of this paper was to show evidence detailing how our Slope Spectroscopy method can be used as an analytic tool for faster analysis while being compared to the current method within the industry being used. Using the SoloVPE System can provide a more cost-effective way to measure in process samples from Chromatography through Filtration and allow the process to continue without losing time sending samples out for analysis.

Q & A

Joe Ferraiolo, Associate Director, Bioanalytics Applications, Repligen, answers readers' questions on Slope Spectroscopy using VPT and the CTech SoloVPE System.

Q Can the SoloVPE System technology currently measure partials?

JF: Unfortunately, no. UV is not a selective enough technology to distinguish partial versus full, rather than the total content of what is. You would certainly see a change in concentration without doubt. However, it is not the job of the UV to properly define it as a partial or if detect if there is residual impurities within the sample.

Q Are there any applications for CAR-T processes with this technology?

JF: Not yet, as it is not something we are focused on near-term. If your current method is a UV-based one, then you could certainly apply Slope Spectroscopy to that.

Q Is the SoloVPE System and software fully GMP compliant? How are the system and software qualified, and what type of support is provided for qualification?

JF: Every new system is purchased with an Installation Qualification (IQ) and an Operational Qualification (OQ) package. The software package that is provided with the system is GMP capable. In addition, we either offer a software validation service to validate the software for you in GMP conditions, or work with your current IT team to see exactly how the configuration should be put in place for your specific group.

Q Can the SoloVPE System be used to test incoming plasmid product as raw material to check purity levels?

JF: The short answer is yes. For our approach, it must be purified material. We are not looking at anything from the harvest, it must be purified.

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Q How does the UV distinguish AAV from host cell DNA from the harvest?

JF: It is a multi-wavelength slope analysis. We are looking at the relationship between the two wavelengths of interest, then doing a complex equation implementing slope-based extinction coefficients or published extinction coefficients from literature to be able to calculate the concentration.

Q Can the SoloVPE System be used for release of GMP finished drug product?

JF: Yes. The majority of our business is in GMP environments related to drug substance and drug product.

Q How long does it usually take for the SoloVPE System to be installed and validated for process development work?

JF: From our perspective as the vendor, it typically takes 1 to 2 weeks to complete IQ and OQ training and software validation. The real work starts with the detailed plans for how each group will implement the system. How many methods are going to be validated could depend on the scope of work planned for each system. Based on our experience, when the project has the appropriate time and resources, we typically see a complete method validation and implementation of our technology within 6–12 months. However, the majority of that is reliant on the resources of the company adopting the technology.

Q What validation support does Repligen provide?

JF: Our Applications Group within Bioanalytics Applications has post-sales responsibilities with every system provided to the market. You will be paired with one of our application specialists to understand exactly how you will be implementing the technology, and then receive the associated method development and validation support to get that implemented within your organization. You would have a one-on-one custom support to help you through any SoloVPE System project.

BIOGRAPHY

Joe Ferraiolo

Associate Director, Bioanalytics Applications, Repligen

Joe leads the bioanalytics applications team and is in charge of the SoloVPE variable pathlength spectroscopy system for at-line applications. He has been with the company

for more than 20 years, with over ten years of development and validation experience in analytical applications. He specializes in UV analysis and leads the development and commercialization of high-value products and flexible solutions that address critical steps in the production of biologics.

AUTHORSHIP & CONFLICT OF INTEREST

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Analytical development insights spanning viral vectors & gene editing

David McCall, Commissioning Editor of *Cell & Gene Therapy Insights*, **speaks to Santoshkumar Khatwani,** Director Analytical Development, Sangamo Therapeutics, Inc.

DR SANTOSH KHATWANI graduated from the University of Kentucky in 2010 with PhD in Chemistry under the guidance of Prof. Sylvia Daunert. The research work focused on the use of generically engineered recombinant protein and enzymes for bio sensing and biomaterial applications. Furthermore, he obtained postdoctoral training at the University of Minnesota (until 2012) under the supervision of Prof T Andrew Taton and Prof Mark D Distefano with focus on the use of site-specific covalent conjugation for synthesis of protein–DNA conjugates and analytical assays for the generating novel engineered enzymes with improved functional attributes. Dr Khatwani then joined BioVision Inc. in 2012 and served under different capacities until 2017 where he oversaw the manufacture, testing and release of several recombinant protein, enzyme and assays for various metabolically important enzymes. Furthermore, Dr. Khatwani joined the field of cell and gene therapy at multiple companies including Thermofisher Viral Vector Services (formerly Brammer Bio, 2017–2018), Asklepios Biopharmaceuticals (2019) and Sangamo Therapeutics (2018–2019, 2019-present) at various leadership roles where he has led the development of various analytical assays including physicochemical, biophysical and biological assays to support the manufacture and release of viral vectors in cell and gene therapy. Currently Dr. Khatwani is serving as Director of Analytical Development at Sangamo Therapeutics with strong focus on developing analytical solutions and CMC in support of product development at early and late phase of the clinical development.

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Q What are you working on right now?

SK: Over the last several years, I have worked in the field of cell and gene therapy (CGT). I have played an integral role in advancing drug development for multiple new molecular entities in the area of analytical development including planning and execution of early to late-stage development for viral vector based gene and cell therapy products. At Sangamo, I have had opportunities to work on multiple clinical and non-clinical products in the pipeline. We have a plethora of research, several preclinical and a few Phase 1/2 and Phase 3 programs. We recently announced that we are starting the preparations for a Phase 3 for a Fabry disease program as one of our wholly owned products. A major part of my work has involved helping the program progress from Phase1/2 to Phase 3 readiness. In our capacity as one of the leaders in the industry, my group's overall focus has been to develop novel measurement systems and methods, to move the industry forward in further understanding and characterizing these products.

Q How has gene therapy analytical development evolved in your experience over recent years? What have been the most significant advances?

SK: In the field of CGT several different types of viral and non-viral vectors are being used for targeted gene delivery. In the viral vector-based approaches, there have been several recent advances in manufacturing processes as well as in analytical development for understanding the product quality. As manufacturing processes have evolved, the analytical methodologies and measurement systems have evolved concurrently. Although many viruses such as lentivirus, adenovirus, and, herpes simplex virus (HSV), are being used as gene delivery systems in the industry, Adeno-associated virus (AAV) has seen major improvements in their designs and increase in its use as a gene delivery system. Several AAV serotypes are being used by various sponsors to specifically target different tissues for different disease indications. AAV is also a smaller virus in size as compared to other viruses, so it is relatively well studied and easier to manufacture (in terms of the expected product yield). It is also a comparatively easier to characterize than other viruses. For AAV, the analytical approaches have also improved dramatically over the years to measure critical quality attributes (CQAs) of these AAV products with respect to viral titer, purity, impurity, and potency.

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Q Sangamo is unusual in terms of the variety of cell-based and gene therapy approaches in the R&D pipeline. Can you discuss considerations and overall progress in analytical development across these areas?

SK: Sangamo is in a unique place because of the way in which we make use of different technologies for gene delivery. At the core of these technologies in developing cutting-edge genomic medicines lies Sangamo's proprietary Zinc finger protein (ZFP) platform which is being investigated for a targeted *in vivo* genome engineering using a viral or non-viral vector delivery system. With regards to cell and gene therapy, a viral vector may be used as a drug product in a gene therapy while act as a drug substance (a drug intermediate) in a cell therapy program. However, the focus for analytical development remains very similar in terms of identifying and characterizing CQAs of these viral vector products. We thoroughly characterize our viral vector products that are being used for *in vivo* gene therapy or *ex vivo* gene editing or cell therapy applications. Furthermore, cell therapy drug product also requires additional separate characterization for CQAs as a final drug product. For that Sangamo has a dedicated cell therapy analytics team focused on developing methods specifically for a cell therapy product.

In regard to recent updates in gene therapy pipeline in our portfolio, Sangamo announced preparations to begin the planning for a for Phase 3 trial for our wholly-owned program targeting Fabry disease. Analytical comparability will be critical to the success of the program where we will need to demonstrate that our manufacturing processes result in products that are comparable. The primary objective of analytical comparability will be to show that early and late stage products are comparable with respect to all CQAs. We will rely on a range of analytical methods, including some advanced technologies to achieve this objective. There are only a few successful examples to date in the field of CGT of analytical comparability. Overall, there is a need to assess new technologies in this quickly evolving field. Some of these technologies have been used in traditional biologics and will need to be adapted specifically to CGT. Technologies such as Next generation sequencing (NGS) with appropriate bioinformatics platform expansion, charge detection mass spectrometry (CDMS), flow cytometry need further understanding as they are being increasingly popular in this field. Overall, the progress is slow and steady, but we still need to continue to focus on putting an additional emphasis on rapid turnaround times, improvement in precision and accuracy for critical assays such as dosing assay and potency.

Q Can you go into more depth on how the analytics piece has evolved over the course of this journey from discovery through to late-stage development?

SK: Many of the programs at Sangamo use AAV to deliver a target gene of interest. Therefore, most of our analytical development efforts have focused on using platform analytical methods to characterize these products. This involves appropriate understanding of

"...currently, there are few methods available to measure the partial capsid accurately. These include analytical ultracentrifugation and charge detection mass spectrometry. Additional work is needed to correctly identify and measure partial capsids that can be accomplished by using short and long read sequencing technologies."

the product pertaining to the stage of the development. In the early stages of development, it is common to use platform methods that you can apply across multiple products. E.g., For AAV products, we have traditionally seen platform methods such as quantitative Polymerase Chain Reaction (qPCR) targeting generic regions (ITR, Poly A and promoters) within AAV genome for viral titer determination and an early version of an infectivity potency assay. In addition, several other methods are also used for measuring critical impurities in the product such as host cell protein and DNA, empty/full capsids, and aggregates.

As product development progresses, manufacturing process gets optimized and concurrently, the analytical methods also have to evolve. At this stage, it could be more appropriate to develop additional product-specific methods. Several product quality attributes such as viral titer, potency, purity levels, and the capsid protein modifications need to be compared across manufacturing process changes to ensure these changes result in a product that is similar or comparable to that produced by the initial process.

Q Specifically, within AAV, what are the most critical missing analytical tools? Where is improvement most pressingly required today?

SK: One of the main challenges in this field is how to measure different variants of AAV capsids such as empty or full capsids in a drug product. In addition, a full AAV capsid could be either partially or completely full, or it could package some other genomic element in the production process. These impurities do not offer pharmacological benefit. In addition, it is important to ensure the manufacturing process results in a consistent product quality. There are several methods to accurately measure empty capsids. Chromatographic methods are accurate and being used to measure empty capsids not only for drug product but also for in-process samples. However, currently, there are few methods available to measure the partial capsid accurately. These include analytical ultracentrifugation and charge detection mass spectrometry. Additional work is needed to correctly identify and measure partial capsids that can be accomplished by using short and long read sequencing technologies.

Another challenge is how to accurately measure the stoichiometry of the AAV capsid proteins. AAV capsids are composed of what is traditionally believed with a 1:1:10 ratio of VP1, VP2, and

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VP3 proteins, based on a theoretical understanding of the molecule. In practice, the ratio could be quite different, so we need to understand how that will impact the overall structure of the AAV and its biological function. There are several analytical tools available to measure VP ratio which differ in their principles of measurement and require electrophoretic or chromatographic separation of these proteins. Finding a way to compare all these methods to give an accurate understanding of what the actual ratio is, and whether that ratio is impacting the biological function needs to be understood. In addition, accurate measurement of critical impurities such as aggregates and packaged non-target sequences is essential. Using traditional size-exclusion chromatography for determination of aggregates has certain limitations on column pore size which can influence the accurate quantitation of aggregates, while development of bioinformatics platform for identification of non-target sequences is essential. Moreover, improvement in infectivity assay precision, selection of stability-indicating methods and further refinement of a dosing assay with <5% RSD may need continued evolution of available technologies.

The final but one of the most important challenges is to understand the post-transcriptional modifications of the viral capsid proteins. It is quite challenging to correlate which modifications in viral proteins are critical to alter the biological function, but there are certain examples in the literature that have shown that mass spectrometry will be a critical tool in AAV analysis in coming future.

Q What are the issues in cost and has any progress been made in controlling that aspect?

SK: The available analytical methods in CGT analytics are still reasonably costly for many of the sponsors. Part of the reason is that some of these are newer technologies. As a technology matures, it would become less expensive, higher throughout and user friendly. Next generation sequencing and mass spectrometry-based characterization are quite costly due to high cost of instrumentations which is why you have to rely on contract laboratories to get these analyses performed.

Q Are there any particular needs or improvements relating to speed to result?

SK: For speed to result, one of the main aspects that we focus on internally at Sangamo is utilizing the high-throughput methods to support and improve decision-making processes for the process development and formulation groups.

As mentioned earlier, many of the analytics are new, but there have been considerable improvements to increase the throughput

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– for example, consider droplet digital PCR (ddPCR). As CGT field was moving away from qPCR towards ddPCR for dosing assays, it was still a low-throughput platform, but now there are new systems available that give considerably high-throughput with increased automation. Many of the assays also use chromatographic separations which are traditionally used as high-throughput fast measurement systems. It is only a matter of time before we have even more tools available for users in the CGT field to allow for faster turnaround times for analytics to aid in improved decision making. In addition, rapid methods to detect viral capsid proteins at lower sample concentrations and volumes will be crucial to the improvement in the speed of analytical measurements.

Q How does the gene editing platform component impact analytical development?

SK: Overall, analytical development relating to genome editing with the zinc finger protein (ZFP) platform still uses similar tools to those we use for other CGT applications. In analytical development group, we are mainly engaged in trying to characterize these viral vector molecules as gene delivery systems. These could be AAV, lentivirus, or indeed, any other virus molecule. Several aspects of gene editing such as determination of %indels and off-target effects are critical to investigate. Important emphasis will be on understanding the mechanism of action (MOA) to develop the potency assay for each platform. Most other analytics could be standardized across different platforms.

Q How and where is the automation of analytics and bringing them in-process impacting CGT bioprocessing today?

SK: There are different types of in-process analytics that are important to consider. For in-process in-line analytics, cell age, growth and health are critical. In addition, the monitoring of critical cellular media, metabolite and essential components concentration can be performed via inline monitoring technologies.

However, it is quite challenging to integrate in-process analytics of AAV to support in-line analysis. Part of the challenge in doing this with AAV products is that many of the technologies that are used to characterize AAV products are not high-throughput. In addition, process impurities interfere with several of analytical assays. So, a fast clean-up processes are needed prior to analyzing AAV product during in-process steps.

Q What is the key to successfully adopting a less siloed approach for potency assay development? And do you have any other best practices on how to approach potency assay development that you can share?

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SK: The siloed approach for potency assay development brings into question what the unified approach should be. A potency assay has to reflect the mechanism of action (MoA) when your product is at the later stages of development, but the work itself must begin at early stage. At the early stage, the strategies can include a platform infectivity assay and a gene of interested expression assay to demonstrate the potency (strength) of the product. In addition, potency assays are susceptible to matrix interference, and if the manufacturing process has not settled down, it can be very difficult to develop a potency assay.

Siloed approaches in potency assay development are quite common as there can be several approaches to developing a potency assay. However, as product development progresses, the assay need to reflect mechanism of action to demonstrate *in vitro* potency of the product This results in further optimization of these assays and/or filtering all available approaches to a unified approach that will comply with regulatory guidance. Strong collaboration efforts are needed among research, nonclinical and analytical and quality control groups to facilitate the development of a unified approach for a potency assay.

Potency assays are one of the most complex assays to develop for any biologic. A potency assay is influenced by several assay conditions and the components, such as the use of appropriate cell line with permissivity for viral vector, cell media for optimal cell growth, any additional growth factor/antibiotics used in the culture conditions, type of final assay read out (plate-based, imaging, chromatography, flow cytometry, etc.). Multiple approaches can be undertaken to demonstrate the potency by the expression (RNA, protein) and mechanism of action (enzyme activity, protein binding, protein–protein interactions, etc.).

Q Finally, can you sum up some key goals and priorities, both for yourself in your own role and for Sangamo Therapeutics as a whole, over the coming 12–24 months?

SK: The key goal for Sangamo analytical development group is to plan and prepare for supporting all late phase programs. In addition, several early stage programs will require development of additional platform methods to support their progress into next phase of the development. This can be achieved by identifying and assessing new technologies in addition to improving current technologies in terms of lower sample volume requirements, high-throughput, improved precision, accuracy, and specificity.

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"This is not a sustainable model."

Healthcare Providers call for sector-wide standards & collaboration to solve widespread cell & gene therapy challenges at hospitals and sites of care

Joe DePinto & Robert Richards

Cell and gene therapies (CGTs) are on the cutting edge of medicine, but their current production and delivery complexity is pushing many Healthcare Providers to a breaking point. CGT operational systems and processes are so numerous and unnecessarily varied that the growth of the field is at risk. At a recent advisory council attended by 16 leading cell and gene therapy healthcare professionals, providers and administrators voiced an urgent need for standards and simplicity to make the growth of CGT sustainable. Among the greatest challenges – workflows and systems that are not standardized; large amounts of uncompensated time lost to data entry and system trainings; low staff morale and high attrition risk due to the need to prioritize processes and training over time with patients; IT and cybersecurity vulnerabilities related to the proliferation of too many portals and digital systems; and excessive operational variability and training requirements for CGT clinical trials that may only enroll a limited number of patients per site. These challenges arise from many different types of CGT products, in all phases of development. Healthcare professionals stated that these operational challenges will limit CGT's ability to scale, may prevent some medical centers from taking on new CGT clinical studies, and will likely become unsustainable as the field provides therapies for more common diseases with larger patient populations. Collaboration among the entire sector, with a special emphasis on the needs of Healthcare Providers and the patients they serve, is urgently required to develop necessary standards and harmonized approaches – and reduce complexity.

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Cell and gene therapies (CGTs) are on the cutting edge of medicine. But in the healthcare settings where patients are treated these transformative therapeutics, the supporting operational systems and infrastructure for CGTs have not kept pace. Hospitals are critical not only to the care of CGT patients, but to the production and delivery of these transformative therapeutics – and growing complexity is making all aspects of CGT care more difficult. Healthcare Providers (HCPs) say that ongoing CGT production and delivery complexity is pushing them to a breaking point and puts further growth of the sector at risk.

[1]

At a recent Clinical Advisory Council attended by 16 veteran cell and gene therapy healthcare professionals from the University of Pennsylvania and other leading academic institutions and organizations across the United States, providers and administrative leaders gathered to share CGT challenges experienced across hospitals and sites of care. The attendees included leading physicians, nursing managers, cell pharmacy decision-makers, and hospital IT and technology strategists, all experienced in working with multiple types of cell and gene therapies at all stages of clinical and commercial development. All share a common commitment to CGT patients and their mission of delivering these life-changing therapies.

With more than 200 years of collective experience in CGT among the attendees, the goal of this initial session was to surface challenges that are common across HCPs and institutions. (Future advisory councils will focus further on solutions, as well as collaborative discussions with biopharmaceutical manufacturers.)

The Clinical Advisory Council, hosted by Vineti, revealed a set of common, urgent challenges in healthcare settings that present a call to action for to the CGT sector. Here are some of the most significant, as described by advisory council attendees:

- Workflows and operational systems are not standardized for critical steps across the CGT patient and product journeys, introducing extra work, unnecessary complexity, and risk.
- HCPs are losing excessive amounts of uncompensated time to "back office" work, including duplicative IT audits and risk assessments, one-off system trainings for each individual CGT product, and repetitive, high-risk manual data entry that all lead to delays in offering therapies.
- Clinical staff is overwhelmed by the need to prioritize processes and training over time with patients, resulting in low morale and attrition.
- IT and cybersecurity vulnerabilities are arising from the proliferation of too many individual manufacturer portals and digital systems.
- CGT clinical trials are bogged down in excessive operational variability and training requirements that require large amounts of clinical staff time but may ultimately enroll only a limited number of patients per clinical site.

The challenges related to use of these therapies are present in both research and commercial settings but are proving to have a greater effect on commercial products. The reason for this is that centers will keep more therapies that have

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similar indications on formulary in the event that one company has a long lead time manufacturing. This, coupled with the probability that some CGTs will advance to second-line therapy amid market competition to offer them, will put pressure on centers to site-certify. Research-phase products don't have those pressures, as the number of patients that can be put on trial is smaller, which in turn reduces the risks of complexity at scale.

In this white paper, we will outline the findings of the Clinical Advisory Council, beginning with a detailed look at current trends in CGT as described by council attendees, followed by the key challenges described by the advisory council and some recommended solutions. The goal of this white paper is to surface CGT challenges being described across hospitals and sites of care, in the interest of encouraging the CGT sector to collaborate on solutions. Independent industry-wide organizations, such as the independent, non-profit Standards Coordinating Body for Regenerative Medicine, have already initiated efforts to work with biopharmaceutical manufacturers in support of HCPs. We hope that this paper will provide information to accelerate such efforts.

CURRENT TRENDS IN CGT

On the scientific side, growth in the CGT sector is rapid and multi-dimensional. There are currently more than 5,000 unique therapeutic products in development [2], and more than 2,260 clinical trials ongoing worldwide [3]. The newest wave of treatments represents "CGT 3.0," building on the dendritic cell and CAR-T cell breakthroughs that have already received regulatory approvals over the last 12 years. Many of the newest approaches rely on manipulating a broader range of cells – such as allogeneic cells from donors or starting material from solid tumors – that are often even more complex to collect, manage, and dose than those used in CAR-T treatments. (Figure 1).

Advisory council attendees described growing patient volumes for both clinical-phase and commercial products that mirror this overall CGT sector growth. More than 60 percent of attendees said their institution experienced increased clinical trials and trial patient volumes in 2021 over 2020 (Figure 2) [1]. More than 75 percent said their institution experienced a similar increase

FIGURE 1

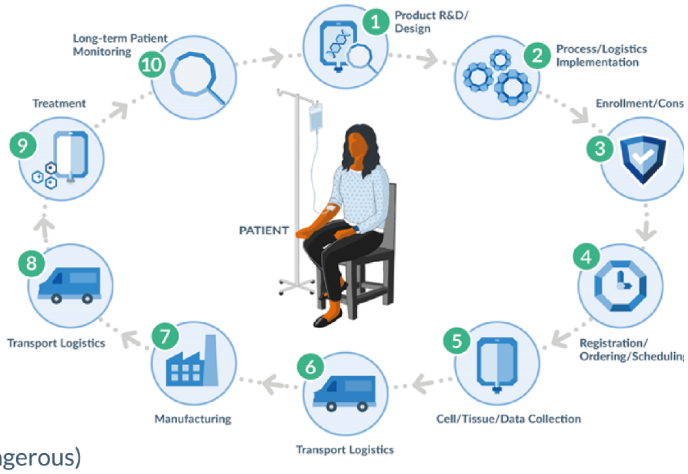
Complexities for healthcare providers

Operational complexities in CGT

Many considerations for clinical sites, including the patient-specific supply chain

Key raw material: human cells (patient and/or donor)

- Variability in the human biology of patients and the cellular starting material
- Complexity
  - GMP starting material is collected in medical centers (regulated process)
  - Multiple locations for collections, depending on cell type
  - Large number of clinical stakeholders
- Circular, patient-centric supply chain
- Unique handling requirements for cells, product
- Increased patient safety risk (mix-ups are extremely dangerous)
- "Real-time" nature of supply chain



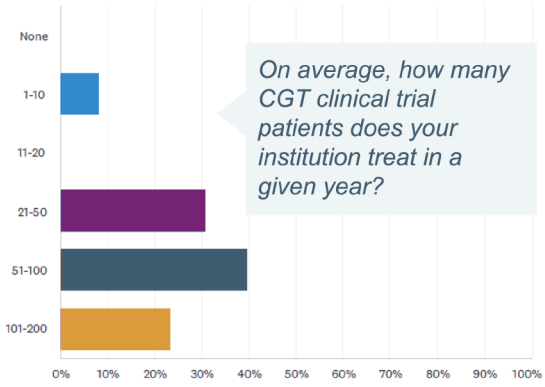
The unique, patient-specific nature of cell and gene therapies introduces a wide variety of new requirements and workflows for Healthcare Providers and sites of care.

FIGURE 2

Growing patient volumes in cell and gene therapy

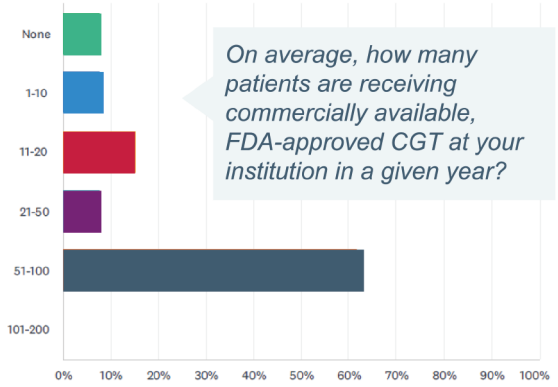
Growth in clinical trials and patient volumes

- 62% of Advisory Council attendees experienced increased clinical trials and patient volumes for clinical trials this year over last year
- Nearly 70% of attendees' institutions treat 20-100 patients per year
- Most support 10-50 clinical trials per year



Growth in patients treated with commercial products

- Five approved products, some with multiple indications
- 62% of attendees are treating 50-100 patients per year
- 77% indicate that patient volumes have increased over last year



Institutions saw growth in the numbers of both clinical trial and commercial patients over the past year [1].

for patients treated with commercial CGTs [1]. This growth has taken place despite the COVID-19 pandemic, which has periodically affected CGT's progress at some medical centers (Figure 3) [4]. However, healthcare professionals have collaborated to find ways to continue their commitment to CGT patients amid the pandemic, with one such consortium stating that the

> "COVID-19 pandemic should not serve as reason to defer CAR T cell therapy for patients truly in need of a potentially curative therapy." [5]

The healthcare professionals attending the advisory council came from a wide variety of roles, specialties, and backgrounds – including physicians, cell pharmacy specialists, apheresis specialists, and program leaders or healthcare technology strategists.

The diversity of roles on the advisory council reflects the realities of CGT care. Healthcare teams serving CGT patients are often sizable and represent a wide variety of skills and specialties – a reflection of the complexity of providing CGT care.

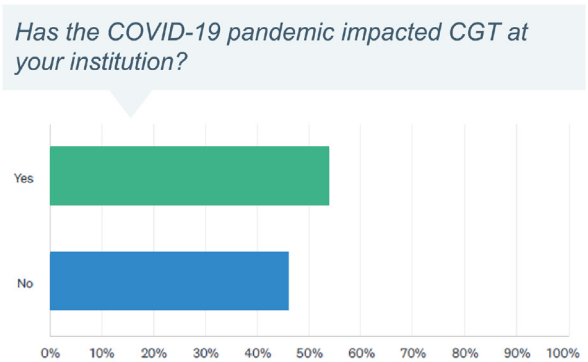
CGT care teams at sites of care can involve dozens of team members. Advisory council attendees described at least 15 specialized roles involved in CGT clinical trials, and at least

FIGURE 3

Effects of COVID-19

COVID affected CGT in 2021, but less than expected

- Institutions adapted quickly
- Clinical trial enrollment was temporarily paused
- Some elective procedures delayed or patients less willing to come on-site for treatment
- Travel restrictions and staff leave played a role



Institutions providing CAR-T cell therapies have worked hard to adapt and serve CGT patients amid the pandemic [1].

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16 involved in CGT commercial products [1]. Roles for both clinical and commercial phases are outlined in the following table (Figure 4).

The clinical and operational complexities of CGT procedures necessitate these large and varied care teams. Some advisory council attendees stated that all the above roles were required to provide CGT patient care at their institutions.

In addition to large-sized teams, the set of professionals involved in key functions can also vary from one hospital to another. Cell shipments, for example, may be handled by one set of specialists at one center and a different set at another – introducing more variability to already complex processes (Figure 5).

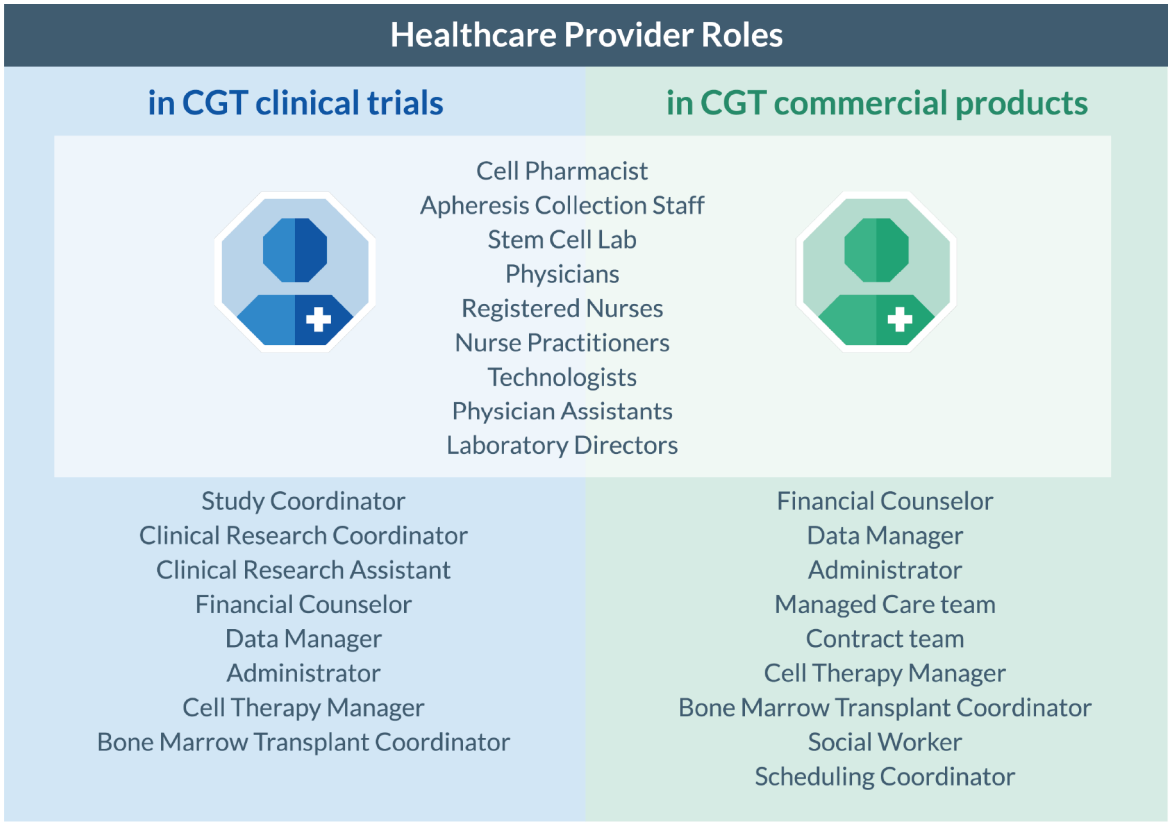
The effects of CGT requirements on HCP staffing (large teams are needed, from a wide variety of specialties), along with the high-touch work that comes with a nascent sector (patient populations that are still relatively small require support from large care teams that are learning as the field develops), have significant implications. Clinical teams must use the correct operational systems, workflows, and processes every time to ensure safe, high-quality care. But ensuring this consistency among many specialists from different backgrounds, all of whom are providing many different CGT treatments to many different patients, can be extremely difficult and time-consuming.

CGT CHALLENGES

The wide variety of specialists providing CGT care is reflective of a wider, fundamental operational challenge facing CGT – excessive variability between products and the processes underlying them. Health care professionals

FIGURE 4

Healthcare Providers in cell and gene therapies



Some roles focus specifically on clinical-phase or commercial products, while others work with CGTs at all phases of development [1].

FIGURE 5

Common tasks, multiple specialists

Schedules the apheresis	Tracks the progress of shipment	Administers CGT Treatments
• Apheresis coordinator, apheresis staff • Nursing coordinator • RN • Donor services • APPs • Apheresis nurse manager • Cell therapy coordinator	• CGT Coordinator • RN coordinator • QA • Physician Assistant • Nursing team • Cell collection/Cell therapy lab • Specialty Lab Director • Lab tech, lab manager • Pharmacists, Admin staff • Nurse coordinator	• Provider/nursing staff • Stem cell lab • Pharmacist • PA/NP with MD support as indicated (if APP not credentialed) • APP or RN • Nurse practitioner • MD • Inpatient nurses, inpatient service (MD, APP, fellow)

From one institution to another, essential CGT operations may be managed by different types of specialists [1].

repeatedly described how the field's supporting operational workflows and technologies, from cell collection protocols to digital portals for product ordering and tracking, often introduce more complexity than they solve. This challenge is largely driven by differences from one product to the next – differences that healthcare professional described as often unnecessary.

Repeatedly, advisory council attendees described working with a confusing jumble of digital and manual systems and processes as they juggled multiple CGT therapies and patients. Some HCP advisors expressed concerns that operational complexity puts patient access at risk. These challenges, they said, threaten to become blockers when CGT products for larger patient populations become widely available. HCPs attending the advisory council spotlighted five specific challenges as the most urgent for providers and patients.

Challenge 1. Workflows & systems that are not standardized for critical steps across the patient journey

These steps include but are not limited to cell collection, cell and product labeling, treatment order placement, and scheduling of cell collections and/or final patient treatments. The process of getting patient to apheresis varies from company to company. Some companies' products are sent out fresh, requiring more coordination of steps such as authorization, case agreements (managed care), hospital review (clearance) prior to collection. Those products that are frozen have more flexibility, but that flexibility puts the institution at risk. For example, an institution may not have a single case agreement executed with the payer. They have to decide whether to continue with collection and recognize that the optics of collection tell everyone that the center is ready to start the episode of care. Complexities in this area alone vary when diving deeper – manufacturing lead times, apheresis/stem cell processing availability, benefits investigation (if needed), pharmaceutical companies varying policies on the collection process (some companies manufacture everything you send, some only use what they need), and the impact to the patient in the event of a failure.

Chain of Identity and Chain of Custody, two required patient- and product-related traceability workflows, are mandated by regulators [6,7] but can be implemented in highly variable ways, HCPs said. To help alleviate

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this tracking burden, some manufacturers have moved towards the use of digital workflow and supply chain orchestration systems, with such digital systems in wider use for commercial products than for clinical-phase therapies. (Figure 6).

However, HCPs described how these digital systems can be of limited assistance if they introduce more variability than they prevent. Some manufacturers choose to implement unique stand-alone systems, which often involve manual processes. Others still rely on paper-based systems, especially for clinical trials, which involve even more manual processes and data entry. Difficulties can quickly arise as care teams try to navigate between multiple systems across multiple therapies and diverse patients, often requiring some mix of digital and manual data entry and management. Duplicate data entry is a common, time-consuming daily task, with HCP teams forced to enter patient and/or treatment data into multiple systems 'all day, every day' [1].

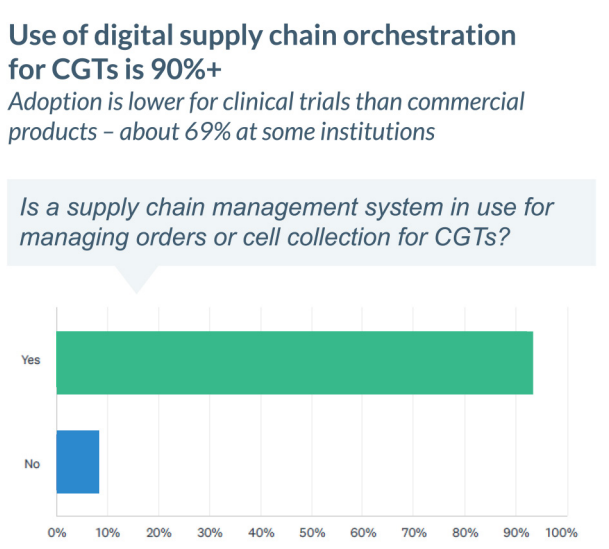
> We have heard of scope creep – you know, the project gets bigger and bigger and bigger. So, portal creep – as every company comes online, there's another portal, right? So sooner or later, that's going to be unsustainable.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

In addition to an over-proliferation of disparate systems and workflow tools, HCPs said that excessive variability has been introduced for processes and Standard Operating Procedures (SOPs) that should have commonality, such as cell collection. Care teams are forced to navigate between multiple processes that are sometimes only slightly different from one another, they said, which can be worse than managing large differences. Small differences between systems and processes, they said, are harder to remember and track, creating risk for both patients and the institution.

FIGURE 6

Digital supply chain orchestration systems



Digital systems are now widely used, especially for commercial CGTs [1].

Challenge 2. Excessive amounts of uncompensated time lost to numerous system trainings for each individual CGT workflow & repetitive, error-prone manual data entry

Many CGT care teams are inundated with time-consuming administrative tasks. Providers repeatedly described how their days are consumed by trainings for each CGT, along with repetitive processes related to data entry, site qualifications, tutorials, and surveys. As products are commercialized, CGT pharma companies have a site certification process that generally consists of legal reviews, IT work and installations (which often includes a specialized, proprietary CGT management portal), audits of institutional policies/procedures, a REMS program, and more. The site certification process is intense and can take months to complete. Beyond that, there is

ongoing work, including operational changes made by the biopharma company (such as portal upgrades, SOP revisions) that affect the sites of care with ongoing training requirements. However, this step-up in work is not always accompanied by increased staffing or gains in efficiency. Some hospital executives may see CGT as a niche field because of its cost and early commercial nature. This, along with the need to break-even, may make resourcing for CGT a challenge.

> We have to throw people at problems... and [we] have sat in on so many meetings where we have MDs, and pharmacists, and a whole host of other individuals sitting in a room with an Excel file up on the wall, people sorting through their phones, trying to find emails, trying to find confirmation numbers. And it is on a weekly basis, or biweekly, depending where we have all these people together, trying to piece these disparate pieces of information together in order to provide the quality of care that the patients deserve.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

Different clinical trial manufacturers may use different electronic data collection systems and require data collection that may be onerous for the clinical staff. Many different electronic data collection systems require unnecessary tutorials, participants said. The problem is not only about the complexity of the job, but also all the additional administrative tasks associated with each individual therapy. This represents not only a resource strain, but also often uncompensated time.

As part of commercial site certification, centers have been asked by biopharmaceutical manufacturers to address these administrative activities by identifying an individual designated as an "authorized representative," or AR, who must juggle large amounts of patient-and product-related information, audits, and numerous portals, bearing an increasing burden as the number of CGTs and portals grows. This workload will quickly become unsustainable for those staff members assigned to manage it.

Challenge 3. Clinical staff are overwhelmed by the need to prioritize processes & training over time with patients, resulting in low morale & staff attrition.

HCP staff bear the brunt of CGT variability and operational challenges. With many care teams inundated with time-consuming administrative tasks, time is taken away from patient care and leads to a need to further invest in resources (staff) to be able to deliver care. The high "back office" workload and related stress, HCPs said, can lead to staff turnover, which in turn requires new staff members to be recruited and trained.

> We have an army of people that are trained in navigating other people's systems, rather than focusing on the quality of care for the patient.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

Many of the advisory council participants described operations-related staffing problems as one of their greatest challenges. Medical centers are bearing an excessive burden and too much risk, providers said, which is an unsustainable model. Not only are staffing needs increased, but members of the care team experience stress, confusion, inefficiency, and burnout, ultimately leading to attrition.

HCPs stressed that they want to help people, not spend time logging tasks or entering the same data into multiple systems manually. Advisory council attendees expressed hope

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that if clinical researchers could be consulted early during study design, there could be alignment between the needs of the company and the institution, which would allow for appropriate scale when a product is commercialized.

> Ultimately it impacts patient experience. Patients deserve full transparency and high-quality care without Herculean efforts to connect data and capabilities.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

Challenge 4. IT & cybersecurity vulnerabilities related to the proliferation of too many portals and digital systems.

Hospitals and healthcare organizations already face daunting cybersecurity requirements and challenges. Patient data and PHI are protected by regulations such as the Health Insurance Portability and Accountability Act (HIPAA) [8] in the U.S. and the General Data Protection Regulation (GDPR) in the EU [9]. In addition, hospitals and sites of care working in CGT must often comply with Good Manufacturing Practices (GMP) regulations, and related rules governing electronic systems such as Title 21 CFR Part 11 and Annex 11 [10].

Such requirements are meant to protect a sector based on sensitive, high-value personal health information and facing frequent security threats. The FBI, Homeland Security, Dept of Health and Human Services warned hospitals of an increased and imminent threat from hackers in the last year [11]. Pharma and biotech companies suffer more data breaches than those in any other industry, with 53% of them resulting from malicious activity [12] .

> ...from a probability standpoint, you're statistically increasing the risk of vulnerability with each and every portal that you have to deal with more and more and more.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

In this environment of already-heightened risk, the proliferation of CGT systems and manufacturer portals becomes even more overwhelming for sites of care. Healthcare professionals said there is an increase in cybersecurity risk and vulnerabilities with each portal that is used – too many people accessing data, requiring user IDs, and requiring training, all of which puts digital systems at greater risk of an access break and puts patient information safety at risk. In addition, varied processes of requesting access to a biopharmaceutical company's portal, along with the management of staff who have access (which is typically left to those who may not understand the risks associated with portal access), increases the likelihood that a potential breach will occur.

> (For) every therapy that comes to market, we go through another training of portals, another cost, and another IT risk assessment. So these things just continue to subtly creep upwards. And they are going to be rate-limiting steps in Centers' ability to offer CGTs.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

Challenge 5. Excessive operational variability & training requirements for CGT clinical trials that require large amounts of staff training and 'back office' time but ultimately enroll only a limited number of patients per clinical site

Given the nascent nature of CGTs, most products are still clinical phase. And, HCPs said, early science is often accompanied by early, unproven, unwieldy operational approaches.

Providers described clinical-phase CGTs as inherently more difficult to implement because they are sponsor-dependent, and their systems are still often paper or are email-based. They said that some small biotech sponsors may also make the process more complex than necessary out of lack of experience with CGT products. Effective coordination and operations often require significant manual intervention by disparate care teams and departments.

[1]

Overall, HCPs stated that for clinical studies, the time and resource investments upfront often take a heavy toll on staff – and ultimately may enroll only a few patients. If not resolved during clinical phase, these complexities can extend through to commercial, putting an institution's ability to continue offering the therapy at risk if the long-term investment proves to be unsustainable. A more standardized approach to clinical trial workflows and processes would go a long way towards making CGT studies tenable – and making commercialized products viable in the long run.

Across the board, HCPs shared that these operational challenges in CGT trigger a wide range of unintended negative outcomes across sites of care:

- Patient experience is reduced due to the care team's ongoing need to prioritize "back office" tasks over spending time at the bedside. Patient access may be reduced if operational complexity makes it too difficult or too costly for institutions to treat more CGT patients.
- Staffing needs are increased when operational complexity, manual data entry requirements, and IT concerns require attention from more healthcare professionals for each patient and product. At the same time, biopharmaceutical manufacturers are recruiting experienced CGT staff from sites of care, adding further pressure to staffing concerns.
- HCPs experience stress, confusion, inefficiency, and an excessive amount of repetitive administrative work, leading to burnout and driving up attrition.
- The transition from clinical phase to commercial is more difficult when one set of research-phase complexities must be replaced by an entirely new set of different but even more complex commercial processes.
- Scaling CGTs to more patients can be limited by operational complexity and administrative costs. Larger institutions are currently better able to recruit greater numbers of patients, but also must choose which clinical trials and commercial products make the best use of staff time and resources. Smaller centers may not have the resources to manage complex

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CGT workflows for larger numbers of patients.

- As a result, development of new therapies and overall sector growth may be hampered. Institutions may be limited in the number of therapies that can be administered. Adoption and participation in clinical trials may be reduced due to the difficulties of running CGT trials.
- To reduce variability and control costs, centers will select what therapies are on formulary, which has the potential to limit access to patients **(Figure 7)**.

Sector growth may be especially challenging in oncology as CGT moves from autologous blood cancer products to other approaches that have the potential to treat greater numbers of patients, such as allogeneic products and products intended to treat solid tumors. Solid tumor CGT products, for example, often have very complex starting material collections, are more likely to require multiple dosing, and the patient populations for some solid tumor indications are larger than seen in blood cancers.

CGT SOLUTIONS

Organizations working towards standards and solutions

- Association for the Advancement of Blood and Biotherapies (AABB)
- The American Society for Transplantation and Cellular Therapy (ASTCT)
- The Standards Coordinating Body for Regenerative Medicine (SCB)

HCPs are keenly aware of the unique requirements of CGTs and have no expectations that all complexity can be removed. It is also important to note that this particular advisory council session brought clinical leaders together to surface challenges occurring across institutions, with subsequent sessions to focus more deeply on specific solutions. However, the discussions of challenges also

FIGURE 7

Summary – key challenges

Challenges with current systems

- Complex web of systems - multiple systems and portals in use for a single patient/product journey and each therapy
- Mixed manual and digital systems increase errors and require duplicate entry
- Systems are not integrated and interoperable
- No transparency and real-time visibility
- Security and data privacy risks

- Rely heavily on dedicated staff and person-to-person communication
- Clinical trials use paper and email-based systems and processes
- Ease of use varies from system to system

Operational challenges

- Lack of standardized processes, requirements, and systems across therapies/trials
- Capacity and scheduling availability/constraints for each step
- Tracking and accessing COI and COC from "vein-to-vein"
- Clinical to commercial transition requires process changes
- Range and number of highly trained HCP personnel required to treat CGT patients
- Roles and touch points - 100s of them - vary across treatments and institutions
- Scaling complex inefficient systems is difficult and expensive
- Clinical trials require specialized staff, more documentation, redundant audits, and rely on inexperienced stakeholders

Top challenges in CGT, as identified by healthcare professionals.

yielded a set of specific improvements that would make the CGT field more sustainable for sites of care. Here are five of the advisors' top recommendations.

- **Standardize workflows and digital systems.** Harmonize processes, systems, and SOPs wherever possible. Connect disparate digital systems to reduce manual data entry and duplicative record-keeping. Key areas recommended for standardization and harmonization are listed in the table below. **(Box 1)**

- **Simplify the clinical phase, and plan for commercial early.** Many HCPs said that clinical trial operations are not sufficiently streamlined to make most studies efficient or enable a smooth transition to the commercial phase. Providers hope to see process improvements embedded in early-phase trials so that scaling is simpler, and they encouraged clinical-phase manufacturers to connect with HCPs and build solutions proactively before challenges arise. Centers that participate in CGT clinical studies would also benefit from being able to start the trial assessment earlier, which would simplify site certification requirements more quickly. Without such changes, they said, centers may start declining to participate in a greater number of trials.

> Get to the manufacturers early in their processes during development and remind them that eventually, if they want to scale up and scale out that they're going to have to work in a standardized ecosystem.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

- **Root out and eliminate small differences.** HCPs repeatedly said that in many cases, small operational distinctions between systems, tools, and products are harder to monitor and may, in some cases, present greater risks to both treatment viability and patient outcomes.

> If it is the same, keep the same. If it can be the same, keep the same. If it is similar, we have a tendency to overlook it. And if it is different, it should be wildly different. It should be able to be identified… Whatever needs to be different needs to be different and explained for us to really appreciate that.
>
> *–Vineti Clinical Advisory Council, 2021*

[1]

BOX 1

Summary – top solutions

- Common key processes should be standardized. These include order placement for CGT treatments, chain of identity, chain of custody, in-process cell and product labelling, and apheresis-based cell collection.
- All manufacturer portals are currently separate from and redundant with the EMR. Integrate CGT systems with EMRs.
- The system landscape is complex and disparate. Information does not flow consistently, requiring significant manual intervention and data entry. Integrate CGT data with a variety of 'back office' hospital workflow, including payor approval, conversion to IRB, and billing.
- Standardize data entry portals, data entry SOPs, audits, and regulatory oversight.
- Create one system that manages clinical processes, no matter if the product is commercial or part of a clinical trial. Portals are not the way to move forward. Each company trains institutions as part of their site certification process – it would be easier if adding portals were more 'plug and play'.

Important solutions to make CGTs sustainable for sites of care, as identified by healthcare professionals.

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- **Reduce costly, demoralizing "back office" demands on providers and staff.** Creating standards, reducing training needs, reducing manual processes and duplicative data entry, and simplifying digital systems will go a long way towards improving HCP morale and opening up more time for patients. If manufacturers aren't willing to make these changes, they should help cover the administrative and staffing costs of CGT, some healthcare professionals said.

- **Solve CGT challenges proactively for allogeneic and solid tumor products.** In the CGTs that are already relatively established, such as the CAR-T products for lymphoma, some HCPs said that solving so many operational challenges this far along in the development of the field feels overwhelming. In less established indications, however, the time to standardize, harmonize, and simplify is now. This is especially important in solid tumor indications, which are often multi-dose treatments with especially complex cell collections and larger patient populations.

CONCLUSION

Healthcare Providers in cell and gene therapies are dedicated to the success of the field and the patients they serve. But for their commitment to yield even greater results over time, urgent action is required on the part of the entire sector.

Systems, workflows, and processes must be standardized and harmonized wherever possible. Disparate systems must be integrated to reduce duplicative manual data entry and the risk of error. Clinical trials should start with eventual commercial processes in mind, and not try to differentiate unnecessarily on workflows and SOPs. Such changes begin now, in advance of allogeneic CGT treatments, products for solid tumor indications, and other approaches for larger patient populations.

Above all, Healthcare Providers should be treated as up-front design partners in the development of CGT systems and workflows. They are working with multiple therapies and diverse group of patients every day. Their perspectives will prevent problems early and allow the entire field of cell and gene therapies to scale and reach more patients.

> Instead of asking 200 transplant centers to conform, maybe these companies can join hands together... and start to standardize and harmonize amongst themselves.
>
> —*Vineti Clinical Advisory Council, 2021*

[1]

As a starting point, we encourage the sector to join the work of key organizations working to create sustainable systems for CGT. Here are three places to start:

- The American Society for Transplantation and Cellular Therapy is conducting an "80/20 Taskforce" to address the roughly 80 percent of operational requests from biopharmaceutical manufacturers that ASTCT members find to be duplicative.

- The FDA-funded work of the independent, non-profit Standards Coordinating Body includes multiple cross-sector standards advancement projects in areas of importance to Healthcare Providers and sites of care, including cell collection, Chain of Identity, and patient data management. Please consider connecting with the SCB and lending your expertise to an SCB working group.

- The Association for the Advancement of Blood and Biotherapies offers multiple resources and workstreams to streamline

the working relationships between HCPs and biopharmaceutical researchers and manufacturers. Learn more at AABB's Biotherapies resources hub.

The successful adoption of cell and gene therapies into mainstream medicine requires ongoing collaboration with healthcare professionals and sites of care. By surfacing challenges across institutions providing CGT care, we hope to encourage the development of new solutions that will ultimately create more patient access to this transformative field of medical science.

BIOGRAPHIES

JOE DEPINTO brings more than 28 years of executive leadership in biotech, pharmaceuticals, specialty pharma, and cell therapy to Vineti. He joins us from Cardinal Health, where he served as the President of Specialty Solutions, leading one of the fastest-growing businesses within the Fortune 14-ranked company. Prior to Cardinal Health, Joe's roles included leadership positions at top pharmaceutical companies, including Johnson & Johnson and Lilly. His core leadership competencies include leading all aspects of strategy, drug development, investor relations, and commercialization with multiple global launches. He also previously served in executive roles at Sunesis Pharmaceuticals, Dendreon, ImClone, and Abraxis.

ROBERT 'ROBB' RICHARDS has over 20 years of experience in oncology, initially with a private practice in Southern New Jersey, and more recently the University of Pennsylvania Health System. He comes to Penn after serving as the IT Manager for the Center for Cancer and Hematologic Disease in Cherry Hill, Regional Cancer Care Associates (RCCA) in New Jersey, Division Chief Operating Officer, and RCCA corporate VP and Chief Information Officer. He has provided oversight in the integration of the South Jersey medical oncologists onboarding to Penn Medicine while working with the Cell Therapy and Transplant program (CTT) and has been the lead in overseeing operationalizing/implementation of CAR-T cell therapy for commercial use. He is currently the Administrative Director of the Cell Therapy and Transplant program at Penn Medicine, with oversight of both commercial and research work, and its expansion in the Penn system. Robb received his BS from Drexel University and both his MS and MBA at St Joseph's University and specializes in oncology informatics.

REFERENCES

1. Vineti Clinical Advisory Council. Vineti, Inc. 2021.
2. Evaluate Pharma, Vineti proprietary database. 2021.
3. Alliance for Regenerative Medicine. Cell & Gene State of the Industry Briefing. 2022.
4. McKinsey Life Sciences. COVID-19 and cell and gene therapy: How to keep innovation on track. 2020.
5. Bachanova V, Bishop MR, Dahi P *et al.* Chimeric Antigen Receptor T Cell Therapy During the COVID-19 Pandemic. *Biol Blood Marrow Transplant.* 2020. 1239–1246.

DOI: 10.18609/cgti.2022.035

6. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs): Guidance for Industry. Silver Spring, MD. The FDA. January 2020.

7. European Commission, EudraLex. The Rules Governing Medicinal Products in the European Union, Volume 4, Good Manufacturing Practice: Guidelines on Good Manufacturing Practice Specific to Advanced Therapy Medicinal Products. London, UK. The European Commission. November 2017.

8. U.S Department of Health and Human Services. Health Information Privacy. Accessed January 2022.

9. GDPR.EU. Complete Guide to GDPR Compliance. 2022.

10. Title 21–Food and Drugs, Chapter I–Food and Drug Administration, Department of Health and Human Services, Subchapter A–General, Part 11 - ELECTRONIC RECORDS; ELECTRONIC SIGNATURES. Silver Spring, MD. April 2020.

11. Evans, Melanie and McMillan, Robert. Cyberattacks Cost Hospitals Millions During Covid-19.

12. Mallempati, Raj. How the Pharmaceutical Industry Can Secure Networks to Avoid Cyberattacks. Forbes 2021 March 18.

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AUTHORSHIP & CONFLICT OF INTEREST

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-

REVIEW

Purification of therapeutic & prophylactic mRNA by affinity chromatography

Julian Grinsted, John Liddell, Emir Bouleghlimat, Ka Yan Kwok, Georgia Taylor, Marco P C Marques & Daniel G Bracewell

In vitro transcribed mRNA is an emerging therapeutic and prophylactic modality with the potential to transform medicine. The drug platform features exceptionally rapid development and versatility of manufacturing processes. Despite the prompt advancement of mRNA from trials to market, purification challenges remain. The cell-free synthesis of mRNA is responsible for the generation of product and process-related impurities, creating the potential for immunogenic effects and decreased translatability into the clinic. Affinity chromatography presents itself as an effective primary capture step for the isolation of functional transcripts from product and some process related impurities. Developing platform processes for the affinity purification of mRNA is hindered by the varying strand lengths of non-amplifying, self-amplifying, and trans-amplifying constructs, with disparities in capacity being observed. Ligand chemistries may contribute to non-specific binding events which remain challenging to characterise. Improved elution and wash conditions may be pursued through novel ligand chemistries, enhanced density and spacing. Regardless of the size or application of the product, the impurities generated by *in vitro* transcription represent a significant obstacle to the safe administration and long-term storage of mRNA. Affinity chromatography is a valuable tool in overcoming these challenges, with current commercially available products relying heavily on oligo deoxythymidine ligand chemistries. Whilst affinity chromatography is highly valuable in the purification of mRNA, the inability to separate key secondary structures such as double-stranded RNA means it remains to be seen if this technology will adopt the same position as protein A does in mAb manufacture.

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The COVID-19 pandemic has shown a rapid response from vaccine companies, manufacturing and delivering mRNA vaccines in record time. Unlike traditional vaccines which rely on complex and inflexible manufacturing processes, mRNA vaccines use the same vaccine backbone for multiple targets with only the expression of the gene of interest, allowing standardised manufacturing with reduced footprints (Table 1). This will enable the manufacturing of different mRNA vaccines using the same production platform. Furthermore, the facilities and manufacturing techniques can be applied to a variety of different products with varying applications, such as vaccines against infectious diseases, cancer immunotherapeutics and protein replacement therapies [1] (Figure 1). However, the global demand for COVID-19 vaccines has placed strain upon global manufacturing and supply chain problems are arising [2]. Therefore, new, or optimised processes are necessary to cope with increased demands of these vaccines. In particular, the purification of mRNA, where the *in vitro* production of mRNA has given rise to unique purification challenges such as low capacity and the removal of immunogenic impurities [3,4].

In 1990, mRNA molecules were successfully synthesised *in vitro* using free enzymes and expressed in mice to produce three proteins: chloramphenicol acetyltransferase, β-galactosidase, and luciferase [5]. Despite this initial success, DNA-based therapeutics were still preferred as mRNA is easily degraded by RNases present in cells and presents overall lower stability at ambient temperatures compared to DNA [6]. Inherently, mRNA based vaccines present safety advantages compared to DNA vaccines: the mRNA cannot interact with the cell genome; the mRNA consists solely of the elements needed for expression of the encoded protein; the mRNA decays within a couple of days and is non-replicative [7]; *in vivo* transfection rates are high due to the fact mRNA only has to cross the cell plasma membrane [8]; For these reasons multiple mRNA vaccine candidates such as the Pfizer-BioNTech BNT162b2 and the ModernaTX mRNA-1273 mRNA SARS-CoV-2 vaccines have been approved by regulatory bodies including the Medicines and Healthcare products Regulatory Agency (MHRA) and the US Food and Drug Administration (FDA) [9].

There are two main types of mRNA, non-replicating mRNA and self-amplifying mRNA (ssmRNA and saRNA, respectively). Both types possess structural similarities, including a 5' cap, 3' and 5' untranslated regions and a polyadenine tail (Poly (A)) [4]. The saRNA contains additional replicons to enable the mRNA to self-replicate, sequences of single-stranded RNA viruses from the genera *Alphavirus, Picornavirus* or *Flavivirus* [10]. These viruses contain a single-stranded, positive-sense genome and can contain regions coding for non-structural proteins (NSPs) (Figure 2A, C). Trans-amplifying mRNA (taRNA) is a new type of mRNA vaccine where the mRNA is split into two transcripts, one encoding a peptide of interest and another encoding virally derived replicative machinery. This system is distinct from saRNA, as

TABLE 1

Comparison of RNA synthesis techniques.

RNA synthesis technique	Advantages	Disadvantages
In vitro transcription	High levels of fidelity and rapid transcription of DNA template [56,57].	T7 polymerase is costly. As a result, some operations may require that the enzyme is manufactured on-site [58].
Oligonucleotide synthesis	The process is cheap and efficient for synthesising short sequences [59].	Only oligonucleotides up to 300 nucleotides long can be synthesised [60].
Cell-based synthesis	The DNA template does not need to be linearized prior to transcription [61].	Transfection of a host organism must occur with the template DNA. Extraction of RNA requires complex procedures [62].

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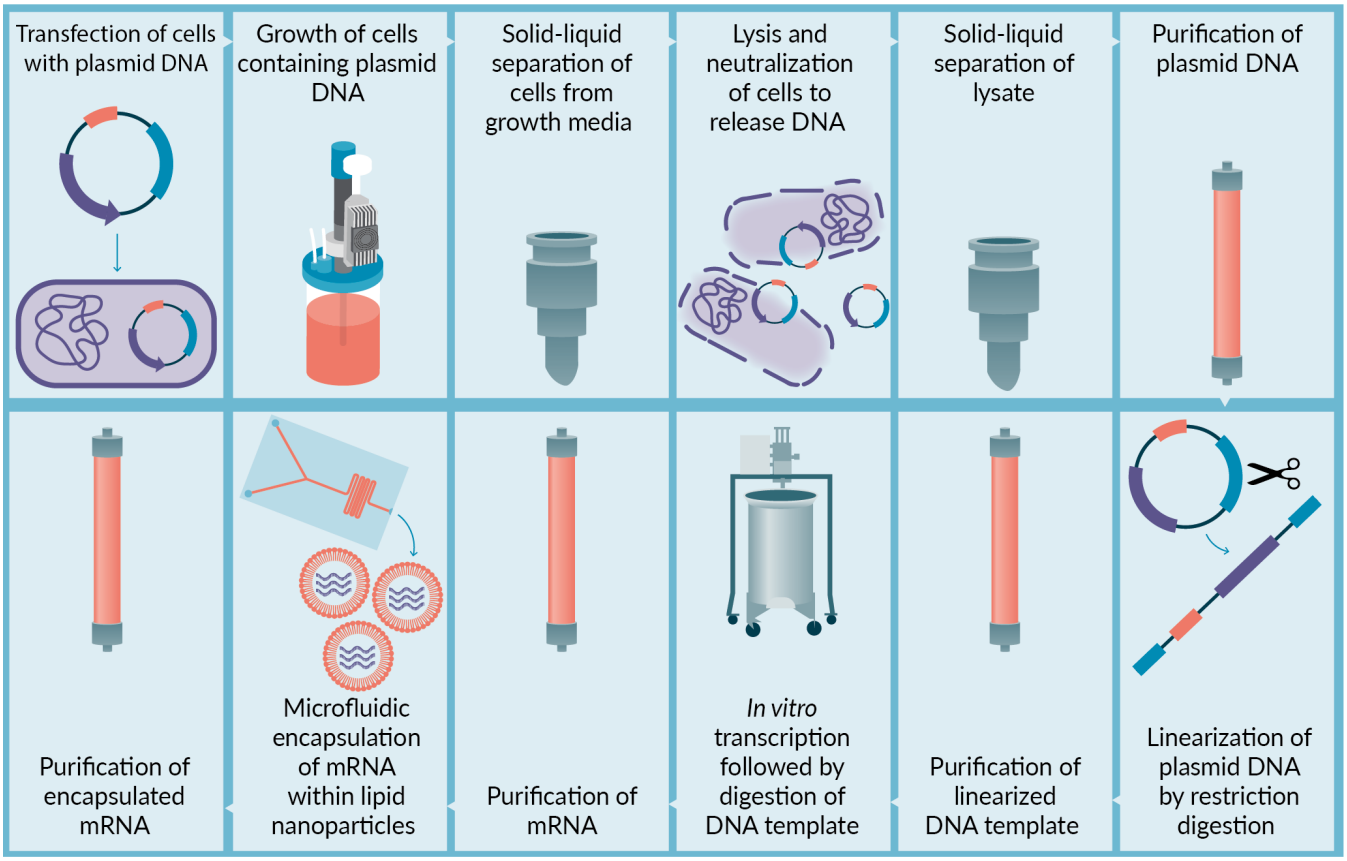
FIGURE 1

A flow diagram of a typical mRNA manufacturing process.



Adapted from [63].

saRNA contains both the gene of interest and replicase on the same strand. Individual taRNA strands are typically shorter than saRNA and are therefore easier to synthesise. Additionally, taRNA displays greater translational efficiency when compared to saRNA [11,12] (Figure 2B).

The mRNA is in transcribed preferentially in cell-free reactions (*in vitro transcription*, IVT) (Table 1) using polymerase enzymes and template DNA [13]. The predominant polymerase is the T7 (T7RNAP) [14], consisting of a single subunit and is highly processive, even in the absence of other transcriptional proteins [15]. T7RNAP exhibits high fidelity, allowing for accurate transcription [68]. In addition to these components, the IVT must also contain nucleotide triphosphates (NTPs), polymerase cofactors e.g., $MgCl_2$, polyamine containing buffer and antioxidants [12]. The IVT product yield ($mol_{mRNA}.mol_{pDNA}^{-1}$) and efficiency ($mol_{mRNA}.mol_{impurities/pDNA}^{-1}$), will have an impact on downstream processing steps. The removal of immunogenic product and process related impurities [16] are essential to ensure that mRNA-based prophylactic and therapeutic agents display acceptable levels of efficacy and safety [17].

IMPURITIES PRESENT WITHIN *IN VITRO* TRANSCRIBED mRNA

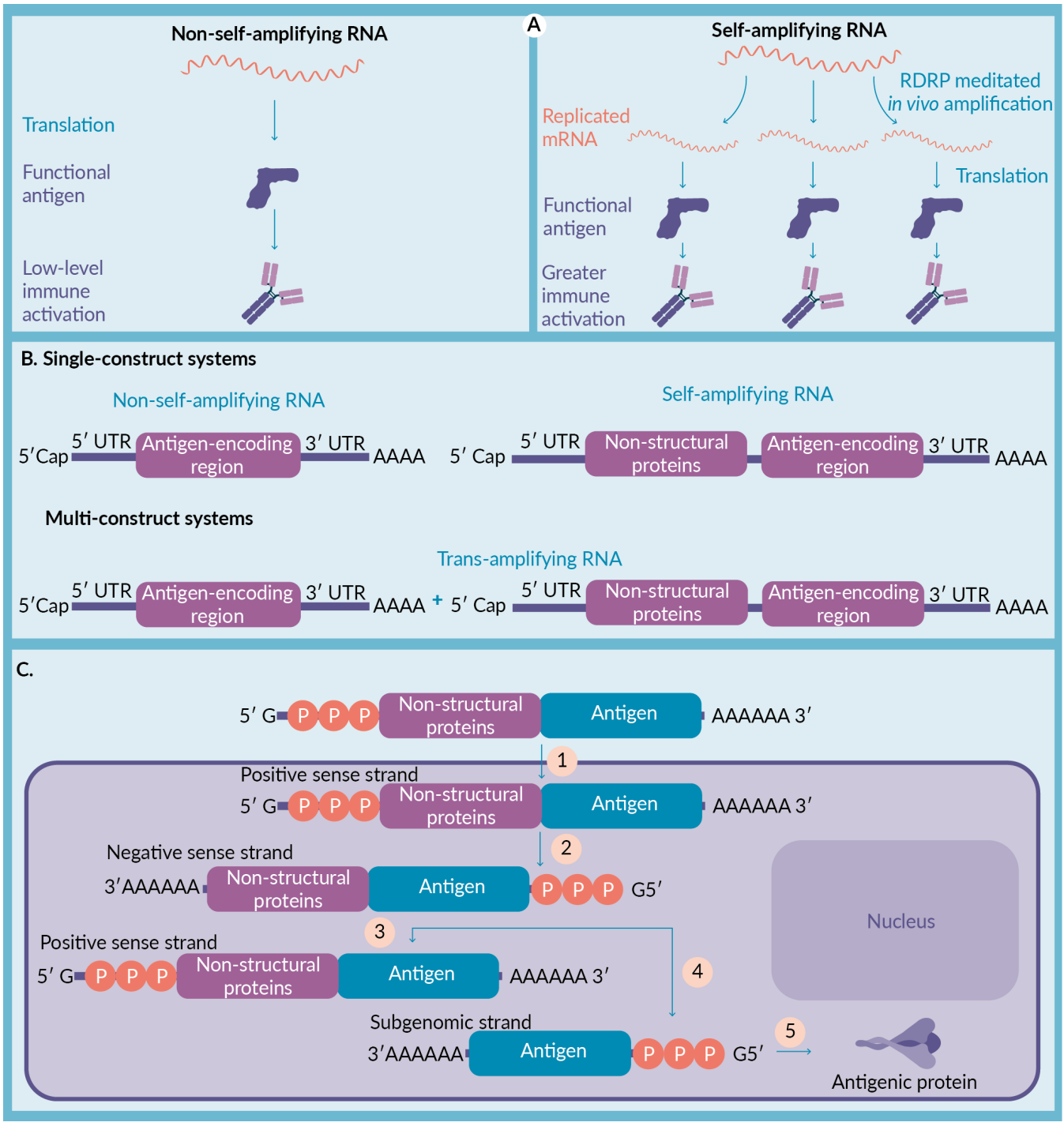
Process related impurities

Template DNA

A key concern surrounding template DNA is the potential for genomic integration if plasmids remain intact in the encapsulated mRNA and infiltrate the plasma membrane of cells upon administration [18]. In addition to the threat posed by large fragments, oligonucleotides produced from enzymatic

FIGURE 2

Different types of mRNA.



A: Self-amplifying RNA vaccines induce enhanced immune activation when compared to non-self-amplifying RNA vaccines. Adapted from [64].
B: Structural comparison of single construct systems (saRNA and ssmRNA) against multi-construct systems (taRNA) in the case of potential mRNA vaccinations. Adapted from [12,65].
C: In situ amplification of a self-amplifying RNA construct encoding an antigenic peptide. 1: Transfection of self-amplifying RNA into cell. 2: Transcription of positive-sense strand to create a negative sense strand. 3: Replication of original positive sense strand via transcription of negative sense strand. 4: Transcription of subgenomic region to create subgenomic strand encoding antigen of interest. 5: Translation of subgenomic strand to produce antigen. Adapted from [66].

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digestion of plasmid DNA (pDNA) may undergo base pairing with partial transcripts to form DNA-RNA hybrid fragments [19].

Plasmids which are produced by microbial fermentation may also contain endotoxins and proteins if they were not removed from the cellular lysate by chromatographic separations prior to IVT. Endotoxin, a lipopolysaccharide constituent of the outer membrane of gram-negative bacteria, has a section that is highly immunogenic (lipid A). Lipid A binds to myeloid differentiation factor 2 and toll-like receptor 4 on the cell surface, initialising signalling pathways, leading to cytokine release and inflammation. As a result of impurities arising from the cell-based synthesis of pDNA, purification steps are necessary prior to IVT [20]. One possible approach to simplify the required purification is the cell-free synthesis of the template DNA [21].

RNA polymerase

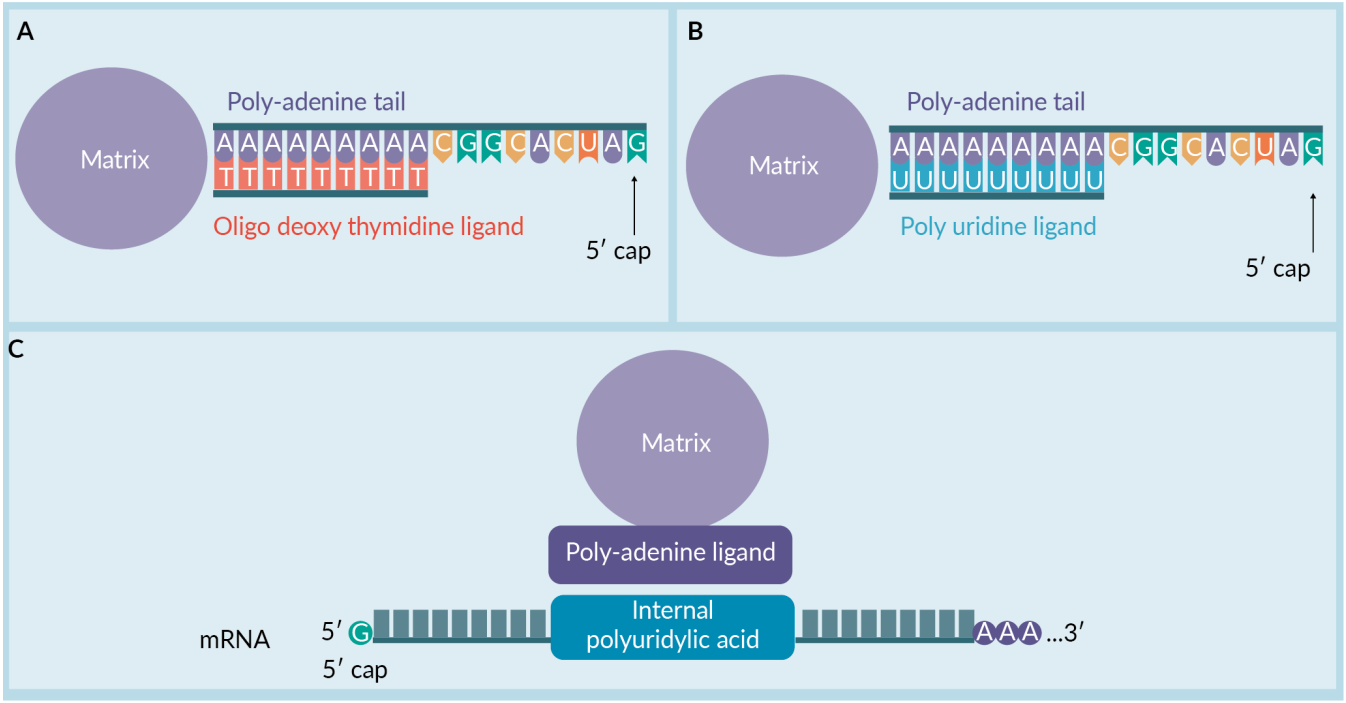
RNA polymerases, primarily T7, but T3 and SP6 may also be used during IVT will remain in solution unless removed. RNA polymerases are produced through cell-based synthesis and may therefore contain endotoxins. Polymerases may be recognised as foreign antigens upon the binding of complementary antibodies, inducing pro-inflammatory cytokines as part of an adaptive immune response and leading to inflammation [22,23].

Free nucleoside triphosphates

Nucleoside triphosphates that are not incorporated into mRNA during IVT may remain in solution. These free nucleotides may activate neuroinflammatory mechanisms within the central nervous system. The free nucleosides can act as agonists by binding to purinergic receptors (P2), classed into two broad categories: P2X and P2Y. P2X are a group of cation channels which selectively bind adenosine triphosphate while P2Y receptors bind adenosine and uridine triphosphate. A diverse range of P2 receptor types are present in the plasma membranes of macrophages, glial cells, and oligodendrocytes [24].

FIGURE 3

Polyadenine tail of mRNA immobilised upon binding.



(A) Polyuridine, (B) oligo deoxythymidine and (C) polyadenine. Adapted from [48,50,67].

Product-related impurities

DNA–RNA hybrid fragments

During IVT, RNA synthesis occurs from the 5' to 3' end with synthesis typically beginning at the T7 promoter region. The polymerase, exhibiting high levels of processivity, will continuously transcribe template DNA in cases where the template is not linearised. This continuous transcription may lead to the formation of excessively long transcripts. Linearization by restriction digestion is therefore mandatory to prevent the formation of these long transcripts, providing that suitable restriction sites exist within the construct (Figure 1) [25].

The digestion of linearised DNA creates oligonucleotide fragments, which may undergo base pairing with RNA fragments which are generated as side products of IVT. The association of these fragments with one another leads to the formation of impurities known as DNA-RNA hybrid fragments. The risks of genomic integration, associated with DNA impurities, warrant the removal of hybrid fragments. In addition, the ssRNA component is associated with activation of toll-like receptors 7 and 8, leading to interferon release [18,26]. The fragments may be removed from the IVT mixture using downstream separation techniques, or alternatively can undergo enzymatic digestion when the deoxyribonuclease, DNase1, is added [19].

Partial transcripts

Incomplete RNA transcripts are generated as a by-product during IVT where during transcription initiation, abortive synthesis events occur. As a result, the RNA polymerase produces short mRNA fragments from the template DNA, between 5 and 11 nucleotides long [3]. ssRNA, including partial transcripts, can be detected by toll-like receptors (TLR) 7 and 8. Upon activation, TLR 7 and 8 can induce the release of type 1 interferon. Additionally, nuclear factor kappa B may be activated, as with the activation of RIG1 and MDA5 in the presence of dsRNA [26].

Double-stranded RNA

Double-stranded RNA (dsRNA) may be formed when partial transcripts, formed from abortive transcriptional events, bind to mRNA, and prime the association of transcriptional apparatus with the mRNA. This induces complementary strand synthesis downstream of the site of the transcriptional apparatus binding [16]. A second mechanism of dsRNA synthesis arises from the production of antisense RNA fragments. These fragments are transcribed from the non-coding DNA strand which is found on double-stranded DNA templates. The annealing of antisense fragments to mRNA can occur through the pairing of complementary base sequences. This leads to the generation of dsRNA [16].

dsRNA removal to very low levels from feed material is necessary because the molecule is highly immunogenic [3]. This is illustrated by the molecules ability to induce a cytokine storm in some cases [27,28]. Despite its immunogenicity, dsRNA holds natural biological purposes within human cellular nuclei [29]. However, the entry of dsRNA into

TABLE 2

Dynamic binding capacities of existing oligo (dT) products.

Product name	Product type	Quoted dynamic binding capacity (mg.mL-1)
Praesto™ Jetted (dT)18-DVB	Beaded chromatography resin	2 (200nt poly (A)) [40]
Poros™ (dT)25	Beaded chromatography resin	0.62 (40nt poly (A)) 4 (2000nt mRNA) 3 (3000nt mRNA) [41]
Dynabeads™ (dT)25	Magnetic beads	0.05 [38]
Sera-Mag™ (dT)14	Magnetic beads	0.11 [39]
CIMmultus™ oligo (dT)18	Monolith	0.18 (2000nt mRNA) [43,44,45]

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the cytosol may induce apoptosis due to its association with viral material [30].

Occurring in all human cells, MDA5 and RIG1 are intracellular receptors. Pathogen associated molecular patterns (PAMPs) can activate MDA5 and RIG1. dsRNA is a PAMP and is often released into the cytosol during viral infection [31]. MDA5 and RIG1 bind differing sizes of dsRNA. Longer strands are bound internally by MDA5, whilst shorter strands are bound at the 5' phosphorylated ends by RIG1 [32,33]. Whilst different sized fragments activate the two receptors, there is overlap in the corresponding signalling pathways. Interferon 1 expression is up-regulated by both MDA5 and RIG1 [31]. A mechanism has also been identified which is dependent on nuclear factor kappa B to stimulate the release of proinflammatory cytokines. Due to the overlapping activities of MDA5 and RIG1, a wide variety of dsRNA strand sizes can be detected through these innate sensing mechanisms [33].

RNase L release within cells is induced by the activation of oligoadenylate synthetase in the presence of dsRNA. Degradation of mRNA may occur in the presence of RNase L. This degradation leads to an inhibition in the translation of mRNA. This mechanism suggests that the removal of dsRNA may contribute to increased levels of mRNA expression. RNase L is also able to cleave dsRNA. The resulting double-stranded fragments may activate intracellular receptors, Melanoma Differentiation Associated Protein 5 (MDA5) and Retinoic Acid Inducible Gene 1 (RIG1) [31].

AFFINITY CHROMATOGRAPHY

Introduction to affinity chromatography

Affinity chromatography was, and continues to be, ubiquitously utilised in the industrial purification of antibodies as a capture step. This is due to its rapid and selective nature [34]. These qualities are also observed in the case of mRNA purification, and it is a highly reliable and consistent primary capture step. The technique supports the use of aqueous buffers and elution may be achieved by simply reducing the salinity of the mobile phase [35]. The technique does not require organic solvents such as acetonitrile in the mobile phase, avoiding the flammability hazard and environmental impact of waste which is produced when compared to reversed phase chromatography [27]. Additionally, mRNA does not require a dedicated tagging step, due to the presence of a poly(A) tail (Figure 2B) [4].

Sodium chloride is used to increase the ionic strength shielding the charge on the ligand and RNA thus allowing the ligand and RNA to bind to each other through base pairing hydrogen bonds. After mRNA is bound to the ligand the salt is removed establishing the original charge repulsion between the ligand and mRNA. This method allows RNA to unbind from the ligand and be eluted and is known as hybridisation affinity chromatography [27]. The 3' poly(A) tail found on mRNA creates an opportunity for purification by base pair affinity chromatography. Additionally, mRNA can be isolated from transfer and ribosomal RNAs, which do not possess a polyadenine tail [36]. Different affinity ligands can be used in the purification of mRNA, such as oligo deoxythymidine, polyadenine and polyuridine (Figure 3).

Purification of mRNA by affinity chromatography has been shown to enhance the stability of the drug substance. This is due to the high levels of impurity removal that can be achieved. This stability is greater than that of mRNA purified through sedimentation. As a result, affinity chromatography is beneficial to the preservation mRNA as well as patient safety [37].

Oligo deoxythymidine

To effectively tackle the bottlenecks associated with large scale mRNA purification and meet the growing global demand, commercially available products are being developed. Novel products would ideally be compatible

with current chromatography platforms, with the current range of oligo deoxythymidine (dT) products aiming to address the low capacity associated with mRNA affinity chromatography (Figure 3A) [12].

Despite rapid development, commercial options for oligo (dT) products are still in their infancy. The main products on the market currently include: Poros (dT)25 and Dynabeads™ (dT)25 by Thermofisher; the CIMmultus™ Oligo (dT)18 monolith and Sera-Mag™ (dT)14 by Cytiva; and Praesto Jetted (dT)18-DVB by Purolite. Both Dynabeads™ (dT)25 and Sera Mag (dT)14 are coated, 1 µm magnetic beads with (dT) ligand coupled onto the surface. The beads boast the high yields and specificity associated with (dT) affinity purification; Dynabeads (dT) has a binding capacity of 10 $\mu g.mg_{resin}^{-1}$, equivalent to 50 $\mu g.mL_{resin}^{-1}$, whereas Sera-Mag (dT) quotes a capacity of 11 $\mu g.mg_{resin}^{-1}$, equivalent to 110 $\mu g.mL_{resin}^{-1}$ [38,39]. The products are ideal for the small-scale purification of polyadenylated mRNA to be used in laboratory techniques, such as RT-PCR and cDNA synthesis. The key benefit of utilising magnetic beads is that the beads can be easily isolated from the supernatant by applying a magnetic field. However, these beads are unsuitable for most large-scale purification platforms as most major purification platforms revolve around a form of fixed bed column chromatography where a packed bed, monolith or membrane would be used. The 1µm bead size would cause large backpressure if used in a packed bed and is unlikely to be considered in industrial applications, except for fluidised bed systems. Table 2 summaries the capacities of existing oligo (dT) products quoted by manufacturers.

The main options for industrially appropriate oligo (dT) products are the Praesto Jetted (dT)18-DVB, Poros (dT)25 and CIMmultus (dT)18 monolith. The first two are resin technologies that utilise a divinyl benzene base matrix and affix the (dT) ligand to the surface with a proprietary linker. Purolite have released a binding capacity of 2 $mg.mL_{resin}^{-1}$ of 200 nt Poly(A) compared to 0.62 $mg.mL_{resin}^{-1}$ of 40 nt Poly(A) on a Poros (dT)25 certificate of analysis [40,41] The Poros (dT) displays 10% breakthrough values of 4 and 3 $mg.mL_{resin}^{-1}$ capacity for 2,000 and 3,000 nt mRNAs, respectively, whilst 1,000 nt mRNAs show a 5% breakthrough of 4 $mg.mL_{resin}^{-1}$ [42]. A clear correlation between mRNA size and capacity is observed, with Poros (dT)25 having lower capacity for larger mRNAs. This indicates that surface crowding is preventing the full utilisation of the bound (dT) ligand. Despite the reduced capacity for larger mRNAs, the resin can be reused for 10 cycles with only a marginal drop in yield.

Commercially available monoliths include the CIMmultus (dT)18 range from BIA Separations. These are Poly glycidyl methacrylate-co-ethylene dimethacrylate monoliths where (dT)18 is immobilised with a C6 or C12 linker chain. The product exhibits a ligand density of 0.5 $mg_{Oligo\ (dT)}.mL_{wet\ support}^{-1}$. There is currently no available data for capacity with any length of mRNA [43,44]. However, a 1 mL CIMmultus™ Oligo (dT) is capable of an 80% recovery when purifying an IVT mixture containing approximately 180µg 2000 nt mRNA, calculating to 0.18 $mg_{mRNA}.mL_{support}^{-1}$ [45]. Whilst the raw numbers for the monolith don't compare well to the packed bed resin alternatives, the pre-prepared nature of the monolith is advantageous for industrial customers who favour a "plug and play" option, when available. Additionally, monolith separations can be completed in a shorter space of time due to the higher rates of convective flow.

Comparing existing products will remain challenging until capacity data for a wide range of mRNA constructs is released. The (dT)18 ligand present on the Praesto Jetted (dT)18-DVB indicates a capacity somewhat like Poros (dT)25, whereas the CIMmultus (dT) displays a lower capacity than Poros (dT)25. The comparison becomes difficult when accounting for differences in the resin and monolith technology. Each technology presents options for mRNA purification at an industrial scale. A second generation of products is required to further push the boundaries in capacity.

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New options could include other base materials, such as agarose. Agarose (dT)20 was prepared using NHS activated Sepharose FF. This achieved a 1.6 mg.mL$_{resin}^{-1}$ capacity with a 900 nt polyadenylated mRNA [46]. However, this could be indicative of the unsuitability of agarose as a base matrix at relatively large pore sizes, given that no agarose products are yet commercially available.

Polyuridine

A capture modality somewhat akin to oligo (dT), polyuridine (poly(U)) targets the poly(A) tail of mRNA for capture via hybridisation (Figure 3B). The ligand consists of a chain of uridine nucleotides and may be immobilised on a medium such as Sepharose. Phillips et al [47] demonstrated high levels of binding specificity between poly(U) and poly(A) containing mRNA. Over 90% of binding in poly(U) agarose columns was complementary. Non-complementary binding was 3% or lower. Only poly(A) tails effectively initiated binding, with internal and non-sequential adenylated sequences not binding to the columns. The technique may be particularly valuable in isolating mRNA with a short poly(A) tail, as only 10 nucleotides in the tail were sufficient for binding to occur to a detectable extent. This suggested a high binding affinity between the target and ligand. This binding frequency increased up to 25 nucleotides, after which binding was independent of poly(A) length.

The high binding affinity of poly(U) presents itself as an advantageous characteristic for the purification of mRNA. However, this property may prevent its implementation in many cases. Berman, Gornaeva and Mazurov [48] showed that an irreversible and non-specific binding of RNA occurred when poly(U) was used on a Sepharose matrix. Strong adsorption of the target may require the use of extreme elution conditions and the addition of compounds which counteract the effects of non-specific binding. Ochoa, Kempf and Egly [49] demonstrated that poly(A) RNA does not exhibit significant binding affinity for Sepharose in the absence of poly(U) when comparable conditions are provided. This suggests that non-specific binding may arise from the ligand itself, or from structures related to the functionality of said ligand. SDS, an anionic surfactant, was shown to be highly effective at eluting poly(A) mRNA from poly(U). This suggested that hydrophobic interactions could be attributed in part to non-specific binding between the target and immobilised ligand.

Chaotropic salts may be an ineffective constituent of elution buffers in the case of poly(U), as their use does not guarantee effective unbinding. Additionally, the potential for the formation of secondary structures in their presence exists [49]. It is unclear why disparities exist in the frequency of non-specific binding events between studies. The exact contributions of binding mechanisms to the unfavourable elution requirements of poly(U) have not yet been fully ascertained. A potential future approach to irreversible adsorption of the target may involve reducing the overall ligand length so that nonspecific binding events are reduced.

Polyadenine

An alternative and seldom utilised mode of affinity separation applies a polyadenine ligand to capture RNA fragments containing internal polyuridine sequences (Figure 3C). This ligand is distinct from both oligo (dT) and poly(U), as separation does not rely on interactions with the poly(A) tail found on mammalian mRNA [50].

Poly(A) is only effective at isolating mRNAs with oligo U sequences. This suggests that some targets may not be suitable for capture by this step as they may lack the necessary poly(U) sequences. Poly(U) sequences are not ubiquitous in naturally occurring cytoplasmic mRNA and approximately 20% of poly(A) containing mRNA also contains internal oligo(U) sequences. The poly(A) tail found on mRNA presents a more broadly applicable

purification opportunity than internal poly(U) sequences if oligo(U) sequences are not deliberately inserted when producing synthetic mRNA [51]. Polyadenine may emerge as an effective method for the isolation of mRNA targets containing internal poly(U) sequences. However, it is not currently utilised at industrial scales for the purposes of therapeutic or prophylactic mRNA purification.

Elution conditions

Extremes in pH or chaotropic agents are capable of disrupting hydrogen bonding and causing elution [52]. Binding affinity between the target and immobilised ligand is affected by both the pH and salinity of the buffer solution. Highly acidic or alkaline conditions induce disruption of hydrogen bonds, therefore reducing the binding affinity between complementary bases. However, mRNA may incur damage under extreme pH values. Cleavage of phosphodiester bonds in RNA is probable at pH > 6 (alkaline hydrolysis) and pH < 2 (acid hydrolysis) [53]. Existing affinity products typically elute mRNA at a close to neutral pH, instead relying on a lowered salt concentration to induce unbinding [38–45].

Binding affinity between bases increases with salinity. Association of positive ions with phosphate groups present on the mRNA has a stabilising effect. This is because the repulsion between the negative phosphate groups is reduced [35].

LIMITATIONS OF AFFINITY CHROMATOGRAPHY

Affinity chromatography as provided by ligands like oligo (dT) provides a method of selectively binding mRNA molecules which contain a 3' poly(A) tail. The poly(A) tail is required to reduce mRNA *in vivo* degradation rates to make effective therapies. This separation method allows mRNA with a poly(A) tail to be isolated from IVT related impurities, including excess nucleosides, residual enzymes, excess capping reagents and buffer components. It will also only separate mRNA molecules with a poly(A) tail so that incomplete transcripts lacking the poly(A) tail, required for *in vivo* stability, are not bound by the media. Oligo (dT) affinity chromatography will not provide a method to separate mRNA species lacking a 5' cap (required to avoid innate immune system activation). Also, it will not separate double-stranded RNA formed through reactions discussed earlier. Double-stranded mRNA is an important critical quality attribute for mRNA, therefore, further polishing separations are required to meet specifications necessary for dsRNA removal.

As well as these quality attribute limitations for oligo (dT) chromatography, there are chromatographic limitations. mRNA molecules as discussed above are large (approximately 4000nt) and in the case of saRNA very large (>10,000nt). These are species with molecular weights of approximately 2MDa–5MDa with the same dimensional range as virus particles. As such, the poly(A) tail utilized in binding an affinity ligand is a small component of a large particle. Hence binding kinetics have the potential to be slow due to steric factors. Binding capacity may be limited, requiring a large volume of affinity media to purify a given amount of mRNA. This will apply particularly to beaded media where mRNA is likely to only bind to surface ligands and very little adsorption occurs to ligands contained within pores. Alternative stationary phase design – membranes, monoliths or nanofibers are likely to achieve higher binding capacities by increasing mRNA access to ligands. A further limitation arises from the need to increase solution ionic strength to achieve binding. Salt precipitation is itself a viable method of separating mRNA and hence there is a fine balance between precipitating mRNA and promoting oligo (dT) ligand binding [3].

Alternatives to affinity chromatography need to consider the two factors discussed above – achieving critical quality attributes in terms of control of product related impurities and the potential for low capacities in bind and elute chromatography. Achieving both these objectives with a single approach is currently difficult. Control of product related

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impurities such as dsRNA has been described using reversed phase high performance chromatography (RP-HPLC) [54]. While this has been shown to be effective in control of dsRNA levels, the approach uses beaded media which will have capacities limited by accessible surface area and requires the use of acetonitrile in the mobile phase.

Effective removal of process related impurities can be achieved by flow-through chromatography. The use of media combining a surface layer, preventing large molecules like mRNA entering the pores of a core containing mixed mode or hydrophobic media has been described using media such as Cytiva CaptoCore chromatography resin [55]. As a flow through media there are no limitations presented by the binding of the mRNA, the limitations are provided by the impurity species. While removal of transcriptional impurities can be achieved, such an approach is unlikely to control levels of dsRNA, which will also flow through.

The inability of affinity chromatography to effectively separate product-related impurities from the target creates the requirement for polishing steps. With each additional unit operation, the overall yield of mRNA is reduced, and process efficiency decreases. It is therefore economically beneficial to utilise separation techniques which distinguish between product-related impurities with high resolution. Yield varies between mRNA constructs and buffer composition, however, CIMmultus (dT)18 is quoted at approximately 80% and Praesto Jetted (dT) 18-DVB at approximately 60% [43,70]. Over-reliance on the poly(A) tail as a basis of separation ignores the potential formation of secondary structures, DNA-RNA hybrid fragments and partial transcripts which may display internal or external poly(A) sequences [3].

CONCLUSIONS & FUTURE PERSPECTIVES

mRNA is a therapeutic and prophylactic modality with the potential for rapid development, cell-free manufacture, and stability once highly purified. Impurities generated through *in vitro* transcription, the dominant synthesis technique, are categorised as process and product related. The removal of both sets of impurities is essential to the safety, functionality, and stability of mRNA in a clinical setting. Critical quality attributes such dsRNA must be removed to suitably low levels to comply with the relevant regulatory guidance. When developing new products, the market authorisation holder may be required to carry out assays such as immunoblotting to prove that impurity species are below detectable levels. Immunoblotting was required by the European Medicines Agency to verify the removal of dsRNA from the BNT162b2 (COMIRNATY®) COVID-19 vaccine [69].

Affinity chromatography is an attractive primary capture step for the removal of product and process related impurities, but current approaches struggle to separate the target from structurally similar product related impurities. The benefits of affinity chromatography include purification at ambient temperatures, the use of aqueous buffers in the mobile phase and elution with a simple salt gradient. mRNA affinity chromatography in industry relies almost exclusively on oligo deoxythymidine ligand chemistries, however, polyuridine and polyadenine ligands have both been implemented at the bench scale.

Oligo deoxythymidine products include beaded resins, monoliths, membranes, and magnetic particles. Polyuridine may be susceptible to strong non-specific adsorption due to hydrophobic interactions, although this may be addressed through alterations in buffer composition. Polyadenine as an immobilised ligand is only applicable to targets with internal uridine sequences and does not rely on the 3' poly adenine tail of mRNA, unlike other modes.

These mRNA affinity-based methods have not yet become the default capture step protein A affinity chromatography is for mAb products. The relatively simple range of process impurities from upstream IVT and the large size of mRNA indicates that options such tangential flow filtration may compete. Equally there is potential to develop novel

affinity ligands to improve capacity, and ideally selectivity for product related impurities. It is also likely there is value in pursuing novel matrix materials that allow greater accessibility to ligands for relatively large products such as mRNA.

Further, as novel mRNA targets and modalities emerge, affinity chromatography will need to adapt to the array of strand lengths and associated impurities which will be encountered during purification. Capacities are typically found to be greater for smaller constructs, interactions between the target and ligand must be characterised and mRNA secondary structures identified to improve media for larger constructs. Perhaps most importantly effective purification also requires removal of product-related impurities, particularly dsRNA due to their immunostimulatory effects.

REFERENCES

1. Jackson N, Kester K, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. npj *Vaccines*. 2020; 5
2. Bown C, Bollyky T. How COVID-19 vaccine supply chains emerged in the midst of a pandemic. *World Econ*. 2021; 2021; 45, 468.
3. Baiersdörfer M, Boros G, Muramatsu H et al. A Facile Method for the Removal of dsRNA Contaminant from in vitro-Transcribed mRNA. *Mol. Ther. Nucleic Acids* 2019; 2020;15, 26–35.
4. Kowalski P, Rudra A, Miao L, Anderson D. Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. *Mol. Ther.* 2019; 27, 710–728.
5. Wolff J, Malone R, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. *Science* 1990; 247, 1465–1468.
6. Suschak J, Williams J, Schmaljohn C. Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity. *Hum. Vaccin. Immunother.* 2017; 13, 2837–2848.
7. Jäschke A, Helm M. RNA Sex. Chem. Biol. 2003; 10, 1148–1150.
8. Yamamoto A, Kormann M, Rosenecker J, Rudolph C. Current prospects for mRNA gene delivery. Eur. J. Pharm. Biopharm. 2009; 71, 484–489.
9. Moore J, Millar B. Improving COVID-19 vaccine-related health literacy and vaccine uptake in patients: Comparison on the readability of patient information leaflets of approved COVID-19 vaccines. J. Clin. Pharm. Ther. 2021;46, 1498–1500.
10. Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and Challenges in the Delivery of mRNA-Based *Vaccines*. Pharmaceutics 2020; 12(2): 102.
11. Beissert T, Perkovic M, Vogel A et al. A Trans-amplifying RNA Vaccine Strategy for Induction of Potent Protective Immunity. *Mol. Ther.*2020; 28(1): 119.
12. Rosa S, Prazeres D, Azevedo A, Marques M. mRNA vaccines manufacturing: Challenges and bottlenecks. *Vaccine* 2021; 39(16): 2192.
13. Beckert B, Masquida B. Synthesis of RNA by in vitro Transcription. *RNA* 2010; 703: 29.
14. Conrad T, Plumbom I, Alcobendas M, Vidal R, Sauer S. Maximizing transcription of nucleic acids with efficient T7 promoters. *Commun. Biol.* 2020; 3(1): 1.
15. Borkotoky S, Murali A. The highly efficient T7 RNA polymerase: A wonder macromolecule in biological realm. *Int. J. Biol. Macromol* 2018; 118(1): 49.
16. Wu M, Asahara H, Tzertzinis G, Roy B. Synthesis of low immunogenicity RNA with high-temperature in vitro transcription. RNA 2020; 26(3): 345–360.
17. Weissman D, Pardi N, Muramatsu H, Karikó K. HPLC Purification of in vitro Transcribed Long RNA. *Methods Mol. Biol* 2013; 969: 43.
18. Xia J, Lu G, Lu J, Zhang J, Feng L, Wang B. Towards an effective mRNA vaccine against 2019-nCoV: demonstration of virus-like particles expressed from a modified mRNA cocktail. *ChinaXiv* 2020; : 2.
19. Stöcher M, Berg J. Removal of template DNA from cRNA preparations by combined oligo(dT) affinity chromatography and DNase I digestion. *BioTechniques* 2004; 36(3): 480.
20. Bohannon J, Hernandez A, Enkhbaatar P, Adams W, Sherwood E. The

DOI: 10.18609/cgti.2022.049

Immunobiology of Toll-Like Receptor 4 Agonists. *Shock* 2013; 40(6): 451.

21. Adie T, Orefo I, Kysh D et al. dbDNA™: An advanced platform for genetic medicines. Drug Discovery Today 2022; 27(2): 374.

22. Chaplin D. Overview of the immune response. Journal of Allergy and Clinical Immunology 2010; 125(2): 3–23.

23. Green M, Sambrook J. Synthesis of Single-Stranded RNA Probes by in vitro Transcription. *Cold Spring Harb. Protoc.* 2020; 2020(1).

24. Weisman G, Camden J, Peterson T, Ajit D, Woods L, Erb L. P2 Receptors for Extracellular Nucleotides in the Central Nervous System: Role of P2X7 and P2Y2 Receptor Interactions in Neuroinflammation. *Mol. Neurobiol.* 2012; 46(1): 96–113.

25. Ohanube G, Obeta U. Generation of mRNA Vaccine: An Analysis of Two Types of Vectors. International Journal Of Pharmaceutical And Phytopharmacological Research 2021; 11(4): 25.

26. Bender A, Tzvetkov E, Pereira A et al. TLR7 and TLR8 Differentially Activate the IRF and NF-κB Pathways in Specific Cell Types to Promote Inflammation. *ImmunoHorizons* 2020; 4(2): 93.

27. Gagnon P, Goričar B, Peršič Š, Černigoj U, Štrancar A. Two new capture options for improved purification of large mRNA. *Cell and Gene Therapy Insights* 2020; 6(7): 1035.

28. Kasumba D, Grandvaux N. Therapeutic Targeting of RIG-I and MDA5 Might Not Lead to the Same Rome. Trends in Pharmacological *Science*s 2019; 40(2): 117.

29. Portal M, Pavet V, Erb C, Gronemeyer H. Human cells contain natural double-stranded RNAs with potential regulatory functions. *Nat. Struct. Mol. Biol.* 2014; 22(1): 89.

30. Gantier M, Williams B. The response of mammalian cells to double-stranded RNA. *Cytokine & Growth Factor Rev.* 2007; 18(6): 363.

31. Brisse M, Ly H. Comparative Structure and Function Analysis of the RIG-I-Like Receptors: RIG-I and MDA5. *Front. Immunol.* 2019; 10(1): 3.

32. Reikine S, Nguyen J, Modis Y. Pattern Recognition and Signaling Mechanisms of RIG-I and MDA5. *Front. Immunol.* 2014; 5: 342.

33. Saito T, Gale M. Differential recognition of double-stranded RNA by RIG-I–like receptors in antiviral immunity. *J. Exp. Med.* 2008; 205(7): 1524.

34. Arora S, Saxena V, Ayyar B. Affinity chromatography: A versatile technique for antibody purification. Methods 2017; 116: 84.

35. Farrell R. Isolation of Polyadenylated RNA. RNA Methodologies 2017; 5: 527–544.

36. Slater R. The Purification of Poly(A)-Containing RNA by Affinity Chromatography. Nucleic Acids; 2: 117.

37. Korenč M, Mencin N, Puc J et al. Chromatographic purification with CIMmultus™ Oligo dT increases mRNA stability. Cell and Gene Therapy Insights 2021; 7(9): 1207.

38. ThermoFisher Scientific. Dynabeads™ Oligo (dT)25 [Internet]. 1st ed. ThermoFisher Scentific; 2020. p. 1–2.

39. 39. Cytiva. Sera-Mag Oligo(dT)-Coated Magnetic Particles [Internet]. 1st ed. Cytiva; 2020. p. 1–2.

40. Purolite. Praesto Jetted (dT)18- DVB [Internet]. 1st ed. Purolite Ltd; 2022. p. 1–2.

41. ThermoFisher Scientific. POROS™ Oligo (dT)25 Affinity Resin [Internet]. 1st ed. ThermoFisher Scientific; 2020. p. 1–8.

42. Flook K. Supporting development of mRNA-based therapies by addressing large-scale purification challenges. Cell and Gene Therapy Insights 2021; 7(5): 489–502.

43. BIA Separations. CIMmultus™ Oligo dT18 1 mL Monolithic Column (C6 Linker) (2 µm channels) [Internet]. 1st ed. BIA Separations; 2020. p. 1.

44. BIA Separations. BIA Separations, 2020. CIMmultus™ Oligo dT18 1 mL Monolithic Column (C12 Linker) (2 µm channels). 1st ed. BIA Separations; 2020. p. 1.

45. BIA Separations. Purification of messenger RNA by affinity chromatography on CIMmultus™ Oligo (dT) column [Internet]. 1st ed. BIA Separations; 2020; 2.

46. Issa, W. J.; Aunins, J. G.; Bancel, S. Ribonucleic Acid Purification. WO2014152031A1, September 25, 2014.

47. Phillips L, Pang R, Park J, Hollis V, Famuyiwa F. Poly(U)-Agarose Affinity Chromatography: Specific, Sensitivity Selectivity, and Affinity of Binding. Preparative Biochemistry 1980; 10(1): 11.

48. Berman A, Gornaeva N, Mazurov V. Biospecific chromatography of poly(A)-containing RNA on poly(U)-Sepharose. Biokhimiia 1978; 43(10): 1830.

49. Ochoa J, Kempf J, Egly J. Interaction of poly(A) with different adsorbents for affinity chromatography of nucleic acids. *Int. J. Biol. Macromol* 1980; 2(1): 33–38.

50. Wood W, Edmonds M. A method for isolation of oligo(uridylic acid)-containing messenger ribonucleic acid from Hela cells. Biochemistry 1981; 20(19): 5359.

51. Korwek E, Nakazato H, Venkatesan S, Edmonds M. Poly(uridylic acid) sequences in messenger ribonucleic acid of HeLa cells. *Biochemistry* 1976; 15(21): 4643.

52. Farrah S, Shah D, Ingram L. Effects of chaotropic and antichaotropic agents on elution of poliovirus adsorbed on membrane filters. *PNAS* 1981; 78(2): 1229–1232.

53. Bernhardt H, Tate W. Primordial soup or vinaigrette: did the RNA world evolve at acidic pH?. *Biol. Direct.* 2012; 7(1): 4.

54. Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res.* 2011; 39(21): 142.

55. Berlanda S, Wen Y, Geall A, Porter F. RNA Purification Methods. WO 2014/140211, 2018.

56. Reid-Bayliss K, Loeb L. Accurate RNA consensus sequencing for high-fidelity detection of transcriptional mutagenesis-induced epimutations. Proceedings of the National Academy of *Science*s 2017; 114(35): 9415–9420.

57. Ali M. Efforts to Develop Rapid Technology of in vitro Transcription and Translation in Vaccine Synthesis in Indonesia. Indonesian Bulletin of Animal and Veterinary *Science*s 2016; 25(4): 1.

58. Gallo S, Furler M, Sigel R. in vitro Transcription and Purification of RNAs of Different Size. CHIMIA *Int. J. Chem.* 2005; 59(11): 813.

59. 59.de Koning M, Ghisaidoobe A, Duynstee H, Ten Kortenaar P, Filippov D, van der Marel G. Simple and Efficient Solution-Phase Synthesis of Oligonucleotides Using Extractive Work-Up. *Org. Process Res. Dev.* 2006; 10(6): 1238.

60. Song L, Deng Z, Gong Z, Li L, Li B. Large-Scale de novo Oligonucleotide Synthesis for Whole-Genome Synthesis and Data Storage: Challenges and Opportunities. *Front. Bioeng.* 2021; 9: 2.

61. Štrancar A. High yield mRNA production process from E.Coli to highly pure mRNA. Presentation presented at; 2020; *Bio Process International.*

62. Tinafar A, Jaenes K, Pardee K. Synthetic Biology Goes Cell-Free. *BMC Biol.* 2019; 17(1): 1,8.

63. Ouranidis A, Vavilis T, Mandala E et al. mRNA Therapeutic Modalities Design, Formulation and Manufacturing under Pharma 4.0 *Principles. Biomedicines* 2021; 10(1): 50.

64. Vogel A, Lambert L, Kinnear E et al. Self-Amplifying RNA *Vaccines* Give Equivalent Protection against Influenza to mRNA *Vaccines* but at Much Lower Doses. *Mol. Ther.*2018; 26(2): 446.

65. Rodriguez-Gascon A, del Pozo-Rodríguez A, Solinís M. Development of nucleic acid vaccines: use of self-amplifying RNA in lipid nanoparticles. *Int. J. Nanomedicine* 2014; 10(9): 1836.

66. Bloom K, van den Berg F, Arbuthnot P. Self-amplifying RNA vaccines for infectious diseases. Gene Therapy 2020; 28: 117.

67. Sau S, Larsen A, Chaput J. Automated solid-phase synthesis of high capacity oligo-dT cellulose for affinity purification of poly-A tagged biomolecules. *Bioorg. Med. Chem.* 2014; 24(24): 5692–5694.

68. Ulrich S, Kool E. Importance of Steric Effects on the Efficiency and Fidelity of Transcription by T7 RNA *Polymerase. Biochemistry* 2011; 50(47): 10343.

69. 2021. Assessment report Comirnaty. 1st ed. [ebook] EMEA, p.40.

70. 2021. Praesto® Jetted (dT)18-DVB. 1st ed. [ebook] Purolite, pp.4, 6.

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CELL & GENE THERAPY INSIGHTS

Innovation Insights

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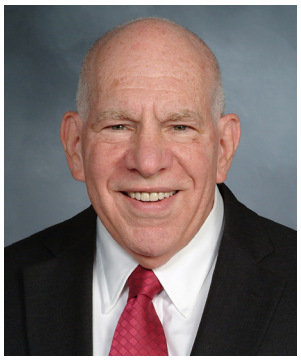
INTERVIEW

David McCall, Editor, *Cell and Gene Therapy Insights* speaks to **Ronald G Crystal,** Professor and Chairman of the Department of Genetic Medicine at Weill Cornell Medical College

Reflections on a life in gene therapy



RONALD G CRYSTAL, MD, is Professor and Chairman of the Department of Genetic Medicine at Weill Cornell Medical College, where he is also Bruce Webster Professor of Internal Medicine and Director of the Belfer Gene Therapy Core Facility. After earning a BA in physics from Tufts University and an MS in physics and an MD from the University of Pennsylvania, Dr Crystal served as Chief of the Pulmonary Branch of the National Heart, Lung and Blood Institute. His initial research at Weill Cornell focused on inflammatory diseases of the lung, shifting in the late 1980s to the field of gene therapy in which Dr Crystal became a pioneer. Recently developed gene therapy programs include the treatment of Friedreich's ataxia, cocaine addiction and Alzheimer's disease.

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Q What are some personal highlights from your 30+ years working in the gene therapy field?

RC: The genesis of my entry into the gene therapy field started in the early 1980s with an idea to treat alpha 1-antitrypsin deficiency by purifying the alpha 1-antitrypsin protein and giving it back to patients. We did that for five patients in 1981 while I was at the National Institute of Health (NIH) working in the Pulmonary Branch. In 1986, we carried out the pivotal study which was approved by the FDA in 1987 and is now used to treat about 10,000 people worldwide. Around that time, I started thinking that rather than giving patients a protein every week that has a half-life of 4.5 days, why don't we just give them the gene?

I was very lucky to be in the gene therapy field right at the beginning. Our first publication in 1987 was one of the first gene therapy papers. The most exciting thing we did was to provide the first example of human virus gene therapy: we were the first to use an adenovirus to treat cystic fibrosis in April 1993.

Q What have been the most significant advances in terms of technology platforms over this period?

RC: The concept of gene therapy has been around for quite a while. The challenge is that you cannot just eat a gene and expect it to go to an organ. You have to deliver the gene in a targeted way and the problem lies in doing so effectively. How can you transfer either DNA to the nucleus or RNA to the cytoplasm, both effectively and safely?

The two strategies that investigators have used over the years are viral and non-viral. The non-viral methods have not worked out very well, and it is obvious why. Viruses figured out millions of years ago that their job is to reproduce themselves, so they are very good at transferring their nucleic acid to either the nucleus or the cytoplasm, depending on the virus. Gene therapists capitalize on this by modifying viruses to be able to transfer therapeutic genes to cells. The challenge in the field now is how to develop viruses that are effective and safe.

The field has evolved. It started with retroviruses, then adenoviruses, then herpes viruses, then adeno-associated viruses (AAV). There have been a few other viruses that people have tried, but the dominant ones have been adenovirus, AAV, and the RNA viruses, which each have very different characteristics. The adenovirus is good for effective short-term expression, peaking at about seven days and lasting a few weeks. The adeno-associated virus is very effective for persistent expression in cells that are not proliferating. RNA viruses include the murine leukemia virus and

"You have to deliver the gene in a targeted way and the problem lies in doing so effectively."

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lentiviruses, which have revolutionized cancer and bone marrow-derived disorders. These are also going to be effective in treating diseases like sickle cell disease.

Q Where are the most pressing innovation gaps in developing novel or improved platforms and enabling technologies for gene therapy?

RC: The challenge for gene therapy has been the same for a long time: how do you do it effectively, safely, and less expensively? No-one has been able to effectively develop a virus that you can give systemically and that can go specifically to one organ. There is no virus (or non-virus) known that can be given systemically, intravenously, and can selectively transfer genes only to the brain, the eye, or the heart. Many people are working on this and there has been some significant advances, but there have not been any quantum leaps yet. It is slow progress for all the viruses that are used in the field, whether RNA or DNA viruses. The strategy is to modify the capsids because it is the capsid of the virus that defines where it goes.

The other problem is that when using these viruses, we are essentially giving foreign proteins to the human host. The immune system does not like that; it evokes immunity against it. At relatively low and medium doses this is not a problem, but when you get to very high doses, the immune and host defense systems start to cause some problems. It is, again, an area many people are working on, and there are a lot of different strategies to try and get around it.

Q It has been a tough year for AAV-driven gene therapy. What are the specific challenges facing the field, and are there any promising strategies or approaches addressing these challenges?

RC: Delivery, immunity, and manufacturing. It is basically the same for all the vector systems, though there are different challenges for each class of viruses. The adeno-associated viruses are good for cells that are not proliferating because they work in an epichromosomal fashion. They are good for the transfer of genes to organs with non-proliferating cells, such as the brain, heart, liver, or skeletal muscle.

Gene therapy is also very good in terms of functioning in compartments. For example, one of the real challenges in the brain is the blood–brain barrier, which makes it difficult to get biologics like monoclonal antibodies or

"The challenge for gene therapy has been the same for a long time: how do you do it effectively, safely, and less expensively? No-one has been able to effectively develop a virus that you can give systemically and that can go specifically to one organ."

"In terms of clinical research, the whole biomedical establishment has been hampered by the pandemic. Patient recruitment has been a challenge"

other proteins inside. With gene therapy, you can bypass that by going directly into the brain and getting brain cells to produce your therapeutic protein. Other examples of compartments include the eye. One of the real advantages of gene therapy is getting high concentrations within the organ of interest by direct administration.

Q You launched LEXEO Therapeutics around about a year ago now. How have you managed the challenges that the COVID pandemic is presenting to biotechnology companies, and the world at large?

RC: The pandemic is causing problems for the academic and commercial worlds not only in terms of people working within laboratories, but also people working within groups and having meetings. You and I are doing this by Zoom, but it would be more effective if we were in the same room together.

In terms of clinical research, the whole biomedical establishment has been hampered by the pandemic. Patient recruitment has been a challenge for everybody, in terms of the safety of travel and people not wanting to be part of experimental studies in the context of an epidemic. Despite these challenges, at LEXEO Therapeutics everything is going very well and programs are moving along. We have two clinical programs and a third about to go into the clinic.

Gene therapy is interesting because it started in the academic world, where a lot of the methodologies were developed. In the academic world, we cannot do Phase 2 or Phase 3 studies as we do not have the resources or expertise to take drugs to registration. The companies are able to do that. They can do it more effectively, as they have the expertise to do it correctly and safely. In the academic world, we can take things from the laboratory bench, move it through the clinic and do the Phase 1 studies. After that, it is much more rational to turn it over to companies to get it to the goal line for approval.

Q Looking ahead to 2022, what breakthroughs do you hope and expect to see in gene therapy, both in general and within the specific disease areas in which you specialize?

RC: I do not think we are going to see any quantum leaps in gene therapy, but we are going to see slow progress and start to see more approved products. The resources going into gene therapy in the commercial world now are very impressive. For someone who has been in the field since the beginning, it is great to see that we now have some approved

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products in the field, and I think we are slowly going to see more products for multiple different companies approved.

The lesson here is that for any of these fields or technologies, it takes an army of people. There has been a huge amount of effort by both academia and industry in gene therapy. Now, we are seeing the fruits of that in terms of approvals and taking these products forward to help people.

Q What are your primary goals and priorities in your own work over the next few years?

RC: We are focused primarily on trying to improve the problems in gene therapy. We are working to improve efficiency, delivery, and safety. The goals are to help understand what the problems are and to develop solutions. The areas that we are interested in working in are hereditary disorders, disorders of the brain, disorders of the heart and vaccines.

Gene therapy started with a focus on hereditary diseases. Over the next decade, the field is going to expand into delivering genes to treat acquired disorders, rather than classic hereditary disorders. Small molecules, monoclonal antibodies, recombinant proteins, and gene therapy are all drug delivery systems of various kinds. It is just a matter of finding what diseases are good targets, and how gene therapy is effective for solving the problems.

Q Do you have any words of advice for those who are just beginning their journey in gene therapy?

RC: You have to decide where you want to make your contributions. Gene therapy is first understanding pathogenesis of disease, identifying the target, and then identifying how to solve that problem. You need to work out what kind of vector to use, what kind of experimental animal models to use and how to show efficacy. There are also the challenges of manufacturing, toxicology, and clinical studies.

For an individual who is interested in beginning their career in gene therapy, they should first decide where their skills and interests are that can contribute to the field. If I was starting out now, I would look into the landscape and choose a laboratory within the academic world to work in and get my feet wet in that area. More and more people are doing this, but many are also going to industry. There are a lot of biotechnology companies now with very clear targets, good technology, and good people behind them. Large pharmaceutical companies are now also developing gene therapy programs. There are a lot of different opportunities for people starting out.

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Clinical Trends

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INTERVIEW

Exploring the translational landscape of regenerative cell therapies

Anthony Atala

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Clinical Trends

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INTERVIEW

Exploring the translational landscape of regenerative cell therapies

David McCall, Commissioning Editor of *Cell & Gene Therapy Insights*, talks to **Anthony Atala**, G. Link Professor & Director, Wake Forest Institute for Regenerative Medicine

ANTHONY ATALA, MD, is the G. Link Professor and Director of the Wake Forest Institute for Regenerative Medicine, and the W. Boyce Professor and Chair of the Department of Urology at Wake Forest University. His work focuses on growing human cells, tissues and organs. Fifteen applications of technologies developed in Dr Atala's laboratory have been used clinically in human patients. Dr Atala was elected to the Institute of Medicine of the National Academies of Sciences (now the National Academy of Medicine), to the National Academy of Inventors as a Charter Fellow, and to the American Institute for Medical and Biological Engineering. Dr Atala is a recipient of the US Congress funded Christopher Columbus Foundation Award, bestowed on a living American who is currently working on a discovery that will significantly affect society; the World Technology Award in Health and Medicine, for achieving significant and lasting progress; the Edison Science/Medical Award; the Fast Company World Changing Ideas Award; the R&D Innovator of the Year Award; and the Smithsonian Ingenuity Award. Dr Atala's work was listed twice as Time Magazine's top 10 medical breakthroughs of the year, and as one of 5 discoveries that will change the future of organ transplants. Dr Atala's work was ranked in 2019 by the Project Management Institute as one of the top 10 most impactful biotech projects from the past 50 years. Dr Atala was named by Scientific American as one of the world's most influential people in biotechnology, by U.S. News & World Report as one of 14 Pioneers of Medical Progress in the 21st Century, by Life Sciences Intellectual Property Review as one of 50 key influencers in the life sciences intellectual property arena, and by Nature Biotechnology as one of the top 10 translational researchers in the world. Dr Atala has led or served several national professional and government committees, including the National Institutes of Health working group on Cells and Developmental Biology, the National Institutes of Health Bioengineering Consortium, and

the National Cancer Institute's Advisory Board. He was a Founder of the Tissue Engineering Society, the Regenerative Medicine Society, the Regenerative Medicine Foundation, the Alliance for Regenerative Medicine, the Regenerative Medicine Development Organization, the Regenerative Medicine Manufacturing Society, and the Regenerative Medicine Manufacturing Consortium. Dr Atala works with several journals and serves in various roles, including Editor-in-Chief of: Stem Cells Translational Medicine; Therapeutic Advances in Urology; and BioPrinting. He is the editor of 25 books, has published more than 800 journal articles and has applied for or received over 250 national and international patents.

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Q What are you working on right now?

AA: Much of our work involves bringing regenerative medicine technologies from the bench to the bedside. This includes the initial hypothesis, extensive basic research, the proof of concept, preclinical studies, the development of the product in our FDA compliant Good Manufacturing Practices (GMP) facility, and the coordination for the delivery of the therapy to patients. As we brought these technologies through to human patients, we realized that manufacturing was very important. It is acceptable to make these products by hand if you are treating a small number of patients in a small trial, but if you want these technologies to be fully translated into the clinic, they need to be scaled-up in terms of manufacturing process and cost reduction. Our most recent work also involves developing better techniques for advancing manufacturing for the field of regenerative medicine, promoting a smoother transition of therapies into the clinic.

Q How would you describe the most significant advances that have impacted the clinical translation of regenerative medicines – firstly, in terms of therapeutic platform evolution?

AA: When we are talking about the field of regenerative medicine in terms of therapeutic platforms, we must go back to how it started in the 1970s. Some of the very first therapy platforms in the space

"Our most recent work also involves developing better techniques for advancing manufacturing for the field of regenerative medicine, promoting a smoother transition of therapies into the clinic."

involved the use of biomaterials alone for tissue regeneration. These biomaterials had to be designed to be bio-compatible with a low inflammatory response. These technologies work very well for bridging small defects that are typically <0.5cm from any edge of normal tissue.

The use of cells for therapy was also being investigated. This needed a lot of work in terms of ensuring that the cells were safe and had the right release criteria. There was a need to manufacture these cells so they could be expanded into large quantities while still retaining their normal functional properties.

The next step in the field was the use of cells and materials together to create tissue engineered structures that could replace specific organ defects. We have seen this develop over time.

"Another area that has advanced rapidly is 3D printing, giving the ability to bring precision and design control around the products. Many of these tools and technologies are advancing rapidly in the current landscape."

Q Secondly, in terms of the evolution of non-clinical tools?

AA: Various non-clinical and translational tools and technologies needed to be developed to get to where we are today. At first, a lot of this work was being done manually. Today, automation of these technologies is occurring, including cell sorting technologies and automated bioreactors to grow cells and combine them with biomaterials.

Another area that has advanced rapidly is 3D printing, giving the ability to bring precision and design control around the products. Many of these tools and technologies are advancing rapidly in the current landscape.

Q Let's turn now to innovation in early phase clinical trial design. How is that impacting the regenerative medicine field?

AA: Regenerative medicine, for the most part, involves biologics. Biologics are not always like devices that can be manufactured in the same manner with the same properties every time. There is variability in terms of both the therapy itself, and the recipient. Therefore, a lot of innovation in terms of clinical trial design involves how to best predict which patients can benefit most from therapies based on their own genetics, epigenetics, and disease process- basically optimizing personalized medicine principles to maximize treatment efficacy.

Q How have regulators' attitudes to regenerative medicine changed over relatively recent times?

AA: It has been a great area of growth because for many of these technologies, there was no pathway for regulation when the field first got started. The regulatory bodies, such as the FDA, have done a great job analyzing the field and moving forward alongside it as regenerative medicine has grown. There has been a proactive process in ensuring these technologies can be translated clinically in a safe and effective manner, while also allowing the field to advance.

Q What are the most pressing challenges facing the regenerative medicine space that need to be addressed by both industry and academia?

AA: A key challenge today is developing the ability to better manufacture these therapies so we can scale them up, have more precision in terms of the production and design, and can lower costs. Another challenge in the field has been establishing the vascularity of the major solid organs. When we try to engineer tissues and organs, for example, there are varying levels of complexity. Flat structures, like skin, are the least architecturally complex. Tubular structures, such as blood vessels, are slightly more complex, followed by even more complex structures such as hollow, non-tubular organs like the bladder or stomach. But by far the most complex structures are solid organs such as the heart or liver, as they have exponentially greater vascularity requirements. Both these factors are still in development and are very important for the final dissemination of these products to a broader patient population.

Q How and where is gene editing impacting your field, and what considerations, opportunities, and challenges does it present for the clinical translation of new regenerative medicines?

AA: Then field of gene editing has presented great opportunities for regenerative medicine, such as providing tools for positively impacting patients with known genetic conditions. In the past, patients with organ failure due to inherited genetic defects could not benefit from tissue engineering technologies that used their own cells, as the engineered tissue would retain its abnormal traits. Gene editing is now allowing the use of the patient's own cells, and these can be genetically modified so a healthy tissue can be created.

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Q Finally, what will be some key goals and priorities for your own work over the short-to-mid-term?

AA: Fifteen applications of our technologies have been advanced into patients to date. Our hope is to continue to increase the number of technologies that we can bring to patients, and to also increase the overall numbers of patients that can benefit from these therapies.

We have also spent a lot of energy developing integrated body-on-a-chip systems using regenerative medicine tools. Using similar strategies to create tissues and organs for patients, we miniaturized the system so it could be applied as a human surrogate for testing. We are now applying these systems for drug discovery, toxicity testing and personalized medicine. We hope to keep advancing this area further using disease modelling and artificial intelligence.

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