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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Downstream Bioprocessing

Guest Edited by Michael Covington

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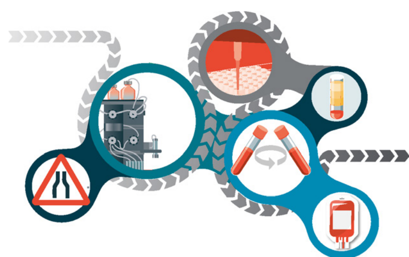

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DOWNSTREAM BIOPROCESSING

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EXPERT INSIGHT

Vector purification: issues and challenges with currently available technologies

David Moss

Genetically engineered cell therapies are emerging platforms that have the potential to address unmet clinical needs. Several strategies have been developed for inserting a gene of interest into the target cell, including novel direct DNA and RNA delivery methods. However, viral-based vectors have so far remained the primary platform for these key transductions. The increasing number of products being registered for clinical trials in recent years has created a need for viable Good Manufacturing Process (GMP) vector production. Here we focus on challenges and new developments in purifying vector products at scales that are suitable for cell and gene therapy treatments.

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Cell and gene therapies rely on the effective delivery of a gene of interest into the target cell and are currently under development for a number of treatments. These include gene replacement for haemophilia [1] and X-lined severe combined immunodeficiency [2] and adoptive T-cell therapies for cancer using chimeric antigen receptors (CARs) or engineered T-cell receptors (TCRs) [3,4]. Typically, gene insertion into the target cell is performed using a vector system based on adenovirus (Ad), adeno-associated viruses (AAV) or retroviruses such as lentivirus (LV), depending on the target cell type and the gene size being transferred. Treatment requirements will also determine vector selection, with Ad capable of transient expression while AAV and LV integrate the target gene [5]. Both LV and AAV

can transduce non-dividing cells, though AAV has a limited target gene capacity (4–6 Kb) compared to LV vectors capable of delivering genetic packages up to 15 Kb [6,7]. The current industry LV production process is based on transient transfection of a host cell line (such as HEK 293) with a third generation self-inactivation (SIN) plasmid system that encodes the target gene and the minimal viral protein components required [8]. Similarly, Ad vector expression systems have undergone their own development to improve safety, and AAV production is via multi-plasmid or helper virus expression systems [5].

Vector production is an active area of research [9], and its development directly impacts the scale and requirement of subsequent downstream purification (DSP). Once vector is produced, DSP must balance the demands of processing (impurity removal, concentration, sterility and formulation) with the need to maintain viability of a complex and large multi-component particle. All this is overlaid with the need to generate a procedure capable of working under Good Manufacturing Process (GMP) standards.

Established vector purification techniques employed in research settings and phase I clinical trials often depend on centrifugation (ultracentrifugation or involving some element of density gradient, e.g. caesium chloride). These techniques are generally labour intensive, difficult to scale-up, and lack process control [3,10]. This can cause significant challenges for meeting yield and GMP requirements when products progress into phase II of clinical trials and beyond.

THE PURIFICATION TOOLBOX

Many vector purification processes were developed from established protein and antibody purification systems and have four key stages: clarification, capture, concentration and formulation. In addition, a DNAase treatment is commonly used to assist in removal of contaminating host cell or plasmid DNA, and a sterile filtration step is typically incorporated. While the order of purification steps and the consumables used may vary, the actual 'toolkit' available for purification is limited and is fundamentally based on selecting vector particles via certain physicochemical characteristics.

CLARIFICATION

Clarification at its basic level is the separation of cells and cellular debris from the culture media, which will contain the vector product (post cell disruption if required). While centrifugation has been used in the past and is still a component of some small-scale purification processes, this is not an easily scalable process and limited in GMP [11]. Fortunately, there are a variety of commercial filter products on the market that are capable of managing a variety of feed stream conditions. These include multi-layered dead-end and depth filters, which can be used in combination. The optimal choice depends on the feed stream being processed and whether the material is generated from adherent or suspension cultures [9]. While the primary action of all clarification media is based on their pore size (typically using a lower limit of 0.2-0.5 µm), some filters also

incorporate a charge factor to aid in impurity removal (such as DNA or viruses). Such filters are likely to negatively impact vector recovery and are best avoided. An emerging technology that could also be used as part of clarification is macro tangential flow filtration (TFF) [12,13].

DNA DEGRADATION

One of the main contaminating factors in vector production is DNA, either from the plasmid used for transfection or from the host cells. While DNA can be removed in several ways, it is common to find a DNA digestion stage as part of the purification steps. This stage reduces any DNA product viscosity or aggregation [5,14], with DNA fragments physically removed via filtration (e.g. TFF) or by active binding (anion exchange chromatography) in later purification steps.

CAPTURE

Chromatography remains a key technology of the capture stage, with the majority of published processes using affinity-based ligands or ion-exchange [5,15–17]. For AAV vectors, there are a variety of serotype-specific binding modalities that effectively work as affinity binders (discussed below) and even Ad has been reported to bind to metal affinity columns [18]. However, no such affinity-based mechanism has been shown for LV.

AAV and LV vector particles have an overall negative surface charge at or near neutral pH, and they interact with the anion exchange medium via varying and multiple charged surface entities [19]. This can mean that product elution requires high conductance levels, which can co-elute bound impurities and decrease vector viability due to osmotic shock [16,20], limiting quality and yield. While this surface charge can be modified by varying the pH, and thus reduce the mobile phase ionic strength required to elute, this is not possible for some vector products due to their fragility [21]. In addition, the relatively large hydrodynamic radii, especially of LV particles, significantly restricts their physical access to binding sites in classic resin media [22]. One method used to address this has been the development of membrane and monolith chromatography formats, both of which have been used in LV purification [23–28].

A more specific and potentially gentler capture method is affinity chromatography. This platform has been actively developed for AAV, with systems such as Capture SELECT™ [29], and heparin and sialic acid-based binding can be used for AAV serotypes [5,15]. These processes do not separate viable and non-viable particles, which is reported to be possible using ion-exchange [30], and as with all purification systems, a combination approach is likely required to maximize viable particle purification. Heparin has an affinity-like interaction with vesicular stomatitis virus G (VSVG)-pseudo-typed particles [31], a common pseudo coat protein used in LV vectors. However, the use of animal-derived heparin for GMP is problematic. Sulfated spherical cellulose (Cellufine™ Sulphate, JNC Corporation) is a heparin mimetic that has been applied successfully to purify other vector and influenza and dengue virus [30,32] and may be worth further

study. Alternatively, an affinity tag could be engineered onto the surface of the vector particle. For vectors enveloped by a host-cell membrane, such as LV, one method might be to express the tag in the host cell plasma membrane so that it is passively incorporated during viral vector budding [33].

CONCENTRATION AND FORMULATION

TFF is a powerful DSP tool, capable of both concentration and buffer exchange, and has been successfully used for viral products [34–36]. This platform is scalable, and there are several commercial vendors providing filter formats and membrane chemistry options. The size of all vector particles means that a larger molecular weight cut-off can be used (typically, +300 kDa) and may indeed be preferable to enhance the speed of filtration [37]. The physical processing in TFF is relatively gentle, though consideration should be given to the membranes' physical format to minimize turbulence and associated shear factors.

Buffer formulation is also a critical factor, both for processing and final product composition. Often, the working range of the buffer is constrained by what the product can tolerate and maintain its viability rather than the processing step. This is a topic specific to each vector and outside the scope of this review.

STERILE FILTRATION

Sterile filtration requires significant consideration. While the best GMP would incorporate sterile filtration into the final filling procedure, this poses a major hurdle for large vector particles. Some virus particles will be actively removed by sterile filtration (e.g. Vaccinia virus, 250-270 nm [38]), and while AAV, Ad and LV vectors discussed here are smaller (AAV, 20-30 nm; Ad, 70-90 nm; LV, 120-150 nm), some remain close to any validated sterile filter threshold (200 nm). Considering LV particle size and the chances of particle aggregation during processing and concentration, the risk of product loss in a sterile filtration step increases as purification progresses [37]. This has led some operators to invest in enclosed and validated sterile systems, which place sterile filtration earlier in the process [3,14], or to remove it from the process entirely [39].

HOW DO WE IMPROVE?

The key processes of clarification, capture, concentration and formulation are currently completed in discrete processing steps. This staged system increases costs, time and potential for product loss. One of the primary drivers in process development is overall simplification to reduce these risks [40].

To define what improvements are needed, it is necessary to understand the attributes of product quality, including impurities, throughout purification. Some analytical techniques can be easily transferred between production platforms (e.g. host cell protein enzyme-linked immunosorbent assay), while others (e.g. biological assays) pose more challenges, often being product specific and requiring their own characterization [41]. Understanding what is being processed and produced through purification will greatly aid process development and manufacturing,

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and the development of timely and robust product assays should continue to be a focus for the industry.

The majority of current DSP techniques rely on separating vector particles based on their physical characteristics, for example size or charge. This means that they will also capture non-viable/empty particles (e.g. contain no DNA/RNA) or cellular vesicles of a similar size and membrane/protein composition [25]. While some work has been published on distinguishing full and empty AAV capsids via there variations in ion-exchange binding [26,30,42], there is currently no system available to make such distinctions for other vectors except for complex and time-consuming electron microscopy. Understanding how to distinguish these empty particles would aid significantly in developing processes to reduce their production and allow for their removal.

The two steps in vector purification that currently present the greatest challenges are capture and sterile filtration. Manufacturers of chromatography media have been active in developing new binding ligands and their supporting matrices, utilizing structures including monoliths, porous filters and nanofibers. Such format change increase access for the larger Ad and LV particles to binding entities and may support the development of single use chromatography. A transition has already been made in biological production and other purification steps to support GMP production [11].

In an interesting twist, the vector exclusion aspect of bead resins has been monopolized in Capto-Core400/700 (GE Healthcare) [43]. Here, the core resin-binding ligands are shielded by a coat that excludes particles greater than 400 or 700 kDa, causing the vector particles to pass around the resin in a flow through mode with impurities entering the resin and binding [44].

A further issue with any chromatography process incorporation into the purification process is aseptic validation. While many of the other process steps employed can be effectively modified to enable aseptic manipulation/processing, chromatography remains a semi-open platform, requiring the inclusion of a sterile filtration step later in the purification path that can have its own detrimental impact on vector recovery. Given the highlighted issues around sterile filtration, a major step forward would be finding a method of generating a closed chromatography system, and several active vector producers have highlighted the need for closed-system processing [3,45].

One of the simplest methods of increasing vector production is the linear scale-up of the production volume, preferably using stir-tank bioreactors. While many of the purification techniques discussed here are scalable, they remain designed for a batch-based process. Therefore, it is worth considering how continuous production would impact DSP and what adaptations may be needed. Technologies that may aid include semi-continuous chromatography and single-pass TFF [46], which while originally developed for antibody purification, could be adapted for vector by modifications to the membranes used.

TRANSLATIONAL INSIGHT

Viral vector purification remains a challenging task. As cell and gene therapies move into later clinical

phases and commercialization, the quality conditions and prerequisites placed on their supporting vector production requirements are increasing rapidly. Industry suppliers are taking heed of the practical issues of working with large vector particles and engaging with end-producers to find technical solutions. Here, addressing current limitations of capture chromatography and its enclosure to aid sterile filtration would significantly enhance the process. However, we expect that major improvements will come from a holistic approach to all processing steps and their simplification. Underpinning all the purification aspects is timely and detailed understanding of how each process step affects the vector product. Rapid and robust analytics are also required, and the analytics must be generated hand-in-hand with purification advances to enable DSP full potential. Working within a GMP environment adds further conditions to what consumables are available, especially if we consider the prominent use of single use platforms, where suppliers and scalable options can be limited [11]. Regarding regulatory oversight and product licence applications, operators in vector production should consider the FDA preferred approaches of Quality by design (QbD) and Process Analytical Testing (PAT) to most efficiently develop and understand there vector purification systems [47]. It is undoubtedly an exciting time to be working in this field.

REFERENCES

1. Pipe SW. Gene therapy for hemophilia. *Pediatr. Blood Cancer* 2018; 65(2).
2. Mukherjee S, Thrasher AJ. Gene therapy for PIDs: progress, pitfalls and prospects. *Gene*. 2013; 525(2): 174–81.
3. Sargent *et al.* Key Considerations in Gene Therapy Manufacturing for Commercialization. *Cell Culture Dish*. 2018.
4. Johnson LA, June CH. Driving gene-engineered T cell immunotherapy of cancer. *Cell Res.* 2017; 27(1): 38–58.
5. Burova E, Ioffe E. Chromatographic purification of recombinant adenoviral and adeno-associated viral vectors: methods and implications. *Gene Ther.* 2005; 12 Suppl 1: S5–17.
6. Counsell JR, Asgarian Z, Meng J *et al.* Lentiviral vectors can be used for full-length dystrophin gene therapy. *Sci. Rep.* 2017; 7: 44775.
7. Kumar M, Keller B, Makalou N, Sutton RE. Systematic determination of the packaging limit of lentiviral vectors. *Human Gene Ther.* 2001; 12(15): 1893–905.
8. White M, Whittaker R, Gandara C, Stoll EA. A Guide to Approaching Regulatory Considerations for Lentiviral-Mediated Gene Therapies. *Hum. Gene Ther. Methods* 2017; 28(4): 163–76.
9. Macchiarulo E, Bassett P, Dudley M. Challenges and progress in lentiviral vector bioprocessing. *Cell Gene Ther. Insights.* 2018; 4(10): 915–23.
10. Jiang W, Hua R, Wei M *et al.* An optimized method for high-titer lentivirus preparations without ultracentrifugation. *Sci. Rep.* 2015; 5:13875.
11. Shukla AA, Gottschalk U. Single-use disposable technologies for biopharmaceutical manufacturing. *Trends Biotechnol.* 2013; 31(3):147–54.
12. al Re. Best Practices for Optimization and Scale-Up of Microfiltration TFF Processes. *BioProcessing J.* 2012(Spring): 30–40.
13. Besnard L, Fabre V, Fettig M *et al.* Clarification of vaccines: An overview of filter based technology trends and best practices. *Biotechnol Adv.* 2016; 34(1): 1–13.
14. Merten OW, Hebben M, Bovolenta C. Production of lentiviral

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vectors. *Mol. Ther. Methods Clin. Dev.* 2016; 3: 16017.

15. Wright JF. Manufacturing and characterizing AAV-based vectors for use in clinical studies. *Gene Ther.* 2008; 15(11): 840–848.

16. McNally DJ, Darling D, Farzaneh F, Levison PR, Slater NK. Optimised concentration and purification of retroviruses using membrane chromatography. *J. Chromatogr. A.* 2014; 1340: 24–32.

17. Kutner RH, Puthli S, Marino MP, Reiser J. Simplified production and concentration of HIV-1-based lentiviral vectors using HYPERFlask vessels and anion exchange membrane chromatography. *BMC Biotechnol.* 2009; 9: 10.

18. Huyghe BG, Liu X, Sutjipto S, Sugarman BJ, Horn MT, Shepard HM, *et al.* Purification of a type 5 recombinant adenovirus encoding human p53 by column chromatography. *Human Gene Ther.* 1995; 6(11): 1403–16.

19. Rodrigues T, Alves A, Lopes A, Carrondo MJ, Alves PM, Cruz PE. Removal of envelope protein-free retroviral vectors by anion-exchange chromatography to improve product quality. *J. Sep. Sci.* 2008; 31(20): 3509–18.

20. Nasimuzzaman M, Lynn D, Ernst R *et al.* Production and purification of high-titer foamy virus vector for the treatment of leukocyte adhesion deficiency. *Mol. Ther. Methods Clin. Dev.* 2016; 3: 16004.

21. Higashikawa F, Chang L. Kinetic analyses of stability of simple and complex retroviral vectors. *Virology* 2001; 280(1): 124–31.

22. Iyer G, Ramaswamy S, Cheng K-S, Sisowath N, Mehta U, Leahy A, *et al.* Flow-Through Purification of Viruses- A Novel Approach to Vaccine Purification. *Procedia in Vaccinology.* 2012; 6: 106–12.

23. Lesch HP, Laitinen A, Peixoto C *et al.* Production and purification of lentiviral vectors generated in 293T suspension cells with baculoviral vectors. *Gene Ther.* 2011; 18(6): 531–8.

24. Bandeira V, Peixoto C, Rodrigues AF *et al.* Downstream processing of lentiviral vectors: releasing bottlenecks. *Hum. Gene Ther. Methods* 2012; 23(4): 255–63.

25. Johnson S, Wheeler JX, Thorpe R, Collins M, Takeuchi Y, Zhao Y. Mass spectrometry analysis reveals differences in the host cell protein species found in pseudotyped lentiviral vectors. *Biologicals* 2018; 52:59–66.

26. Krajacic M, Ravnikar M, Strancar A, Gutierrez-Aguirre I. Application of monolithic chromatographic supports in virus research. *Electrophoresis* 2017; 38(22–23): 2827–36.

27. Venkatachalam ARK, Kiener TK, Balraj P, Kwang J. Concentration and purification of enterovirus 71 using a weak anion-exchange monolithic column. *Virology* 2014; 11(99).

28. Zimmermann K, Scheibe O, Kocourek A, Muelich J, Jurkiewicz E, Pfeifer A. Highly efficient concentration of lenti- and retroviral vector preparations by membrane adsorbers and ultrafiltration. *BMC Biotechnol.* 2011; 11:55.

29. M Toueille LD, Attebi E, Cartigny J *et al.* Development of purification steps for several AAV serotypes using POROS™ CaptureSelect™ AAVX affinity chromatography. *Cell Gene Ther. Insights* 2018: 637–46.

30. Qu G, Bahr-Davidson J, Prado J *et al.* Separation of adeno-associated virus type 2 empty particles from genome containing vectors by anion-exchange column chromatography. *J. Virol. Methods* 2007; 140(1–2): 183–92.

31. Segura MM, Garnier A, Durocher Y, Coelho H, Kamen A. Production of lentiviral vectors by large-scale transient transfection of suspension cultures and affinity chromatography purification. *Biotechnol. Bioeng* 2007; 98(4): 789–99.

32. Kanlaya R, Thongboonkerd V. Cellufine sulfate column chromatography as a simple, rapid, and effective method to purify dengue virus. *J. Virol. Methods* 2016; 234:174–7.

33. Mekkaoui L, Parekh F, Kotsopoulou E *et al.* Lentiviral Vector Purification Using Genetically Encoded Biotin Mimic in Packaging Cell. *Mol. Ther. Methods Clin. Dev.* 2018; 11:155–65.

34. Nehring D, Schweizer M, Cichutek K, Czermak P. Integrated inline filtration: A method to produce highly concentrated retroviral vector titer supernatant. *Desalination* 2009; 245:614–20.

35. Cooper AR, Patel S, Senadheera S, Plath K, Kohn DB, Hollis RP. Highly efficient large-scale

lentiviral vector concentration by tandem tangential flow filtration. *J. Virol. Methods* 2011; 177(1): 1–9.

36. Miyake K, Miyake N, Yamazaki Y, Shimada T, Hirai Y. Serotype-independant Method of Recombanant Adeno-assocated Virus (AAV) Vector Production and Purification. *J. Nippon Med. Sch.* 2012; 79(6): 394–402.

37. Carvalho B, Silva RJS, Moleirinho MG *et al.* Membrane-Based Approach for the Downstream Processing of Influenza-Like Particles. Biotechnology Journal. 2019; 14:1–12.

38. Berting A, Goerner W, Spruth M, Kistner O, Kreil TR. Effective poxvirus removal by sterile filtration during manufacture of plasma derivatives. *J. Med. Virol.* 2005; 75(4): 603–7.

39. Ausubel LJ, Sharma A, Shakeley R *et al.* Production of CGMP-Grade Lentiviral Vectors. *Bioprocess Int.* 2012; 10(2): 32–43.

40. McCarron A, Donnelley M, Parsons D. Scale-up of lentiviral vectors for gene therapy: advances and challenges. *Cell Gene Ther. Insights*. 2017; 3(9): 719–29.

41. van der Loo JC, Wright JF. Progress and challenges in viral vector manufacturing. *Hum. Mol Genet.* 2016; 25(R1): R42–52.

42. Kramberger P, Urbas L, Strancar A. Downstream processing and chromatography based analytical methods for production of vaccines, gene therapy vectors, and bacteriophages. Hum *Vaccine Immunother.* 2015; 11(4): 1010–21.

43. Healthcare G. Capto™ Core 700. Data file 28–9983–07 AA. 2012.

44. Healthcare G. The use of Capto™ Core 700 and Capto Q ImpRes in the purification of human papilloma virus like particles. Application note 29–0983–01 AB. 2014.

45. Stanton D. Cell and Gene Therapies 2018: State of the Industry Report. KNect365 *Life Science* 2018.

46. Ayturk E. Understanding Single-Pass Tangential Flow Filtration and the New Era of Bioprocessing. PALL.

47. Sheppard V. Cell-Culture–Based Influenza Vaccine Manufacturing. *BioProcess Int*. 2018; 16(9): 34–45.

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CELL & GENE THERAPY INSIGHTS

DOWNSTREAM BIOPROCESSING

SPOTLIGHT

EXPERT INSIGHT

Developing a quality control program for advanced therapy medicinal products in Europe

Margarita Codinach

Advanced therapy medicinal products (ATMP) are complex medicines based on gene therapy, somatic cell therapy, and tissue engineering. These products are arising as novel and promising therapies for a wide range of different clinical applications.
The quality control system, which operates independent from production, is a key element during manufacturing and ensures that the finished product obtained has been produced with the quality and safety required. Control strategy includes the sampling plan, test methods and acceptance/rejection criteria for raw and starting materials, intermediates and finished products. Furthermore, it has an important role in stability testing and microbiology monitoring of production areas, equipment and personnel.
The quality control system has implemented quality management tools such as risk-based approach and change control programme in their working routine. However, some other quality control aspects like availability of ATMP reference materials or proficiency testing to ensure the reliability of the ATMPs analytical measurements still remain under development.

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Advanced therapy medicinal products (ATMP) are complex medicines based on gene therapy, somatic cell therapy, and tissue engineering or combinations thereof. These products are arising as novel and promising therapies for a wide range of different clinical applications from orthopedic [1,2] and ocular disorders [3,4] to neurologic diseases [5,6] and serious hematopoietic malignancies [7,8]. In Europe, they must be manufactured according to Good Manufacturing Practices (GMP) as stated in the Regulation (EC) No 1394/2007) and the Directive 2001/83/EC [9,10].

Monitoring of manufacturing process is essential not only during process validation but also during production of ATMPs for clinical application. The quality control system operates independent from production and ensures that the finished product obtained has been produced with the safety, quality and efficacy required [10].

An approved and well-defined strategy of assays applied to raw materials, biologic starting materials and finished product as well as in-process controls is the key aspect of the quality control programme for ATMPs. The control strategy includes the sampling plan, the test methods and acceptance/rejection criteria for each step of the manufacturing process. Following the quality-by-design (QbD) ideology, understanding of the product (target product profile) and the process (critical process parameters) allows for the design of a control strategy based on the critical quality attributes of the product [11].

The nature of the biologic starting materials, level of complexity of the manufacturing process and autologous versus allogeneic use may determine the strategy of implemented tests. Moreover, short ATMP shelf-life, limitation in ATMP availability or administration for medical need immediately after ATMP preparation may make it impossible to perform the release tests directly on the finished products. In those instances, real-time release testing may provide an alternative for end-product testing as part of the batch release decision.

ATMPs have the same testing requirements as other medicinal products of chemical, biologic or biotechnological origin in which each batch has to be controlled. Keeping in mind that most autologous treatments have a very small batch size (even just one unit) process controls have an important impact on cost of manufacture of the ATMPs.

According to International Conference on Harmonization Q2 Guidelines [12] and the European Pharmacopeia [13], analytical testing methods used to assess quality and safety should be also validated. Specificity, linearity, precision (repeatability and intermediate precision), robustness and accuracy are aspects to be evaluated. A specific personnel training program has to be developed and documented as well as an appropriate maintenance, calibration and qualification equipment policy.

Methods to assess safety include sterility tests, mycoplasma tests, adventitious agent detection, replication-competent virus tests and quantification of endotoxin level. Sterility tests for starting materials, intermediate products and finished ATMPs have to be done according to European Pharmacopeia (Eur. Ph.) Monograph 2.6.1. Samples are cultured with enriched media

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FIGURE 1

Quality control program cycle.

(thioglycollate and soya-bean medium) to detect bacteria and fungi during a minimum of 14 days. Culture media must prove their fertility by positive growth promotion tests. Moreover, every matrix examined during the process must submit to method suitability tests to verify that the product does not interfere with the assay [14].

Other rapid microbiological methods may be considered for cellular products - for example, automated culture systems such as BacT/Alert (Biomerieux), Bactec (Becton Dickinson) and VersaTREK system (TREK Diagnostic Systems), as described in Monograph 2.6.27. Moreover, nowadays other technologies like Flow Cytometry are also being evaluated as alternatives for rapid microbiology testing.

Mycoplasma contamination detection is carried out according to Eur. Ph. Monograph 2.6.2 using either culture method or nucleic acid amplification technique (NAT). Specificity, limit of detection and method robustness are the most important elements of NAT analytical procedure validation.

Endotoxin quantification may be tested through different methodologies following Eur. Ph Monograph 2.6.14 and 5.1.10 Guideline: Gel-Clot (limit or quantitative test), turbidimetric kinetic, chromogenic kinetic, chromogenic end-point or turbidimetric end-point methods. Administration route, volume of the ATMP infused and patient body mass define the endotoxin limit and consequently, the Maximum Valid Dilution for that specific test. In addition, sample pH, cell concentration and presence of interfering substances or cellular debris are critical aspects to be considered not only as a part of the product characterization, but also when setting up the endotoxin test [14].

Furthermore, adventitious agent detection assays are required for ATMPs of allogeneic treatments to ensure the absence of viral contamination [15]. Lytic and/or haemadsorbing viruses are detected after inoculation of a normal human diploid cell line (such as MRC-5) and a monkey kidney cell line (such as Vero) with the ATMP samples. Cell lines are examined regularly for signs of a cytopathic effect over a culture period of 14-28 days. Alternatively, molecular diagnostic by PCR testing are being used to demonstrate absence of virus contamination. Testing for replication-competent virus with a validated test at the level of the viral production system or, alternatively, in the transduced cells in gene therapy-based products is also mandatory in both autologous and allogeneic therapies [16].

On the other hand, controls to evaluate identity and purity are also critical in ATMP characterization. Cell count may be performed manually (hemocytometer), but automatically is recommended since assays used to quantify and characterize the cells also need to be validated [17,18]. Flow cytometry assays to evaluate cell count, viability and immunophenotype are the most widely used [19]. However, absence of clumps in the sample must be examined to avoid false results in cell counting. Antibody concentration, staining volume and time definition, internal controls requirement (isotype, fluorescence minus one control, etc.) or the need for erythrocyte lysis are critical factors during the protocol set-up. Likewise, it is also very important to characterize, and control finished product-related impurities of biological origin as well as other residuals (for example: DMSO, trypsin, etc.).

Potency, the quantitative measure of biological activity based on the attribute of the product, is another key parameter of ATMP characterization and should reflect the clinical mechanism of action [20]. A lot of different specific tests are implemented to evaluate the ATMP potency such as *in vitro* proliferation and cytotoxicity assays, interferon-gamma release assay, immunomodulatory function, colony-forming efficacy, differentiation capacity to specific lineages, etc. or even flow cytometric assays to determine presence of specific molecules as surrogate markers [21–24]. *In vivo* assays for potency may also be useful especially when experimental animal models are available. A combination of multiple methods may be needed to adequately define the potency of these products during the development because of the complexity of ATMPs.

The quality control program also has a relevant role in stability testing. Shelf-life must be defined not only for the finished ATMPs,

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but also for intermediates subjected to storage and active substances. Stability validation must be performed using real storage (container, temperature range) and transport conditions. In addition, it must be considered that ATMPs usually have a short self-life when the stability study timeline is defined. Finally, environmental monitoring of production areas, equipment and personnel must be executed and analyzed by the quality control team.

Quality management system tools such risk assessment may determine the strategy of assays implemented and the validation strategy for each analytical method [25,26]. For example, we could consider in-process sterility testing on a sample taken 48–72 hours prior to final harvest, a Gram stain test of finished ATMP for product release, or even a product release based on a "negative-to-date" result (intermediate with negative sterility test for short shelf-life ATMPs). Another example of risk-based approach would be the validation of NAT method for mycoplasma contamination using only the most probable species to be present in the product of the seven species described in Eur. Ph. if this has been considered acceptable by the regulators. The quality control strategy is dynamic and may change throughout the lifecycle of the product. Any change in analytical methods or in the validated status of facilities, systems, processes or equipment should be assessed for risk to product quality prior to implementation and be documented accordingly, and an evaluation and monitoring plan completed after change implementation.

Lastly, it is important to mention that reference materials for ATMPs are very limited [27]. Several articles have been published during recent years pointing out the importance of reference standard existence of mesenchymal stromal cells to ensure the reliability of the analytical measurements [28–30], and some companies are working extensively on that direction – for example, ATCC MSC lines from different origins are already commercially available. Likewise, proficiency testing for ATMPs is also under development. In the meantime, we propose collaboration among quality control labs to perform comparability studies by exchanging ATMP samples.

REFERENCES

1. Codinach M, Blanco M, Ortega I *et al.* Design and validation of a consistent and reproducible manufacture process for the production of clinical-grade bone marrow-derived multipotent mesenchymal stromal cells. *Cytotherapy* 2016;18(9): 1197–208.
2. Aditya B, Reza M, Pouya M, Wasim SK. A Systematic Review And Meta-Analysis of Clinical Trials of Mesenchymal Stem Cell Therapy for Cartilage Repair. *Curr. Stem Cell Res. Ther* 2018;13(3): 215–25.
3. Zhao Y, Ma L. Systematic Review and Meta-analysis on Transplantation of Ex Vivo Cultivated Limbal Epithelial Stem Cell on Amniotic Membrane in Limbal Stem Cell Deficiency. *Cornea* 2015;34(5): 592–600.
4. Mishan MA, Yaseri M, Baradaran-Rafii A, Kanavi MR. Systematic review and meta-analysis investigating autograft versus allograft cultivated limbal epithelial transplantation in limbal stem cell deficiency. *Intl Ophthalmol.* 2019.
5. Uccelli A, Laroni A, Brundin L *et al.* MEsenchymal StEm cells for Multiple Sclerosis (MESEMS): a randomized, double blind, cross-over phase I/II clinical trial with autologous mesenchymal stem cells for the therapy of

multiple sclerosis. *Trials* 2019;20(1): 263.

6. Staff NP, Jones DT, Singer W. Mesenchymal Stromal Cell Therapies for Neurodegenerative Diseases. *Mayo Clinic Proceedings* 2019;94(5): 892–905.

7. Pettitt D, Arshad Z, Smith J, Stanic T, Holländer G, Brindley D. CAR-T Cells: A Systematic Review and Mixed Methods Analysis of the Clinical Trial Landscape. Mol. Ther. 2018;26(2): 342–53.

8. Horwitz ME, Wease S, Blackwell B *et al.* Phase I/II Study of Stem-Cell Transplantation Using a Single Cord Blood Unit Expanded Ex Vivo With Nicotinamide. *J. Clin. Oncol.* 2019;37(5): 367–74.

9. Regulation (EC) No 1394/2007 of 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 [online] ec.europa.eu/health/files/eudralex/vol-1/reg_2007_1394/reg_2007_1394_en.pdf.

10. Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, 2017 [online] eur-lex.europa.eu/legal-content/EN/TXT/?qid=1410944582971&uri=CELEX:02001L0083–20121116

11. Salmikangas P. Design and optimisation of a quality target product profile for ATMPs. The Organization for Professionals in Regulatory Affairs 2019.

12. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; Validation of analytical procedures: Text and methodology Q2(R1) 2005.

13. European Pharmacopoeia (Ph. Eur.) Chapter 2.6.1: Sterility; 2.6.27: Microbiological control of cellular products. 9th Edition. 2018.

14. Rustichelli D, Castiglia S, Gunetti M *et al.* Validation of analytical methods in compliance with good manufacturing practice: a practical approach. *J. Transl Med.* 2013; 11:197.

15. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; Q5A (R1) Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (CPMP/ICH/295/95). 1999.

16. Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014). 2018.

17. Codinach M, Lloret M, Torrents S *et al.* A practical approach of method validation for cell counting by flow cytometry in advanced therapy medicinal products. Cytotherapy 2017;19(5): S111.

18. Gunetti M, Castiglia S, Rustichelli D *et al.* Validation of analytical methods in GMP: the disposable Fast Read 102® device, an alternative practical approach for cell counting. *J. Transl Med.* 2012; 10:112.

19. Radrizzani M, Soncin S, Lo Cicero V, Andriolo G, Bolis S, Turchetto L. Quality Control Assays for Clinical-Grade Human Mesenchymal Stromal Cells: Methods for ATMP Release. In: Gnecchi M, editor. Mesenchymal Stem Cells: Methods and Protocols. New York, NY: Springer New York; 2016. p. 313–37.

20. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; Q6B: Note For Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. (CPMP/ICH/365/96). 1999.

21. Bravery CA, Carmen J, Fong T, Oprea W, Hoogendoorn KH, Woda J, *et al.* Potency assay development for cellular therapy products: an ISCT review of the requirements and experiences in the industry. *Cytotherapy* 2013;15(1): 9–19.

22. Guthrie K, Bruce A, Sangha N, Rivera E, Basu J. Potency evaluation of tissue engineered and regenerative medicine products. *Trends Biotechnol.* 2013; 31(9): 505–14.

23. Castella M, Boronat A, Martín-Ibáñez R *et al.* Development of a Novel Anti-CD19 Chimeric Antigen Receptor: A Paradigm for an Affordable CAR T Cell Production at Academic Institutions. Mol. Ther. Methods Clin. Dev. 2018; 12:134–44.

24. Priesner C, Esser R, Tischer S, Marburger M *et al.* Comparative Analysis of Clinical-Scale IFN-γ-Positive T-Cell Enrichment Using Partially and Fully Integrated Platforms. *Front. Immunol.* 2016; 7:393.

25. Lopez F, Bartolo CD, Piazza T *et al.* A Quality Risk Management Model Approach for Cell Therapy Manufacturing. *Risk Analysis* 2010; 30(12): 1857–71.

26. Viganò M, Budelli S, Lavazza C *et al.* Tips and Tricks for Validation of Quality Control Analytical Methods in Good Manufacturing Practice Mesenchymal Stromal Cell Production. *Stem Cells Intl* 2018;2018:3038565-.

 DOI: 10.18609/cgti.2019.123

27. Bravery CA, French A. Reference materials for cellular therapeutics. Cytotherapy 2014;16(9): 1187–96.

28. Tanavde V, Vaz C, Rao MS, Vemuri MC, Pochampally RR. Research using Mesenchymal Stem/Stromal Cells: quality metric towards developing a reference material. *Cytotherapy* 2015;17(9): 1169–77.

29. Viswanathan S, Keating A, Deans R,*et al.* Soliciting strategies for developing cell-based reference materials to advance mesenchymal stromal cell research and clinical translation. *Stem Cells Develop.* 2014;23(11): 1157–67.

30. Deans R. Towards the creation of a standard MSC line as a calibration tool. *Cytotherapy* 2015;17(9): 1167–8.

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CELL & GENE THERAPY INSIGHTS

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Responding to the challenges of flow cytometry in GMP product testing, a technical evaluation of the Accellix platform

Kevin W Johnson, Conor O'Malley, Steve Kunitake, Amanda Trent, Aaron Gassman, Jyostna Ivatury, Anna Brown and Geoff Hodge

Flow cytometry is a powerful tool used in the research and development of cell and gene therapy products. With this tool the researcher can gain valuable insight into the phenotype and function of populations of individual cells and how those cells respond to perturbations in their respective environments. In the development of cellular medicine, flow cytometry is used for assessment of culture health, phenotypic characterization of in process culture and final product, as well as functional characterization to quantify the effect of potential process changes as well as indicate the labs' capability of making a safe and effective product on the lab bench.

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Issues can and do arise with this valuable technology; yet, when development efforts are successful, the product proceeds onto clinical development and the need to translate research assays into a regulated environment is imperative. Cross-site reproducibility and the relative scarcity of a labor pool trained in this technology within the GMP testing environment are examples of the challenges facing the implementation of flow cytometry-based assays broadly to inform cell product characterization and release. Manufacturers of flow cytometry instrumentation must rise to these challenges in order to make their technology broadly applicable to the rigors required for this type of testing. To this end, Accellix Inc. has developed the Accellix Platform, a measurement and analysis system composed of a compact benchtop flow cytometer instrument and a single-use cartridge. Herein we report the results of a technical evaluation of the Accellix platform's automated sample staining and data acquisition functionalities. We will assess its performance in key assay metrics of linearity and precision by repeatability, in order to assess its capabilities for its proposed implementation in GMP processes, e.g., cell phenotype enumeration, cell population characterization, process quality control (QC), etc., that are critical in the manufacture of a T-cell immunotherapy product.

BACKGROUND & ACCELLIX OVERVIEW

Personalized, cell-based therapy is emerging as a paradigm-shifting strategy in healthcare. To fully harness the potential of cutting-edge cellular engineering, stringent process controls must be put into place that monitor and measure critical parameters of the cell product at specific points in the manufacturing process, from patient sample acquisition to drug product release.

In order to increase accessibility to a wide range of cell and gene therapy products, streamlining and simplification of process and analytical procedures will be necessary as to reduce timelines and costs associated with the manufacture of these promising therapies. Of the analytical procedures employed in cell therapy manufacturing, flow cytometry has proven to be one of the most intractable methods to be streamlined and simplified. Traditional flow cytometry is the heretofore "gold standard" for cell phenotyping, enumeration and characterization. Yet, the challenge of incorporating the method into a commercially viable cell therapy manufacturing workflow remains, due to the complexity and time-intensive nature of the operation and maintenance of traditional flow cytometers. Standardization and maintenance of instrumentation, requirement for fluorescence compensation, reagent variability, and subjective expert data analysis are all features of traditional flow cytometry that constitute significant challenges when considering its candidacy as a platform for robust analytical methods which can pass the stringent validation procedures of a regulated manufacturing environment.

The Accellix System is designed to offer a streamlined workflow solution by migrating traditional flow cytometry assays onto a relatively small footprint instrument. The system combines automated sample preparation within a single-use microfluidic cartridge, customized ambient-stable reagents, high sensitivity fluorescent event detection, and assay-specific auto analysis (which is out of the scope of this particular study). Sample processing, including fluorescent staining and fixing of the cells, RBC lysis (if needed), and sample dilution are executed wholly within a closed microfluidic cartridge. To initiate the assay, a lab user pipettes patient sample directly into a customized, dried reagent formulation; this fluorescent staining of the cells potentially allows for significant gains in intermediate precision over traditional flow cytometry assays and should be tested in future studies.

EXPERIMENT RATIONALE & OVERVIEW

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) has set guidelines for determining if an assay is validated for its intended use. The criteria to consider for complete validation of a quantitative assay include assessment of Specificity, Linearity, Range, Accuracy, Precision, Robustness and Stability [1]. We evaluated the linearity and precision of the Accellix System to establish proof of concept and to determine if this technology warrants further development for our analytical procedures. We used the off-the-shelf T-cell cartridge and a custom cartridge designed to identify our construct, an antibody-coupled T-cell receptor (ACTR) composed of the extracellular domain of CD16 linked to a CD3ζ signaling domain and to a costimulatory domain. ACTR-expressing T cells are universal and can be flexibly paired with desired therapeutic antibodies to target tumor antigens. The performance of the Accellix assays were benchmarked against an in-house conventional polychromatic flow cytometry procedure. For testing material, we first evaluated Beckman Coulter's Cyto-trol™ reagent, a QC reagent of fixed, lyophilized cells with stable cell populations which can be compared across instruments as well as to the manufacturer's certificate of analysis (COA). We then proceeded to test previously frozen, ficoll-purified PBMC from healthy donor apheresis and our post-harvest ACTR T cell product to represent the beginning and end of our manufacturing process, respectively. In our manufacturing process, a donor subject's leukocytes are collected via leukaphereis and purified to PMBCs via density gradient centrifugation. T cells within these PBMC's are activated and transduced with the ACTR construct *ex vivo* and then expanded by continued culture. These samples are thawed, cryoprotectant is washed out with fresh media and cells and split for analysis on each platform. For manual flow cytometry, the media is washed out and cells are resuspended in a staining buffer consisting of Dulbecco's Phosphate Buffered Saline and Bovine Serum Albumin before being stained with a panel to identify T cells and high-level subsets. (see Table 1 for an overview of the panels used in each instrument). For samples run on the Accellix instrument, thawed/washed

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TABLE 1.

Overview of experiments used to compare Accellix to traditional cytometry.

Cells Tested	Samples (n)	Replicates	Traditional Cytometry Antibody Panel (Clone)	Accellix Cartridge Panel (Clone)	Endpoints
Coulter Cyto-trol™	1	2-3	CD45 (HIT3) CD3 (UCHT1) CD4 (RPA-T4) CD8a (RPA-T8)	CD45 (HIT3) CD3 (UCHT1) CD4 (RPA-T4) CD8a (RPA-T8)	CD3 (% of CD45) CD4 (% of CD3) CD8 (% of CD3)
PBMC and ACTR T-cells	5 of each	3	Viability (7AAD)[1] CD45 (HIT3) CD3 (UCHT1) CD4 (RPA-T4) CD8a (RPA-T8)	Viability (DiYO-3™) CD45 (HIT3) CD3 (UCHT1) CD4 (RPA-T4) CD8a (RPA-T8)	
ACTR T-Cells/ MOCK controlled mix dilution series	1 series of 6 dilutions	2	Viability (7AAD) CD45 (HIT3) CD3 (UCHT1) CD16 (B73.1)[1]	Viability (DiYO-3™) CD45 (HIT3) CD3 (UCHT1) CD16 (B73.1)[2]	ACTR T-cells (CD3+CD16+)[2]

Four types of samples were used to assess performance of the Accellix. Coulter CytoTrol™ cells, PBMC, ACTR T-cells and MOCK transduced T-cells were used to test reproducibility and linearity. Panels with identical antibody clones, but different fluorochromes optimized for each instrument, were tested with endpoints of CD3, CD4, CD8 and ACTR T-cell relative frequency in each sample.
7-Aminoactinomycin D
The extracellular domain of ACTR is characterized by the corresponding portion of human FCRIII receptor or CD16, detectable by this clone

sample in media was added directly to their dry reagent tube consisting of the same antibody clones used for manual cytometry, though these were spectrally optimized for the Accellix. All samples were run in triplicate to assess repeatability of the assays run on the platform. Figure 1 shows a representative comparison of the different gating strategies for a PBMC sample on the two platforms.

In order to assess dilutional linearity, proportional frequencies of each endpoint need to be generated and tested. To this end we used spiked known proportions of ACTR T-cell product into cells that had gone through a similar manufacturing process but had not been transduced with the construct (MOCK cells) as a diluent to generate known concentrations of transduced cells. Briefly, MOCK cells and a representative T-cell product were normalized to the same concentration and a two-fold serial dilution series of six samples was generated by diluting ACTR drug product cell suspension into the MOCK cell suspension.

The resulting data from these experiments were converted to FCS files and manually gated in FlowJo™ (Treestar Software) flow cytometry analysis software in order to obtain relative frequency of the CD3, CD4 and CD8 population to assess precision by repeatability, and to obtain frequency of ACTR in serially diluted samples to assess linearity. The automated analysis function of the Accellix will be evaluated in future work with the system. Dead cells were identified in each assay platform when fixed cells were not used and eliminated from the analysis. Table 1 outlines the panel used in the Accellix cartridges vs. our current flow cytometry procedure.

RESULTS

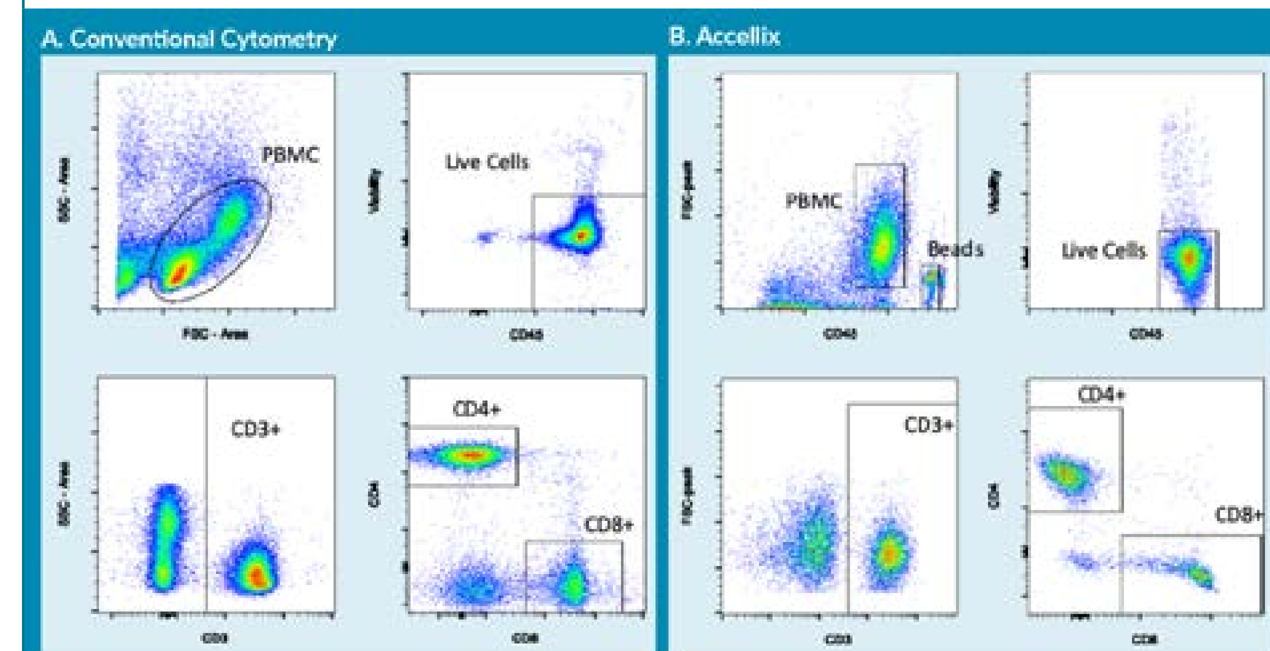
Repeatability

When comparing frequencies of T-cell populations in the Cyto-trol™ cells, Figure 2 shows both platforms were able to meet the manufacturer's specifications for the cell ranges expected in the control product for CD3 and CD8. Measured CD4 cells were slightly above this range; however, when compared to our conventional cytometry data, the Accellix data closely agreed for all measured populations.

When T-cell population frequencies obtained by each of the two methods are compared by linear regression, the slope of the correlation curve for CD4 and CD8 indicate a bias between the technologies; however, a high correlation is observed over the range of samples (Figure 3). The range of T cells in these samples represents as low as 50% of viable lymphocytes in the PBMC samples to nearly 100% of cells representative of our T-cell product. CD3 shows good correlation over this range ($R2 = 0.9020$; $p < 0.0001$) and both CD4 ($R2 = 0.9339$; $p < 0.0001$)

FIGURE 1

Comparison of gating strategies.



Differences in gating strategies between the Accellix and Conventional Cytometry for identifying T-cell subsets from a PBMC sample. In Conventional Cytometry (A), after doublet discrimination PBMCs are identified on a Forward Scatter (FSC) vs. Side Scatter (SSC) plot. Lice cells are identified as CD45+ and Viability dye (7AAD)-. T cells are subsequently Identified as CD3+ and CD4+ and CD8+ are gated as subsets of CD3+. On the Accellix (B), Cells are Identified as CD45+ and discriminated from QC beads by their higher FSC and lower fluorescence in the same CD45 channel. Live cells are then similarly identified as CD45+ and Viability dye (DiYO-3™negative). Similar subsequent gating strategy is then followed to identify T cells, and CD4+/CD8+ subsets respectively.

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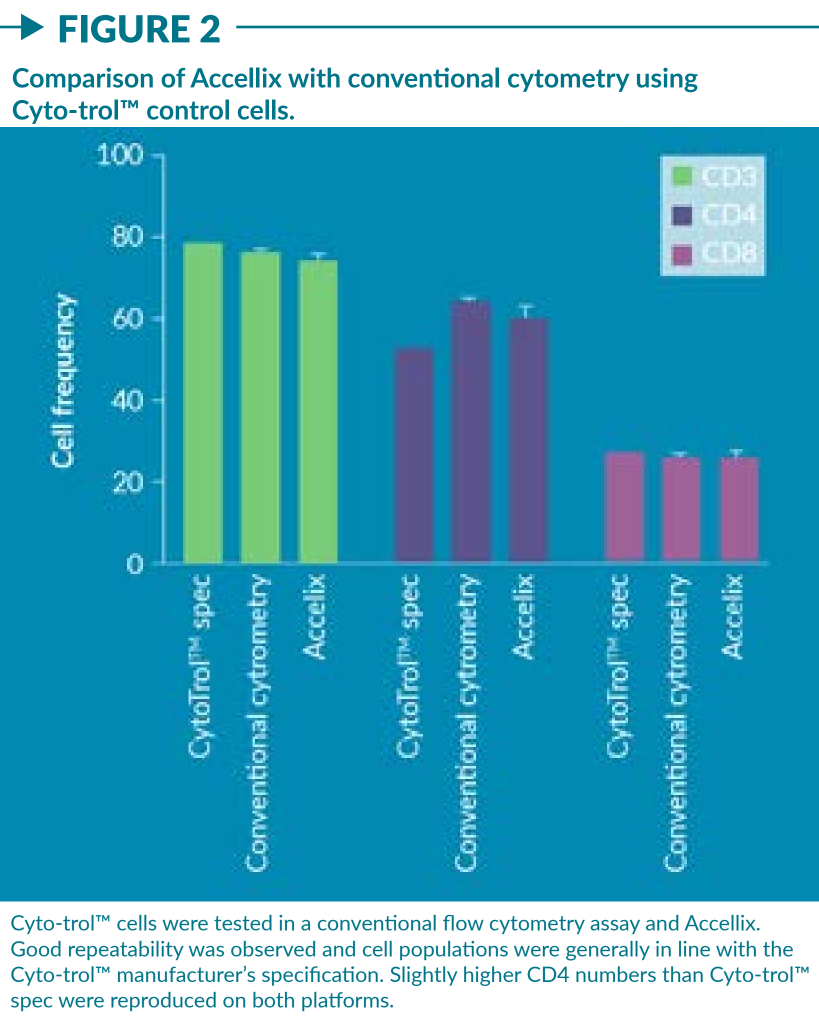
FIGURE 2

Comparison of Accellix with conventional cytometry using Cyto-trol™ control cells.



Cyto-trol™ cells were tested in a conventional flow cytometry assay and Accellix. Good repeatability was observed and cell populations were generally in line with the Cyto-trol™ manufacturer's specification. Slightly higher CD4 numbers than Cyto-trol™ spec were reproduced on both platforms.

and CD8 (0.9644 $p < 0.0001$) show excellent correlation over a range of as low as 20% to up to 80% of T-cells.

In order to assess repeatability, the coefficient of variation (CV) was calculated from the replicate samples run on each platform. Figure 4 shows excellent repeatability on both platforms and comparable results. It should be noted that a benchmark of acceptable variability for a flow cytometry assay is 20% [2]. Agreement between the two methods, as measured by relative percent difference (RPD) between the values calculated from each instrument, are summarized in Table 2. Good reproducibility was observed with values generally under 30% and average RPD for all samples at 5%, 13% and 19% for CD3, CD4 and CD8 respectively. RPD values above 30% were observed for two out of ten samples for CD8, for samples three and ten, which represent samples with the lowest initial cell concentration and as result, the lowest number of events acquired on the Accellix.

Linearity

When comparing ACTR frequency measurements determined by the Accellix to expected concentrations, data presented in Figure 5 demonstrate excellent linearity with an R^2 value of 0.9987 ($p<0.0001$) for ACTR T cells and RPD values (Table 3) of less than or equal to 12.1% throughout the entirety of the dilution series. These data represent good dilutional linearity of ACTR T-cell frequencies as low as 1.5%.

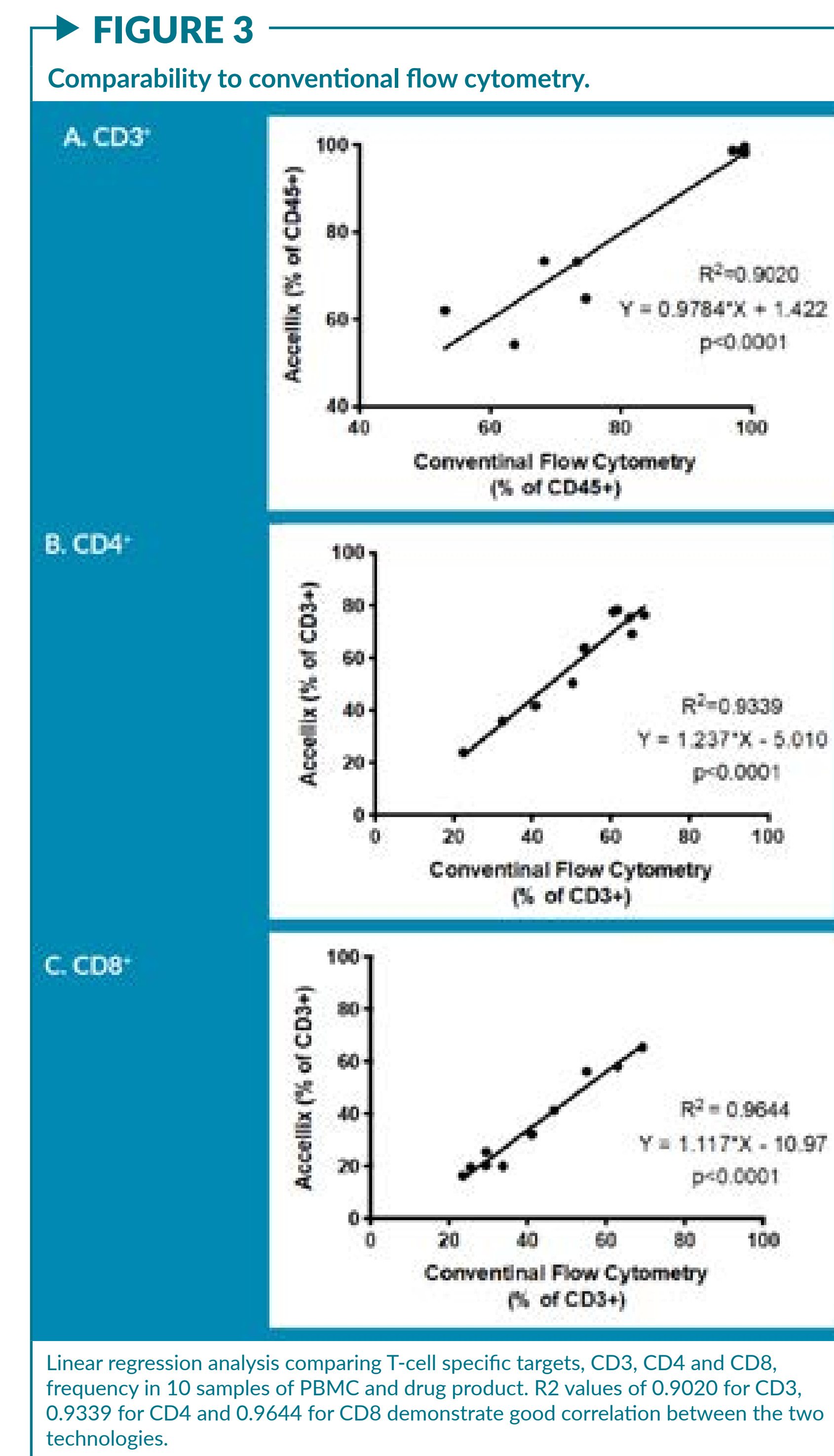
TRANSLATION INSIGHT

The data from these studies taken together show an initial proof-of-concept for this platform, which can be further evaluated for use in GMP manufacturing assays. Manual analysis of the results shows a platform with the potential for providing data to the regulated environment of T-cell manufacturing. Further evaluation would be needed to fully validate this platform, particularly in regard to is performance on extended data sets showing intermediate precision, reproducibility, range, and quantitation limits. Particular attention should be paid to assessing optimal cell input; this should be evaluated and appropriately controlled for each assay migrated onto the Accellix cartridge. Absolute cell counting and automated data analysis are also available features of this platform, though they are not assessed in the current study.

The Accellix Platform is best suited to provide automated, in-process, single cell insights on individual samples that can be encountered in the typical cell therapy manufacturing QC lab. To provide these insights, the current assay configuration on Accellix requires a preparation/execution/analysis cycle of approximately 35 minutes for a single sample as opposed to 3 hours or more estimated for a typical GMP sample requiring instrument startup and QC, samples preparation/staining and analysis. For extensive studies conducted in basic research, where testing multiple conditions in a single experiment may be desired, this combination of duration and single-cartridge throughput may make the Accellix platform less suitable than other flow cytometric platforms. Accellix

FIGURE 3

Comparability to conventional flow cytometry.



Linear regression analysis comparing T-cell specific targets, CD3, CD4 and CD8, frequency in 10 samples of PBMC and drug product. R2 values of 0.9020 for CD3, 0.9339 for CD4 and 0.9644 for CD8 demonstrate good correlation between the two technologies.

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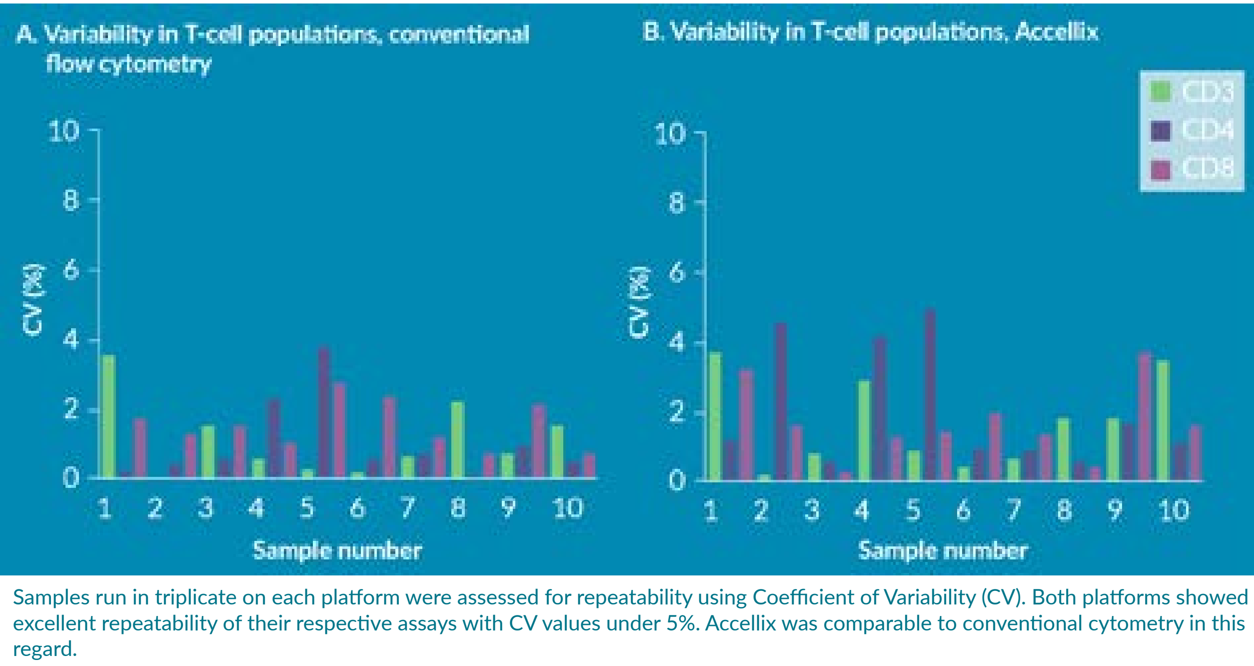
FIGURE 4

Repeatability comparison of conventional cytometry to Accellix.



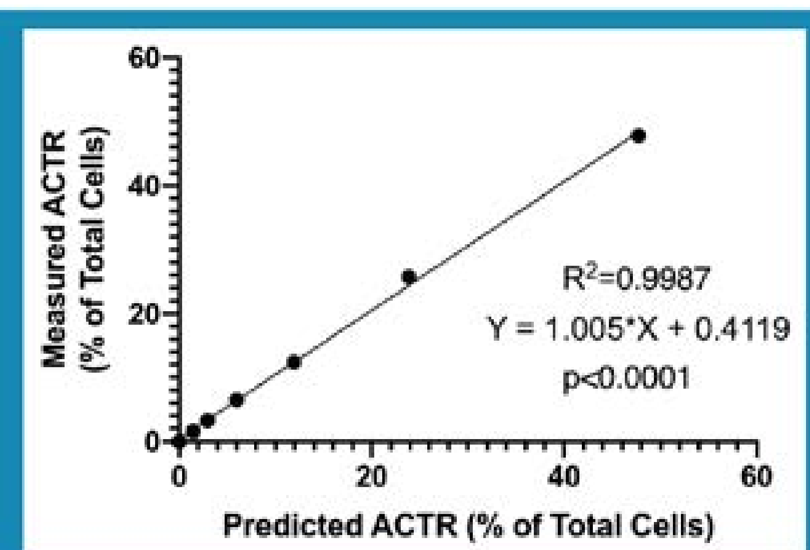
Samples run in triplicate on each platform were assessed for repeatability using Coefficient of Variability (CV). Both platforms showed excellent repeatability of their respective assays with CV values under 5%. Accellix was comparable to conventional cytometry in this regard.

will not, and apparently does not aim to, replace traditional flow cytometry in this regard. To fully evaluate the Accellix Platform, a more extensive validation study (with a large number of samples) would require a significant amount of time, assuming the current one-cartridge-at-a-time throughput of the Accellix Platform. It is noted that a higher throughput could be obtained by operating multiple Accellix instruments in parallel, or by ensuring that the next generation of the instrument can process multiple cartridges simultaneously. It is not clear at this time if this feature is under development for future versions.).

Potential gains in the simplification and standardization of flow cytometry assays make the Accellix System worth further evaluation for use as an assay platform in the GMP manufacturing environment. We plan to perform further evaluations in regard to its performance in additional assay qualification endpoints, as well as to assess the Accellix's capability to automate data analysis and provide absolute counts for single platform enumeration of cell subsets.

FIGURE 5

Linearity of ACTR+ cells in a controlled mixed dilution series.



Linear dilution series analyzed on the Accellix platform. A linear regression analysis showing excellent dilutional linearity when the measured frequency of ACTR+ cells is compared against the expected concentration of each dilution. An R2 value of 0.9987 was observed.

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TABLE 2

Reproducibility between Accellix and conventional cytometry.

Sample number	CD3			CD4			CD8		
	Conventional cytometry (% CD3)	Accellix (% CD3)	RPD* (%)	Conventional cytometry (% CD4)	Accellix (% CD4)	RPD* (%)	Conventional Cytometry (% CD8)	Accellix (% CD8)	RPD* (%)
1	68.2	72.7	6.5	61.7	78.3	26.9	23.5	16.4	30.4
2	98.9	98.7	0.2	22.4	24.0	7.0	69.2	65.4	5.4
3	53.1	61.0	14.8	60.6	77.7	28.1	33.7	20.0	40.7
4	98.4	98.8	0.4	32.4	35.9	10.8	62.9	58.1	7.7
5	98.9	99.6	0.7	40.7	41.8	2.8	54.9	56.1	2.2
6	97.1	98.7	1.6	65.2	69.0	5.8	29.4	25.6	12.9
7	98.9	98.5	0.4	50.3	50.3	0.1	46.7	41.4	11.4
8	63.7	54.2	14.9	53.2	63.7	19.8	41	32.1	21.8
9	74.6	64.8	13.1	68.4	76.4	11.7	25.5	19.6	23.0
10	73.3	73.3	0.0	64.7	75.4	16.5	29.4	20.4	30.6
Mean RPD (%)			5.3			12.9			18.6

Direct comparison of the two technologies. Matching samples were run via conventional flow cytometry and on the Accellix using their T-cell cartridge. This table represents good reproducibility between the platforms with RPD values generally under 30% and average RPD of 5%, 13% and 19% for CD3, CD4 and CD8 respectively. RPD of above 30% were observed in two out of ten samples for CD8.
RPD: Relative percent difference.
*RPD = (Accellix result - conventional cytometry result)÷ conventional cytometry result

TABLE 3

Comparison of measured to predicted values of the dilution series

Fold dilution	Measured frequency (%)	Predicted frequency (%)	RPD (%)
Neat ACTR T-cells	47.8	47.8	N/A
2×	25.7	23.9	7.6
4×	12.4	11.9	3.9
8×	6.5	6.0	9.4
16×	3.3	3.0	9.6
32×	1.7	1.5	12.1

An initial frequency from Neat ACTR T-Cells was used to extrapolate predicted frequencies of the dilution series. These values were used to determine RPD of the measured to the predicted frequency for each dilution.
RPD: Relative percent difference.

AUTHORSHIP & CONFLICT OF INTEREST

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REFERENCES

1. International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (Q6B), March 1999 [online] www.ich.org/products/guidelines/quality/article/quality-guidelines.html
2. Wu DY, Patti-Diaz L, Hill CG. Development and validation of flow cytometry methods for pharmacodynamic clinical biomarkers. *Bioanalysis* 2010; 2(9): 1617–1626.

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CELL & GENE THERAPY INSIGHTS

DOWNSTREAM BIOPROCESSING

SPOTLIGHT

INNOVATOR INSIGHT

Optimizing the clarification of industrial scale viral vector culture for gene therapy

Bharath Raghavan, Mike Collins, Stephanie Walls, Alexander Lambropoulos & Silke Bergheim-Pietza

Viral-based vector systems such as lentivirus (LV) and adeno-associated virus (AAV) are widely used and show great potential for delivery of genetic material to target cells in gene therapy. Downstream processing of LV and AAV offers its own unique challenges to generate clinical products of high titer, high potency, and high purity. For AAV, downstream challenges include the undesired production of empty capsids, and the process typically requires a cell lysis step, which generates a significant amount of host cell contaminants. In the case of LV, downstream challenges include low virus stability due to the presence of a fragile lipid envelope layer, as well as sensitivity to pH variations, salt concentrations, and shear stress. The objective of this work was to identify an efficient clarification strategy to remove a wide range of impurities found in typical adherent and suspension based viral vector cell culture. These include host cells, cell debris, aggregates, and cell culture media components. The clarification step needs to combine high throughput for impurity removal, high product yield, and ease of scale-up to prepare for downstream operations. Our testing evaluated various depth filters, prefilters, and bioburden reduction membrane filters, made up of different organic and inorganic materials, to clarify the viral vector cell culture. Both AAV and LV processes can be adherent or suspension based, each having their own challenges. In this work, we compared clarification options in terms of throughput and recovery for adherent LV and suspension AAV viral vector feed streams.

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INTRODUCTION

Gene therapies are some of today's most promising patient treatments, where gene-modifying technologies are used to repair, correct, or add new functions to the body's own cells. After years of research, the industry is seeing a rapidly increasing pipeline of gene therapy products and a few commercially available products in the USA. The pipeline is very strong, with over 1000 products in clinical trials, and with approximately 90 in Phase 3 [1]. The products must all undergo process development, where the challenge is to manufacture functional product economically, with sufficient quantity and quality. LV and AAV are the most commonly used viruses for therapeutic purposes due to their specific functional properties.

One of the methods in gene therapy uses expression systems capable of making viral vectors in quantities suitable for therapeutic use. The most commonly used systems are based on transient transfection in either adherent or suspension cell culture.

Yield is an important parameter when evaluating a viral vector gene therapy process. Each process step potentially reduces the amount of active viral vectors. The first process step after cell culture is the removal of cells, cell debris, and other impurities to reduce biological burden as much as possible. The easiest and most economical technology to clarify the cell culture is filtration. The chosen filter or filter combination should demonstrate high throughput and high yield.

This study not only describes how different filter materials for cell culture clarification influence yield, but it also demonstrates a strategy to define an efficient and scalable method for clarification. The study investigates the feasibility of filters made from cellulose, polymers, or inorganic material such as glass fiber to clarify LV produced using HEK293T cells in adherent format, or AAV produced in HEK293 cells grown in suspension. The results that are shown demonstrate the influence of filter materials and construction on throughput and yield during the clarification step, and will help illustrate a strategy to define the most efficient and scalable filtration steps.

MATERIALS

Cell culture properties

To cover a broad range of processes, two types of cell culture were used.

Lentivirus

Lentivirus product was produced with HEK293T cells in an adherent cell culture bioreactor. The harvested post-transfection solution had a turbidity of up to 20 nephelometric turbidity units (NTU). Figure 1 shows an iCELLis® 500+ bioreactor.

FIGURE 1

iCELLis 500+ single-use fixed-bed bioreactor.

Adeno-associated virus

The adeno-associated virus product was produced using HEK293 cells in a suspension cell culture bioreactor. The suspension cell culture was harvested after the cells were lysed and had a turbidity around 430 - 540 NTU. Figure 2 shows an Allegro™ STR bioreactor.

Filter choice

Depth filters, prefilters, and bioburden reduction membrane filters were tested with the described cell cultures.

Depth filters

The primary clarification step removes large debris and macromolecular complexes from the harvesting bioreactor. One of the most common technologies for primary clarification is depth filtration. Depth filters remove contaminants through means of direct impaction, entrapment, and adsorption. These modes of separation occur on the surface of the filter, as well as within the matrix of the depth media.

Depth filters are made with different combinations of cellulose, perlite, diatomaceous earth, and resin binders. These filters have high solids loading capacity due to the depth of the filter media, which allows for the removal of a broad size range of debris. Removing the debris allows for higher throughputs to be achieved on the next process step – bioburden reduction.

The Seitz depth filters were tested in a single layer format and in dual layer combinations. The compositions of the filters used in this study are shown in Table 1.

Depth filters are known for high particle retention capacity. The particles are retained and bound to the filter material. This feature is ideal for high turbidity solutions. Therefore, they were used for the filtration of the AAV suspension cell cultures.

Prefilters & bioburden reduction membrane filters

The filter media is made of polymers such as polyethersulfone (PES), nylon, polyvinylidene fluoride (PVDF), and inorganic material such as resin-bonded glass fiber (GF). The polymeric membrane filters and the glass fiber prefilters are generally thinner than the cellulose based depth filters and show a more limited, but nevertheless sufficient particle retention capacity. In this study, a variety of filters were used to test both LV and AAV cell cultures. Table 1 shows the different filters used for each, as well as their retention ratings and primary material of construction.

FILTRATION TRIAL METHODOLOGY & RESULTS

Lentivirus

In the first stage of evaluation, all filters listed in Table 1 for the LV process, except the Supor EAV,

FIGURE 2

Allegro STR 200 single-use stirred tank bioreactor.

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TABLE 1

Filter types and retention ratings of the filters tested in these studies.

Process	Filter	Media material of construction	Retention ratings	Filter type
LV	SuporLife®	PES	0.45 µm	Bioburden reduction membrane filter
LV	Fluorodyne® II DBL	PVDF	0.45 µm	Bioburden reduction membrane filter
LV	Ultipor® N66	Nylon 66	0.45 µm	Bioburden reduction membrane filter
LV	PreFlow™ UB	Resin-bonded GF	0.45 µm	Prefilter
LV	Supor® EAV	PES	0.2 µm	Bioburden reduction membrane filter
AAV	Seitz Bio 10	Cellulose, resin	0.2–0.4 µm	Depth filter
AAV	Seitz V100P	Cellulose, perlite, resin	2–4 µm	Depth filter
AAV	Seitz HP PDH11 (K700P plus V100P)	Cellulose, diatomaceous earth, perlite, resin	2–15 µm	Depth filter
AAV	Seitz HP PDK11 (K900P plus V100P)	Cellulose, diatomaceous earth, perlite, resin	2–20 µm	Depth filter
AAV	Seitz HP PDP8 (T1500P plus K700P) plus Bio 10 in series	Cellulose, diatomaceous earth, perlite, resin	0.2–30 µm	Depth filter

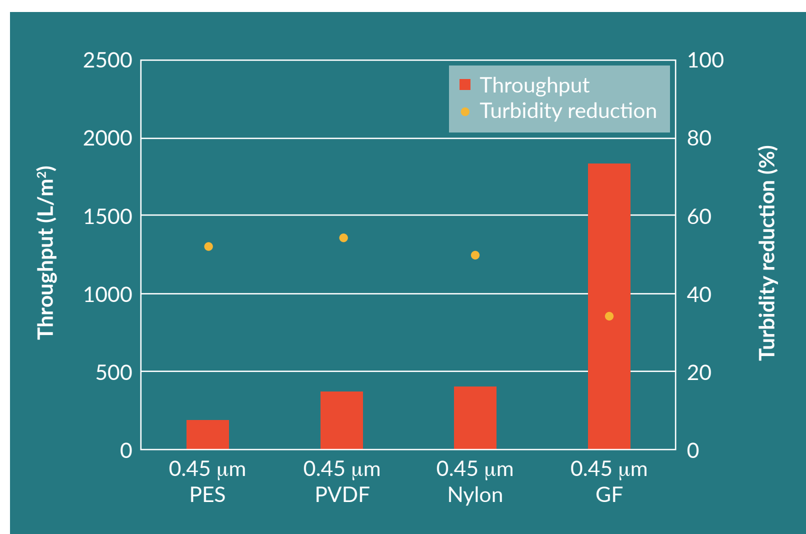
were tested. The cell culture feed turbidity was 7 NTU and the filtration experiments were performed at a constant pressure of 0.5 barg. Throughput, turbidity reduction, and viral vector yield were measured for each filter. Figure 3 compares the throughput and turbidity reduction of the different filters tested. Figure 4 shows the corresponding infectious particle recovery for the various filters.

Comparing the results of the prefilters and membrane filters, the GF filter achieved a throughput that was 5–10 times higher than the other filters. The GF filter had an infectious particle yield close to 100%.

Since the GF filter is a nominally rated 0.45 µm prefilter, the inclusion of an additional bioburden reduction membrane filter as a second filtration step is required. A variety of membrane filters in series with the GF filter were tested. The testing was performed

FIGURE 3

Throughput (L/m²) achieved on the bench and turbidity reduction (%) for LV process.



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with the PVDF 0.45 µm, the nylon 0.45 µm, and the PES 0.45 µm membrane filters. Additionally, a nominally rated 0.2 µm PES was tested in parallel with the following filter trains (0.45 µm GF prefilter + 0.45 µm nylon membrane, 0.45 µm GF prefilter + 0.45 µm PES, and 0.45 µm GF prefilter + 0.45 µm PVDF membrane).

The cell culture for the second run had a feed turbidity of 4 NTU and the filtration experiments were performed at a constant pressure of 0.5 barg. Figure 5 compares the throughput and turbidity reduction for the different filters. Figure 6 shows the corresponding infectious particle recovery for the various filters.

Comparing the results of the different filter combinations, the GF plus PVDF filter train achieved the highest throughput and highest infectious particle yield. This combination had an acceptable turbidity reduction.

Each filter combination, other than the 0.2 µm PES, utilizes two filters in series. Even though the throughput of the 0.2 µm PES was the lowest, this is a feasible option as well, considering only one filter is being used. The turbidity reduction and infectious particle yield for the 0.2 µm PES is similar to the other filter combinations.

FIGURE 4

Yield (%) of infectious particles post-filtration for LV process.

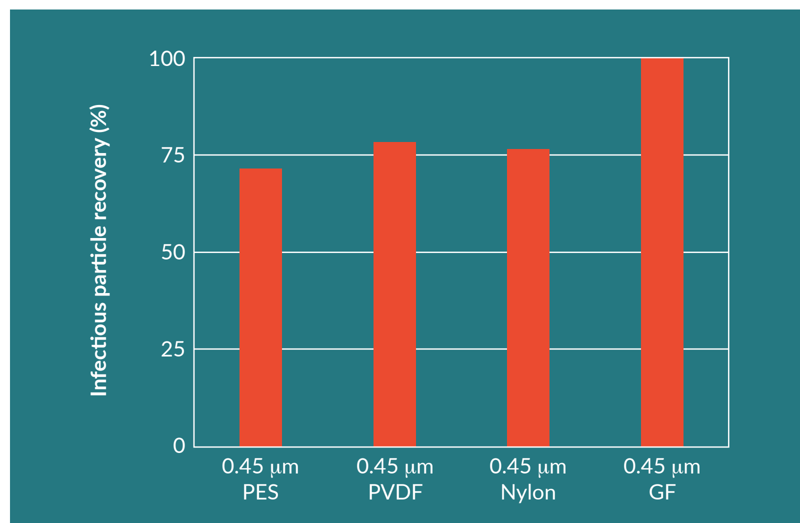
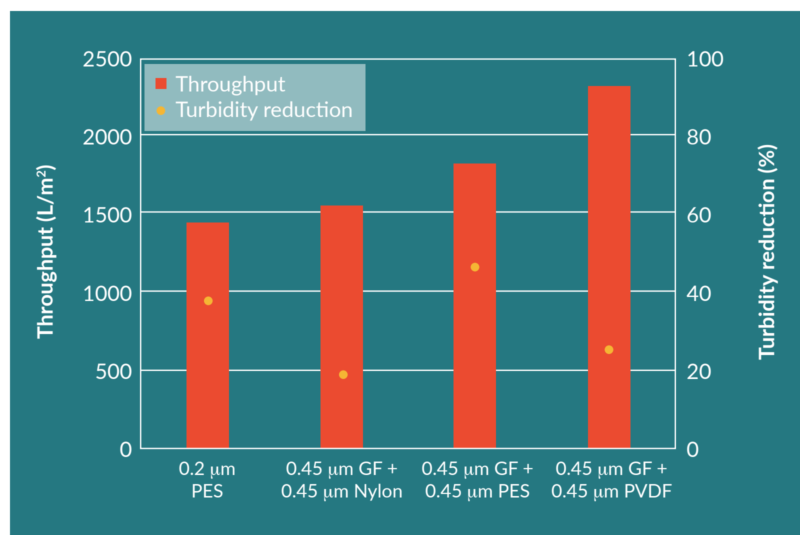


FIGURE 5

Throughput (L/m²) achieved on the bench and turbidity reduction (%) for LV process.



Cost/efficiency analysis for LV filtration

The combination of the GF prefilter and the PVDF membrane filter showed the best results with respect to throughput and product yield. However, it is a two-step filtration process. From an economical and operational point of view, a one-step filtration process with the nominally rated 0.2 µm PES membrane filter can be considered as well. Comparing 254 mm (10 in.) capsules, the PES membrane filter with 1.06 m^2 effective filtration area

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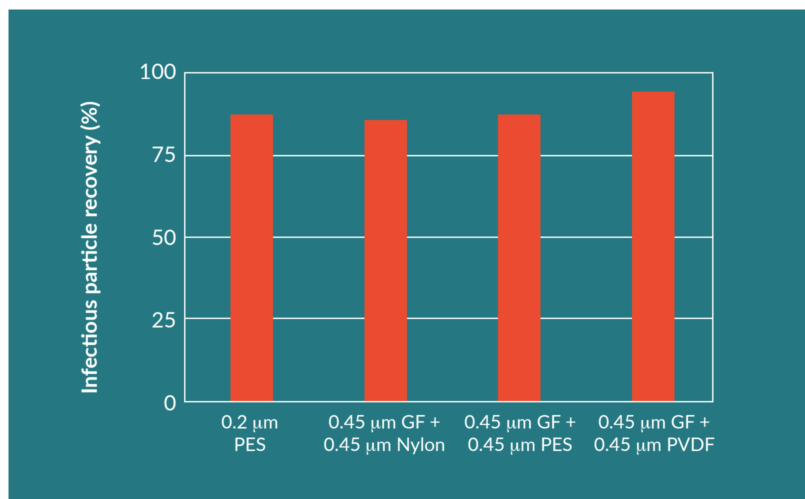
FIGURE 6

Yield (%) of infectious particles post-filtration for LV process.



(EFA) provides a significantly higher surface area than the GF prefilter with 0.68 m² EFA, and the PVDF membrane filter with 0.55 m² EFA.

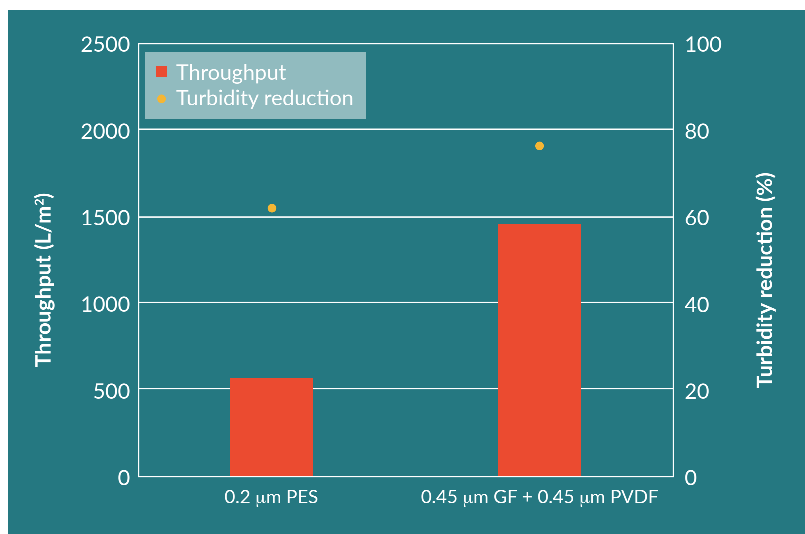
To evaluate the influence of surface area per 254 mm (10 in.) filter capsule, another test was performed. The combination of the GF prefilter and the PVDF membrane filter was tested in parallel with the 0.2 µm PES membrane. The cell culture feed had a turbidity of 14 NTU and the experiment was performed at a constant pressure of 1 barg. Throughputs and turbidity reduction were measured for each filter, shown in Figure 7. Figure 8 shows the corresponding infectious particle recovery for the various filters. Normalizing the filters to determine the theoretical volumes that could be processed by a 254 mm (10 in.) capsule are shown in Figure 9.

The difference in throughputs seen in Figure 7 is compensated for by the higher area per 254 mm (10 in.) module for the 0.2 µm PES membrane filter. The processed volumes per 254 mm (10 in.) module (Figure 9) are much closer to each other than throughputs from Figure 7.

Disposables for a single step filtration can potentially cost less than disposables for a dual step filtration. For this reason, both listed options are viable, but throughput, yield, and cost need to all be considered when making a choice.

FIGURE 7

Throughput (L/m²) achieved on the bench and turbidity reduction (%) for LV process.



Adeno-associated virus

The AAV suspension cell cultures in this study required a lysis step to release the virus from the cells prior to clarification. The combination of cells in suspension and the lysis step results in a significantly higher feed turbidity than an adherent cell culture process. For this study, the first AAV cell culture tested had a turbidity of 430 NTU. The filtration experiments were stopped at a predetermined terminal differential pressure. Due to the higher feed turbidity, depth filters were selected for primary clarification step. Throughput and viral vector yield were measured for each filter. After depth filtration, each solution was filtered

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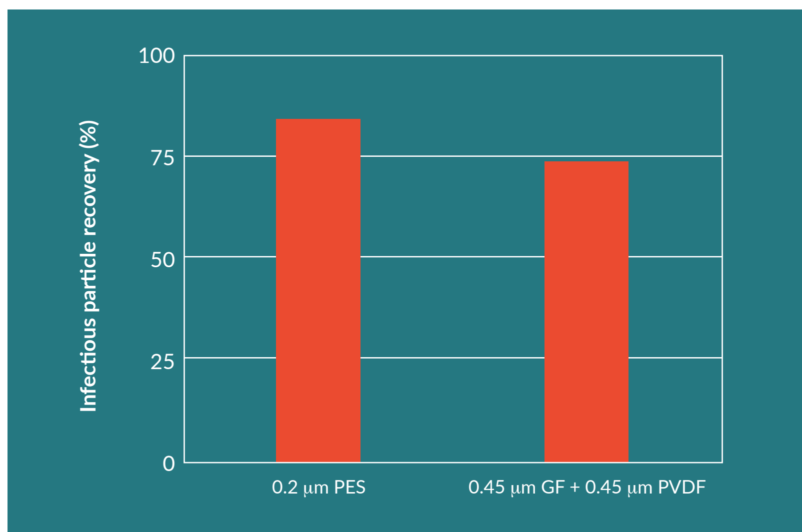
FIGURE 8

Yield (%) of infectious particles post-filtration for LV process.



through a 0.2 µm membrane filter to reduce potential bioburden.

Figures 10 & 11 show throughputs and viral vector recovery with different depth filter options.

The Seitz HP PDH11 depth filter (Seitz K700P in series with Seitz V100P) had a high recovery similar to the Seitz Bio 10 filter. It also had the highest throughput of all three depth filter options. The Seitz K700P layer retained contaminants in the range of 6 to 15 µm and protected the finer Seitz V100P layer of the filter. This was evident when comparing the throughputs between the Seitz V100P alone versus the Seitz HP PDH11.

The Seitz Bio 10 filter showed the highest yield. Since the retention rating of the Seitz Bio 10 filter ranges from 0.2 to 0.4 µm, a second filtration test was performed to determine if a suitable coarser depth filter could protect the Seitz Bio 10 layer and improve the throughput without reducing the viral vector yield.

For the second test, an AAV cell culture with a feed turbidity of 540 NTU was used. The filtration experiments were stopped when the filter system reached a

FIGURE 9

Volume (L) that can be processed by a 254 mm (10 in.) filter capsule for LV process.

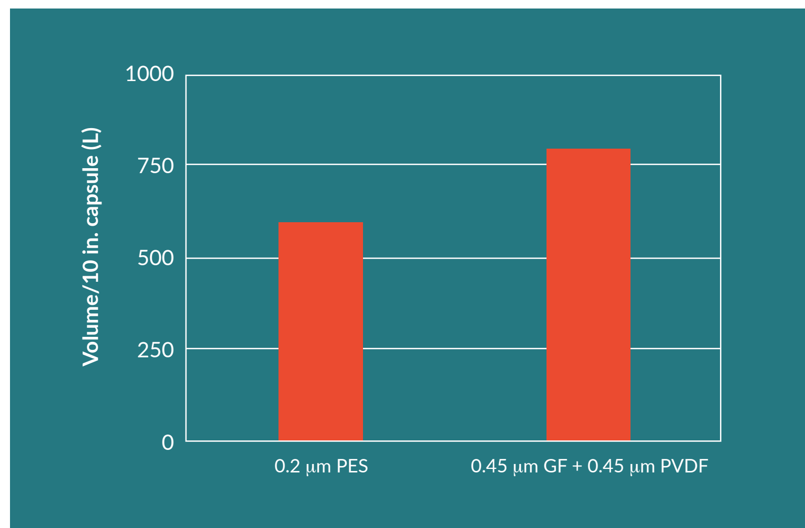


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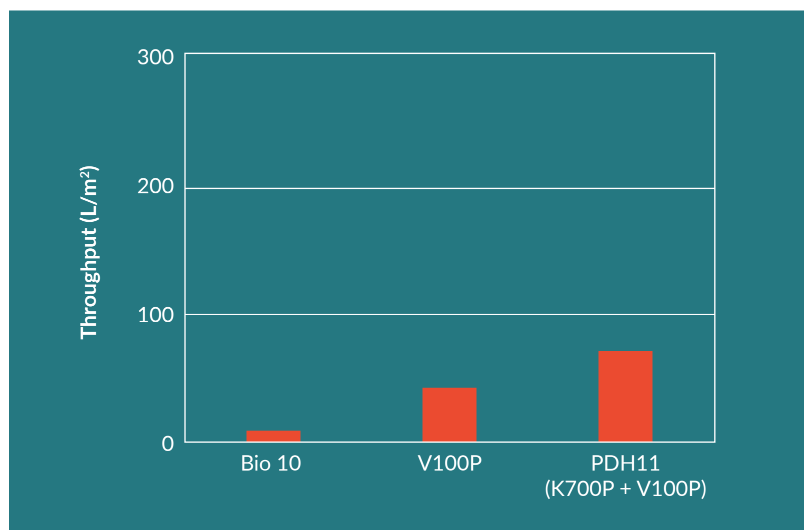
FIGURE 10

Throughput (L/m²) achieved on the bench for AAV process.



predetermined terminal differential pressure or no more feed material was available. **Figure 12** shows the throughput for each filter combination that was tested, while **Figure 13** shows the viral vector yield post-filtration.

The combination of a Seitz HP PDP8 dual layer filter in series with a Seitz Bio 10 filter resulted in the highest throughput and the highest viral vector yield. The Seitz HP PDP8 dual layer filter is made up from a Seitz T1500P upstream, coarse layer and a Seitz K700P downstream, finer layer. The Seitz HP PDP8 protected the finer Seitz Bio 10 single layer filter and improved the throughput on the Seitz Bio 10. This is evident when comparing the throughput of the Seitz Bio 10 in **Figure 10** versus the throughput of the Seitz HP PDP8 and Seitz Bio 10 combination in **Figure 12**.

The throughput of the Seitz HP PDP8 and Seitz Bio 10 combination was approximately five times higher than the Seitz V100P filter alone. The throughput of the dual layer Seitz HP PDK11 filter was approximately 4 times higher than the Seitz V100P filter alone.

Cost/efficiency analysis for AAV filtration

From an economical perspective, the filter area per capsule and the number of filtration steps are used to determine the 'best' filter

FIGURE 11

Viral vector yield (%) for AAV process.

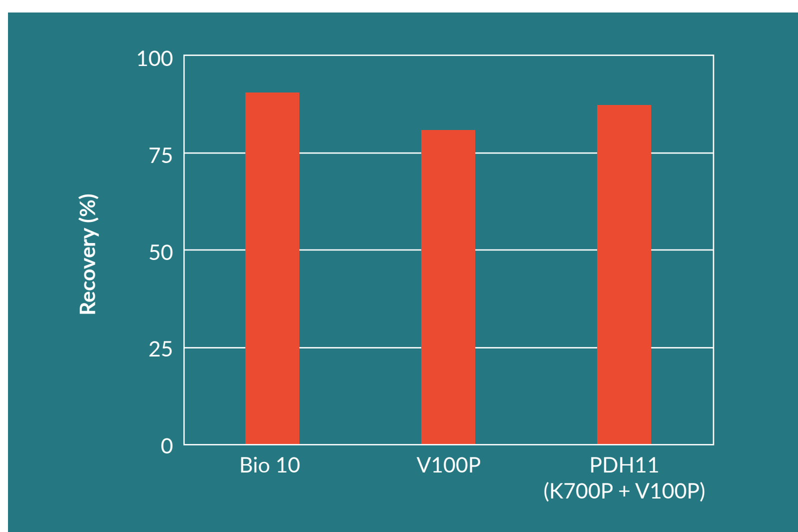


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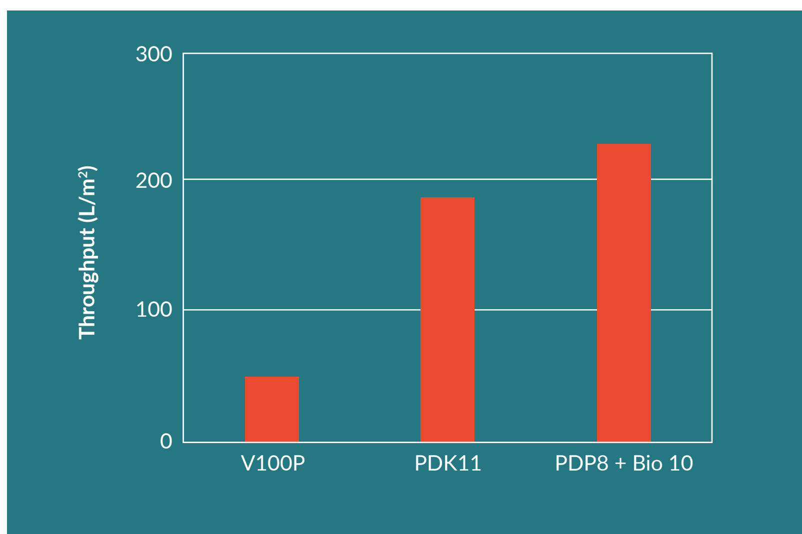
FIGURE 12

Throughput (L/m²) achieved on the bench for AAV process.



system. Dual layer and single layer capsules look the same and have identical outer dimensions. Dual layer capsules, such as the Seitz HP PDH11, PDK11 and PDP8 media, contain half the EFA compared to the same size single layer depth filters such as the Seitz Bio 10 and V100P.

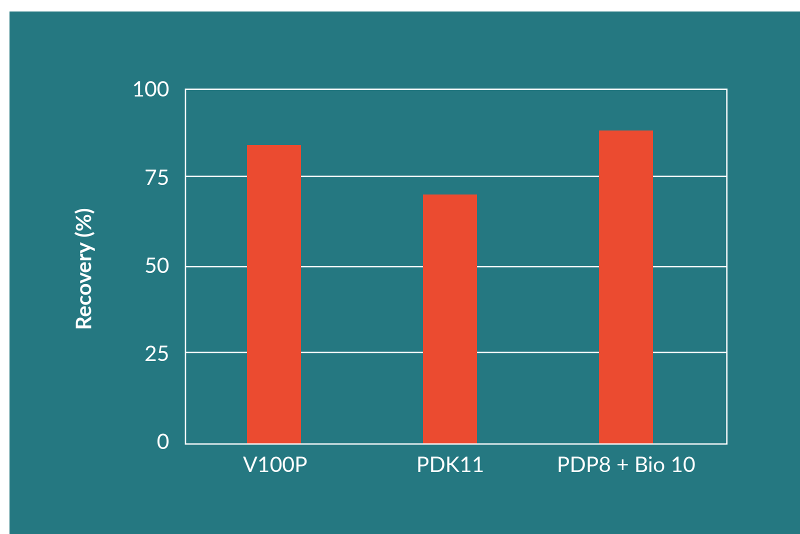
This implies that the throughput or yield of a dual layer combination needs to be higher than that of a single layer depth filter to make economic sense. This is the case for the Seitz HP PDK11 filter in comparison with the Seitz V100P filter. The Seitz HP PDK11 dual layer filter had approximately four times the throughput of the single layer Seitz V100P filter, while having an acceptable viral vector yield.

The combination of the dual layer Seitz HP PDP8 filter in series with the Seitz Bio 10 media is a two-step filtration. The cost of a two-step filtration can be higher when compared with a one-step filtration (the Seitz HP PDK11 dual layer filter). This implies that the throughput or yield of a two-step filtration needs to be higher than that of a single step filtration to make economic sense.

For the two-step filtration consisting of Seitz HP PDP8 and Seitz Bio 10, the throughput was approximately five times greater than the Seitz V100P (single step, single layer filter). This filter train also provided the highest yield,

FIGURE 13

Viral vector yield (%) for AAV process.



meaning this combination provides the best overall performance.

CONCLUSION

For the study shown here, an adherent based LV cell culture process and a suspension based AAV cell culture process were used to define an effective clarification strategy.

Depth filters, prefilters, and bioburden reduction membrane filters were considered for each application. The evaluation criteria included the following: throughput, turbidity reduction, volume that could be processed per 254 mm (10 in.) filter capsule, product yield, and the overall economics of the various proposed solutions.

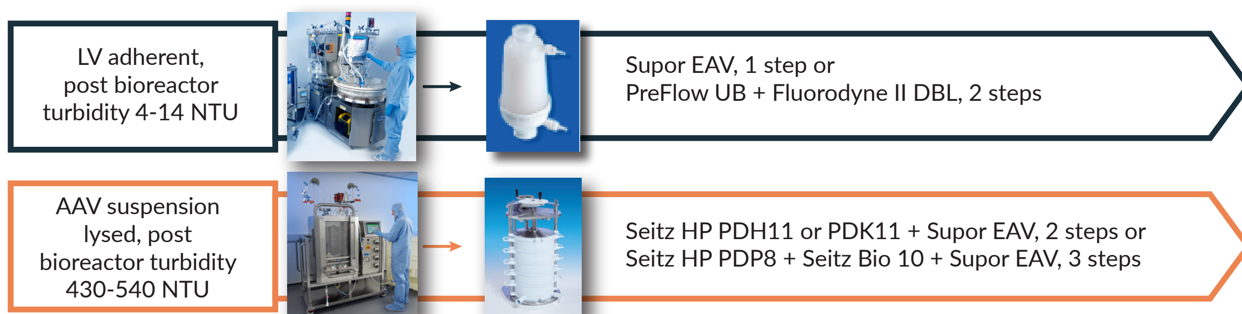
For the clarification of the adherent LV process, the PES Supor EAV 0.2 µm filter and the combination of the PreFlow UB 0.45 µm GF prefilter in series with the Fluorodyne II DBL 0.45 µm PVDF membrane filter performed best among the filters tested, in terms of throughput and yield. The combination of the PreFlow UB filter in series with Fluorodyne II DBL filter generated a higher throughput than the Supor EAV filter alone. However, since it is a two-step filtration, disposables could potentially be more expensive than disposables for the single step filtration. For this reason, the method of clarification needs to be evaluated on a case by case basis where throughput, yield, and cost are all considered.

For the clarification of the suspension AAV feed, the dual layer, single step filter options of Seitz HP PDH11 and Seitz HP PDK11, as well as the triple layer, dual step combination of the Seitz HP PDP8 in series with the Seitz Bio 10, can all provide a viable clarification option for these applications. Overall, the combination of the Seitz HP PDP8 filter in series with the Seitz Bio 10 filter showed the highest throughput, the highest yield, and is potentially the most economic option, albeit a two-stage process that introduces a somewhat more complex operation. The method of clarification needs to be evaluated on a case by case basis where throughput, yield, and cost are all considered.

Figure 14 shows the filter guide which gives an overview about the appropriate filter choices for each application.

FIGURE 14

Filter guide for clarification of adherent cell culture producing LV and suspension cell culture producing AAV.



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REFERENCE

1. ARM 2018 State of the Industry: https://alliancerm.org/sites/default/files/ARM_SOTI_2018_FINAL.pdf

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REGULATORY PERSPECTIVE

Sterility sampling of cell and gene therapy products

Timothy Wood

This article discusses justification and rationale for determining appropriate and representative sterility test sampling for cell and gene therapy products. This article is intended to help manufacturers ensure that the sterility test sample meets current regulatory expectations, is appropriate for the material being tested, scientifically sound and representative of the batch while also preserving product material for intended patients. Regulatory perspectives for sterility sampling of cell and gene therapies by necessity will differ from the compendial chapters of USP ⟨71⟩ and Ph. Eur. 2.6.1 [1,2]. Most notably this difference is found in Ph. Eur. 2.6.27 which contains more specific sample size guidance for these products while the US biological product regulation in the 2012 revision of 21 CFR 610.12 removes all specifics regarding sample size [3,4]. However, applicable regulations and industry guidance all agree on key points that the sample must be scientifically sound and representative of the entire batch. To assist in meeting this requirement this article presents some differences to consider between cell and gene therapy products and manufacturing processes to that of typical pharmaceutical drug products. These differences include: the stages of manufacturing where aseptic controls are required; product shelf-life; and the number of units produced or batch volumes. Furthermore, unique sampling and release strategies are discussed that impact cell-based products. If reducing the vein-to-vein time is important, some advantages are presented for having the sampling and the sterility test itself initiated by manufacturing. As part of product risk, surveillance data from the platelet industry is presented to better understand the levels of contamination that have potential impact to patients and how that relates to sample size and positive detection. Finally, the probabilities of sampling success and positive detection from smaller cell-based batches is contrasted from the sampling probabilities presented for large pharmaceutical batches. Knowing and applying the differences between typical cell-based batch sizes or

volumes versus pharmaceutical drug batch sizes may be substantial for determining an appropriate representative sample and sample size.

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TERMINOLOGY

Under more recent terminology from the FDA cell and gene therapy products are known as Regenerative Medicine Advanced Therapies (RMAT); and in the EU Advanced Therapy Medicinal Products (ATMPs) [5]. FDA guidance in the USA refers to the term HCT/Ps (Human Cells, Tissues, and Cellular and Tissue-Based Products) as defined in 21 CFR 1271 [6]. HCT/Ps contain or consist of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human patient. Gene therapies seek to modify or manipulate the expression of a gene or to alter the biological properties of living cells. In this paper the terms cell-based products or cell-based preparations are used to define both cell and gene therapy products.

REGULATIONS & GUIDANCE ON SAMPLE SIZE

This section reviews the regulatory documents and industry guidance applicable to cell-based products.

21 CFR 610.12 sterility test for biologics & FDA

21 CFR 610.12 is the regulation covering sterility testing for US biological products. The 2012 amended 21 CFR 610.12 sterility test requirement no longer specifies a minimum sample size or sample volume but only that it "must be appropriate to the material being tested". Further language in the FDA's final rule to the amendment states that sterility sample sizes must be "scientifically sound and representative" [7].

USP ⟨71⟩ harmonized compendial test

The compendial method per harmonized USP ⟨71⟩ and Ph Eur 2.6.1 is specific on the minimum volume of samples and number of test units based on the overall batch size. Minimum sample sizes are shown in Tables 1 & 2.

These tables do not address cell-based products with small batch sizes or where there are very few units in a batch. However, it does state that other quantities can be used, if they are "otherwise justified and authorized". The compendial test tables, when given as a percentage, lists sample sizes ranging from 2 to 10%.

Ph. Eur. 2.6.27

Recognizing that most cell-based preparations have small batch sizes this chapter determines sterility sample size as a percentage of the

TABLE 1

Minimum quantity to be used for each medium.

Quantity per container (liquids)	Minimum quantity to be used (unless otherwise justified and authorized)
Less than 1 mL	The whole contents of each container
1–40 mL	Half the contents of each container, but not less than 1 mL
Greater than 40 mL, and not greater than 100 mL	20 mL
Greater than 100 mL	10% of the contents of the container, but not less than 20 mL

TABLE 2

Minimum number of articles to be tested in relation to the number of articles in the batch.

Quantity per container (parenteral preparations)	Minimum quantity to be used (unless otherwise justified and authorized)
Not more than 100 containers	10% or 4 containers, whichever is the greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less

total batch volume. The chapter states:

"A sample that is representative of the characteristics of the cell-based preparation is tested. The sample is added to the culture medium as soon as possible. For cell-based preparations where the total volume (V) of the batch is between 1 mL and 1 L in a single container, the following table **(Table 3)** indicates the inoculation volume to be used."

Regarding sample composition and size, this chapter further states:

"The test sample must be **representative of all of the components** of the cell-based preparation and be **taken from the final preparation**. Where this is not possible, surrogate testing may be performed, for example on the liquids last in contact with the cells being processed."

"Microbial contaminants may be found either inside or on the surface of cells or other components of the cell-based preparation and, such as culture or transport media, are analyzed."

"Due to constraints surrounding the use of a single donor or manufacturing-related capacities, the sample volume available for testing at the end of the production process may be limited. Nevertheless, with regard to the sampling error, which may lead to microbial contamination not being detected, the sample size must be sufficient to ensure suitable sensitivity and specificity of the chosen test method."

It should be noted that the sample minimums from Ph Eur 2.6.27 represent the total sample size which is subsequently split between different media types (e.g., aerobic and anaerobic bottles incubated at appropriate temperatures).

TABLE 3

Total cell-based preparation volume (mL)	Total inoculum volume (divided between aerobic and anaerobic bottles)
$10 \leq V \leq 1000$	1 per cent of total volume of preparation to be tested
$1 \leq V < 10$	100 µL
$V < 1$	Not applicable

One article published by FDA authors in the 2009 AABB Cellular Therapy Standards regarding sample size states:

"The FDA recognizes that for cellular therapy products...the lot size may be prohibitive for following test sample volume requirements given in CFR and USP test methods. It is expected that, for many products, approximately 10% of the product be allotted for sterility testing..." **[8]**

From these guidance and publications, in terms of percentages, a range of at least 1% up to as much as 10% of the batch appears to be the range that is suitable for most cell-based product testing. The exception is for very small batches where use of surrogate sample in place of the final product might be justified, or where the product volume is so small that final product testing is not applicable.

WHAT IS A REPRESENTATIVE SAMPLE?

All the major guidance and regulations state that the sterility sample must be '*representative*' of the whole batch. For example, the FDA 2004 aseptic processing guidance states:

"It is important that the samples represent the entire batch and processing conditions. Samples should be taken:

- at the beginning, middle, and end of the aseptic processing operation

- in conjunction with processing interventions or excursions" **[9]**

The current revised draft of EU Annex 1 guidance for medicinal products says:

"Samples taken for sterility testing should be representative of the whole of the batch but should in particular include samples taken from parts of the batch considered to be most at risk of contamination, e.g.,:

- at the beginning and end of the batch and after any significant intervention" **[10]**

What constitutes a representative sample differs between typical pharmaceutical or biopharmaceutical drug products and most cell-based products. One key difference is the stage or stages in the manufacturing process where dependence upon ISO 5 or Grade A environments is required for contamination control. Traditional drug products incorporate a sterilization step, usually sterile filtration prior to final filling. The aseptic processing portion is often dedicated to just the final fill and as such the 'beginning, middle and end' concept applies more explicitly to the final product fill. In contrast most cell-based products by nature cannot undergo sterilization processes therefore the entire production stream from starting cell collection to the final product usually requires aseptic processing in ISO 5 (Grade A) environments. Moreover, a number of these processing steps may be patient specific and dependent on manual manipulations. Representative sampling of the entire batch then must ensure all these aseptic manipulations are captured, especially those that may present the highest risk (e.g., transduction, multiple expansions, incubation, or harvest). Sampling of 'beginning, middle, and end' then better applies for example, at the initial cell collection as the 'beginning', cell harvest as 'middle', and final product as 'end'. In some cases, the final fill might constitute a rather small element of the total

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batch and thus present low risk, especially if the number of units are small, the exposure duration is short, or the final product total volume is very small. In these cases, the use of in-process microbial contamination testing representing the major components and processing conditions of the batch, together with the availability of larger volume of material to test may provide greater sensitivity and specificity of detection than final product testing could provide.

QUALITY CONTROL TESTING & RELEASE STRATEGIES

The FDA cell and gene therapy guidance documents concerning short shelf life products recommends an in-process test for microbial contamination at the point of cell harvest:

> If your product has a short dating period and must be administered to patients before sterility test results of the final product are available, then you will need to develop an alternate approach to provide sterility assurance. As an alternative approach, we recommend that you perform all of the following tests:
>
> - in-process sterility testing on a sample taken 48 to 72 hours prior to final harvest or after the last re-feeding of the cultures
> - a rapid microbial detection test such as a Gram stain or other procedure on the final formulated product
> - sterility testing compliant with 21 CFR 610.12 on the final formulated product [11,12]

While this is called a sterility test, it is really a test for process contamination that will allow for microbial growth to occur and be detected either by visual examination or other means prior to product release and administration.

Ph. Eur 2.6.27 provides a similar approach stating an intermediate "negative to date" read strategy for short shelf-life products:

> 'Negative-to-date' is understood as an intermediate reading of a test method (2.6.1 or an automated growth-based method) that has not yet been completed. Where cell-based preparations have limiting shelf-lives, 'negative-to-date' results may be used as the readout, where justified..." [3]

For products that are cryopreserved and short shelf-life is not a primary concern, the sterility sampling may be performed from the frozen container units as the final preparation. However, this still presents a challenge if the number of cryopreserved units is small. For example, if five final product units are filled, allocating just one unit to sterility is already 20% of the final batch or two times the minimum percentages listed in compendial tables. Furthermore, cryopreservation can have a potential negative impact on recovery of contaminants in a sterility test. It has been reported that cryopreservation may result in bioburden reduction or possible die off for certain types of organisms which might result in false negatives [13]. Thus, there could be some component, material, process, or other source of product contamination that would be masked by testing from a frozen product.

To preserve the full number of final units for therapeutic use, other QC release tests and product retains are sometimes drawn

from the final formulation prior to cryopreservation. Typically, these samples will be drawn by manufacturing operators at that point in the batch, including sterility into a separate sterile container. Rather than pulling this sterility sample and sending to QC or a separate contract laboratory, there are some advantages including reducing total vein-to-vein time for having the sterility test initiated by manufacturing themselves, especially if using a rapid automated method. For example, the sterility sample would be aseptically removed from the final formulation and directly transferred to sterility culture media rather than aliquoted to a separate container. This procedure can have several aseptic process and timing advantages if full test results are desired more rapidly. These include:

- The test is initiated immediately. For growth-based methods, as soon as the sample contacts the culture medium it allows for potential microbial contaminants to acclimate. In so doing the time to test completion may be reduced by up to a day or longer if the sample had to be shipped to a contract testing laboratory
- Avoids use of a separate sterility sample container and handling, transferring, and aseptic manipulations by QC or by the contract laboratory:
- If using in-house automated methods this avoids the need for having an aseptic sterility suite or sterility test isolator in the QC laboratory
- May represent the product or process at worst-case condition as potential impacts due to storage time or temperature are eliminated
- The manufacturing environment already meets appropriate aseptic conditions for sterility testing including environmental monitoring controls
- Operators should already be familiar with aseptic techniques and qualified in aseptic processing

While these advantages are important there are some points and risks to consider. Among these, with possible responses, are:

- There is a potential safety and regulatory concern that the sterility sample may not represent the product in the container configuration intended for patients

Response: While true, there can be favorable advantages of sampling a larger bulk, or in some cases using a surrogate sample as referenced in Ph. Eur. 2.6.27 because there is ample material to draw from which may increase method sensitivity.

- There is a potential conflict of interest wherein a portion of a QC product release test is not under the Quality function

Response: This only concerns the sample transferring portion of the test. Anyone performing this function must be trained in proper aseptic techniques and this training should be performed by, or under direct oversight, by QC. Periodic auditing or unannounced observation by quality might be warranted. Monitoring of the sterility cultures for growth and sterility test interpretation is still maintained under the QC function.

- The sterility sample manipulation should be included in process simulation protocols since it is an

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aseptic step in the manufacturing of the batch

Relationship between bacterial load & species virulence on transfusion reactions

A significant element regarding product safety risk analysis is the likelihood and level of adverse patient reactions in the event the product was contaminated at the time of administration. To better understand this risk, Jacobs *et al.*, 2008 collected data on the severity, or lack thereof, from septic transfusion reactions observed in the platelet industry [14].

In general, it was observed from surveillance that virulent species and bacterial counts greater than 10^5 CFU/mL were the contamination levels associated with severe transfusion reactions. However, bacterial loads less than 10^2 CFU/mL, consistent with sterility test suitability challenge, and up to 10^4 CFU/mL were not associated with severe or even moderate patient reactions.

This information may be very useful in cell-based product safety risk assessment. Depending on the administered volume and route of administration, this data suggests that a product with an infectious level dose should be readily detected by sterility testing methods. The recent publication of USP chapter ⟨1071⟩ *Rapid Sterility Testing of Short-Life Products: A Risk Based Approach* references the platelet industry studies [15]. Regarding method limits of detection, the chapter states that a threshold of at least 10^3 CFU/mL would predict 95% of all platelet contamination cases and a detection threshold of 10^2 CFU/mL would detect all cases (100%). Per the compendial methods, a contamination level of 10^2 CFU is considered the maximum threshold for sterility suitability testing. Therefore, a smaller sample size that is sufficient to provide a high likelihood of positive detection at these thresholds, may be justified. Further evaluation for the likelihood of sampling success and positive detection is presented below.

Sampling success

Sampling success is defined as the estimated probability that true contamination in the product batch would be positively represented within the test sample and thus detected by the test method.

The 2004 FDA aseptic processing guidance states:

> Sterility tests are limited in their ability to detect contamination because of the small sample size typically used.… if a 10,000-unit lot with a 0.1% contamination level was sterility tested using 20 units, there is a 98% chance that the batch would pass the test. [9]

In 2011, Sutton published a table demonstrating the insensitivity of the 20-unit sterility test from an infinite supply representing large pharmaceutical batches (Table 4) [16]. Given a known frequency of contaminated units, the

TABLE 4

Frequency of contaminated units in the batch	Probability of successful detection
0.001	0.0198–2%
0.005	0.0952–9.5%
0.01	0.1813–18%
0.05	0.6321–63.2%
0.1	0.8647–86.5%
0.5	1.0000–100%

probabilities of successful detection from a 20-unit test are shown:

USP ⟨1071⟩ provides calculations showing how reducing the sample size by the number of units tested reduces the test sensitivity. Tables 5 & 6 from chapter ⟨1071⟩ illustrate this for a 20-unit test of a pharmaceutical product and a 6-unit test of compounded sterile preparation [15].

$p = (1 - p)^{20} = q^{20}$

p = proportion of contaminated containers in the batch

q = proportion of non-contaminated containers in the batch

These tables demonstrate the weakness and insensitivity of sterility sampling for the detection of low-level contamination in large batches.

A different model and more applicable for cell-based products is to estimate the probabilities of sterility detection in terms of the contamination (CFU) levels within a total volume, rather than by the contamination rate of individual units. The probability of sampling success and thus positive sterility detection requires:

1. Known total product volume
2. The sterility sample size volume
3. A given contamination or bioburden level (CFU) in the product

Probability is determined using the following calculation:

Probability that at least one discreet particle (in this case one or more CFUs), is removed in a sample volume from the total product volume with a given contamination CFU concentration.

Equation*:*

X = at least one CFU is collected in the withdrawn test sample.

Poisson probability equation: $Pr\{X \geq 1\}$, or

$= 1 - [\,(V - v)\,/\,V\,]^{n}$

Where,

V = total product volume

TABLE 5

The probability that a 20-unit sterility test passes given an increasing contamination rate for a drug product.

	Contaminated items in the batch (%)					
	0.1	1	5	10	20	50
P	0.001	0.01	0.05	0.1	0.2	0.5
Q	0.999	0.99	0.95	0.9	0.8	0.5
Probability (p) of drawing 20 consecutive sterile items	0.98	0.82	0.36	0.12	0.012	<0.00001

TABLE 6

The probability that a 6-unit sterility test passes given an increasing contamination rate for a CSP.

	Contaminated items in the batch (%)			
	1	5	10	20
P	0.001	0.01	0.05	0.1
Q	0.999	0.99	0.95	0.9
Probability (p) of drawing six consecutive sterile items	0.995	0.94	0.73	0.53

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TABLE 7

CFU in total batch volume	Total batch volume (ml)	Sterility sample volume (ml)	Sample size %	Sample success probability %
10^1	100	1	1	10
		2	2	18
		3	3	26
		4	4	34
		5	5	40
10^2	100	1	1	63
		2	2	87
		3	3	95
		4	4	98
		5	5	99
10^3	100	1	1	100
		2	2	100
		3	3	100
		4	4	100
		5	5	100

v = withdrawn test sample aliquot volume

n = number of CFU in the total product volume [17]

Using this equation, the probability of sampling success for a 100 mL batch volume with various sample sizes and given contamination levels are shown in Table 7.

The sampling success probabilities at a contamination level of 10^2 CFU ranges from 63 to 99% for sample sizes of 1 to 5%, respectively. If the product batch were contaminated at 10^3 CFU or higher, indicative of microbial proliferation and potential infectious dose, the sampling success and thus positive sterility detection is essentially assured at all sample sizes down to 1% due to the contamination level. Studies have shown that growth-based sterility methods are capable of detection limits down to 1 CFU [18,19] therefore these probabilities correlate to actual method detection.

These estimated probabilities, combined with the relationship between contamination level and patient reaction in the platelet industry, suggests that sterility sample sizes down to 1% of total volume would be suitable in most cases for sterility testing of cell-based products. While the success probabilities for lower levels of contamination may not appear great at first, they are far better than the examples given for larger pharmaceutical batches. Furthermore, a contamination level of 10^1 CFU is well below what has been suggested as the level of an infectious dose [14,15]. Regardless, it is understood that product sterility assurance is not based on testing alone, but rather the application of, and compliance to, aseptic processing contamination controls.

CONCLUSIONS

In conclusion a sterility sample size of at least 1% of the total batch volume, in compliance with Ph. Eur. 2.6.27, can be an appropriate and justified sample size for many cell and gene therapy products. If enough sample material is available, increasing the

sample size would increase the probability of detecting contamination and enhancing product and patient safety. Final product sterility testing may not be applicable or necessary for products with very small batch size.

For short shelf-life products, in-process testing such as at cell harvest with “negative-to-date” release strategy can provide greater batch representation and sterility assurance prior to release.

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REFERENCES

1. General Chapte ⟨71⟩ Sterility Tests. USP 36 / NF 31. U.S. Pharmacopeia: 2013.
2. European Pharmacopeia Chapter 2.6.1 Sterility Tests. European Pharmacopeia 5.0, European Directorate for the Quality of Medicines (EDQM). Strasbourg France: 2005.
3. European Pharmacopeia Chapter 2.6.27 Microbiological Examination of Cell-Based Preparations. European Pharmacopeia 9.2, European Directorate for the Quality of Medicines (EDQM). Strasbourg France: 2017.
4. 21 CFR 610.12 General Biological Products Standards: Sterility, Final Rule. U.S. Food and Drug Administration. 2012.
5. Regulation (EC) No 1394/2007 of 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).
6. 21 CFR 1271 Human Cells, Tissues, and Cellular and Tissue-based Products.
7. Amendments to Sterility Test Requirements for Biological Products - Final Rule U.S. Food and Drug Administration: 2012: http://www.federalregister.gov/articles/2012/05/03/2012-10649/amendments-to-sterility-test-requirements-for-biological-products
8. Kennett S, Porter C, Bloom E, Wonnacott K. The FDA Perspective on the Manufacturing, Production, and Processing of Regulated Cellular Therapies. Cellular Therapies: Principles, Methods, and Regulations. Areman EM, Loper K (Eds). Bethesda, MD, USA, AABB, 2009; 13–25.

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9. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice. U.S. Department of Health and Human Services. U.S. Food and Drug Administration. 2004.

10. https://ec.europa.eu/health/sites/health/files/files/gmp/2017_12_pc_annex1_consultation_document.pdf

11. Guidance for Industry: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). U.S. Department of Health and Human Services. U.S. Food and Drug Administration. April, 2008.

12. Guidance for Industry: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Cell Therapy Investigational New Drug Applications (INDs). U.S. Department of Health and Human Services. U.S. Food and Drug Administration. April, 2008.

13. Kipp F, Linnemann E, Fischer RJ, Sibrowski W, Cassens U. Cryopreservation reduces the concentration of detectable bacteria in contaminated peripheral blood progenitor cell products. *Transfusion* 2004; 44: 1098–103.

14. Jacobs MR, Good CE, Lazarus HM, Yomtovian RA. Relationship between Bacterial Load, Species Virulence, and Transfusion Reaction with Transfusion of Bacterially Contaminated Platelets. Clin. Infect. Dis. 2008; 46(8): 1214–20.

15. USP PF 44(5) In-Process Revision, General Chapter ⟨1071⟩ Rapid Sterility Testing of Short-Life Products: A Risk-Based Approach. U.S. Pharmacopeia.

16. Sutton S. The sterility tests. In: Moldenhauer J (Ed.). Rapid Sterility Testing. Bethesda, MD: PDA/DHI Publishing; 2011 :7–28.

17. Probability of N or more from uniform distribution: https://www.physicsforums.com/threads/probability-of-n-or-more-from-uniform-distribution.289562/

18. Du Moulin GC, Kielpinski G, Prinzi S *et al.* Detection of Microbial Contamination for Cellular Therapy Products: Validation of an Automated Microbial Detection System. Encyclopedia of Rapid Microbiological Methods. Vol. II. Parenteral Drug Association. 2005.

19. Wood T. Validation of the BacT/Alert® Microbial Detection System as an Alternate Rapid Sterility Test for Dendreon Active Cellular Immunotherapy Products. Encyclopedia of Rapid Microbiological Methods. Miller (Ed.) Vol. IV. Parenteral Drug Association.

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CELL & GENE THERAPY INSIGHTS

DOWNSTREAM BIOPROCESSING SPOTLIGHT

INTERVIEW

Innovation and issues in hPS cell bioprocessing

CHUCK MURRY is a physician-scientist at the University of Washington, where he directs the Institute for Stem Cell and Regenerative Medicine. He grew up in North Dakota, where his family raised horses, ate competitively and honed punning skills at the dinner table. After completing his bachelor's degree in chemistry at the University of North Dakota, he entered medical school at Duke University with plans of becoming a surgeon. A series of events initiated by poor planning skills left him hooked to heart research, however, leading him to enter a PhD program in experimental pathology and dreaming of developing new treatments for heart disease. After completing his MDPhD training at Duke he transitioned to the University of Washington for residency training in pathology, followed by clinical and research fellowship training. Having spent 31 continuous years in formal educational programs, he was no longer employable in the private sector, and he began his career as a member of the UW School of Medicine faculty. As a faculty member, Murry pioneered the use of stem cells to regenerate the heart and understand heart development and disease. His group is one of the world's leaders in heart regeneration, with plans to begin first-in-human clinical trials in 2020. At the UW Murry helped found the Center for Cardiovascular Biology and the Institute for Stem Cell and Regenerative Medicine. He has received numerous awards along the way, including a Presidential Early Career Award in Science and Engineering, election to the Washington State Academy of Sciences, fellowships in the American Association for the Advancement of Science and the American Institute for Biomedicine and Engineering, as well as winning multiple awards for outstanding teaching from UW medical students. He serves as an elected member of the Board of Directors for the International Society for Stem Cell Research, where he has led efforts to bring stem cell discoveries into the clinic, as well as to shut down predatory stem cell clinics. He is married to his sweetie of 39 years, Rene Murry, whom he met in a blood bank. They have two brilliant and beautiful daughters and two vertically challenged dogs.

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Q What you are working on currently?

CM: The principle interest of our group at UW is in the biological process of stem cell-based regeneration of the heart.

Heart disease remains the number one killer in the world and a large part of the reason it is so devastating is the fact that the heart has very little intrinsic regenerative ability. The cell type it cannot replace is the cardiac muscle cell. Our notion is to take human stem cells of the most primitive, powerful type – pluripotent stem cells (hPSC) – grow them in large numbers, differentiate them into cardiac muscle cells, and then ultimately transplant them into patients who have suffered heart attacks in order to try to re-muscularize the heart wall and restore pump function.

Q Can you take us through the bioprocessing requirements for these hPSC – what are the particular considerations for these cells?

CM: We start out with a line of hPSC – these can be either embryonic stem cells (eSC) or their reprogrammed cousins, induced pluripotent stem cells (iPSC). For our initial first-in-human study, we'll be working with embryonic stem cells.

From the 30,000-foot viewpoint, the process starts out with a very early passage vial of cells. We then expand these under GMP to create a master cell bank before creating a working cell bank from individual vials of cells. These steps are carried out under conditions of adherent culture, where the cells are stuck to the plastic substrate. We then switch to suspension cultures for scale-up purposes, taking a vial from the working cell bank and making what we call a seed bank through a large-scale expansion of the undifferentiated stem cells in stirred tank bioreactors.

Finally – and this is the part I find most exciting – we take this seed bank material and seed it again into a stirred tank bioreactor, where we induce their differentiation to form cardiomyocytes.

Q Where is new downstream bioprocessing innovation most needed in this particular field? What novel solution(s) would be on your wishlist?

CM: The thing we are having a lot of trouble with right now is that much of this work has never been done before. Consequently, some of it we do by brute force and some of it we just have to invent for

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ourselves. In terms of what we have to invent for ourselves, one of the key areas is the assessment of genomic integrity.

The regulatory agencies would all like products that are stable and this would include stability of the genome. But there are no proper guidelines telling us what a desirable or stable genome actually is. What we would really like to see here is simply the field coming together to standardize, so that we can properly understand just what is it we need to do along the way to creating a genomically suitable cellular therapy.

"...a large part of the reason [heart disease] is so devastating is the fact that the heart has very little intrinsic regenerative ability.."

The regulators' concerns are well founded, of course. For instance, if there are mutations in cancer-causing genes, cell therapies could actually start growing tumors – nobody wants that. Similarly, depending on what tissue it is you're trying to regenerate, you might actually have mutations that cause diseases in that organ. Take the heart, for example – what if we had mutations that caused electrical instability causing arrhythmias, or mutations that cause mechanical problems meaning we would be introducing cells equivalent to those that cause cardiomyopathy?

That's certainly one of the things I'm spending a lot of time working on right now: how to assess the genomic integrity of our cellular materials.

Q What evolution would you like to see occurring in flow cytometry specifically?

CM: We, like most people in cellular manufacturing, rely extensively on flow cytometry but we're principally using 20, 25-year-old technology. The tools may have improved somewhat over the years in terms of their throughput, their multispectral bandwidth and so forth, but really, it's the same kind of thing as people were using when I came into residency training nearly 30 years ago.

We would like to see improvements in throughput, improvements in sensitivity, and in the ability to readily correlate things like the structure of individual cells with the analytical readouts – where they appear on the flow cytometry plots, and so on. There's certainly a lot of improvements that could happen in flow cytometry!

Q Regarding automation, what is your view on how and where it should be applied in cell therapy bioprocessing?

CM: There's a lot of manual labor involved in cell culture and that's undesirable for a number of reasons. For one thing, it becomes tedious for the workers and induces repetitive motion injury, so from a workplace health standpoint, issues such as tendonitis become problems. And of course, it's a great source of variability and contamination.

> "...what if we had mutations that caused electrical instability causing arrhythmias, or mutations that cause mechanical problems..."

So I think there's a lot of room to introduce automation, but we don't do much of it just yet. As I mentioned, we're using stirred tank bioreactors, but these things are manually primed, manually loaded, manually inoculated with cells… I think all of this would lend itself to robotic, microfluidics-based systems which could offer a much greater degree of precision.

Media changes are an ordeal to go through right now and again, it's a source of variability if the cells are allowed to settle too long – they can become hypoxic, for example, and get stressed out. They recently found something like this may had happened that caused a whole run to be abandoned.

Additionally, getting towards the fill-finish stage where you have billions of cells coming off a run, it's a very non-trivial thing to get them evenly distributed and cryopreserved. At the moment, we have a team of highly skilled human beings involved in this stage and they do a terrific job. But it really is hard work to actually get things properly vialed up and into the freezer so they can be chilled at a controlled rate. Just watching people going through this, one thinks there must be a better way to improve this work process.

Those are some of the areas where I would like to see automation coming in and playing a role.

Q Finally, what's in store for you over for the next 12–24 months?

CM: Our next 12–24 months are going to be the most exciting time in our program's 20-something year history, because they will see the commencement of our first clinical trials.

We've just signed a deal for commercial agreement with a company called Sana Biotechnologies, based here in Seattle, and we're going to be moving our entire heart regeneration group out of the University and into this biotech company.

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We'll be setting up cell manufacturing inside of this biotech company and we'll then work with the regulatory agencies to get approval for a first-in-human clinical trial, which we hope will take place in the first quarter of 2021.

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Commercial insight: cell and gene therapy

Providing a critical overview of the sector's commercial developments – M&As, licensing agreements & collaborations, financial results, IPOs and clinical/regulatory updates, with commentary from our Expert Contributors.

CELL THERAPY

Mark Curtis. Financial Portfolio Manager, Emerging Technologies, Lonza AG, Switzerland

Three years after taking a major stake in Blue Rock Therapeutics, Bayer came forward in August to buy out the remaining joint venture with Versant. The Series A round, which seeded the company in 2016, was $225 million. Bayer posted another $240 million to take the remaining equity in the company. With the additional milestones the deal values Blue Rock around $1 billion. The deal, along with Bayer's newest company formation, Century Therapeutics, puts Bayer on the map as one of the industry's leaders in induced pluripotent stem cell technology. Blue Rock will continue to operate as an individual entity. In other news on the deal side, Celgene paid immatics $75 million to opt in to T-cell immunotherapies for solid tumor indications. Immatics, a Germany-based biotech, has a unique pipeline of autologous and allogeneic cell-based immunotherapies. The company also has a high through-put screening platform for the discovery of cancer-specific peptides.

GENE THERAPY

Richard Philipson. Chief Medical Officer, Trizell Ltd, UK

In the field of gene therapy, reliable and reproducible manufacturing of the product can be one of the most difficult nuts to crack. Many companies have fallen by the wayside following manufacturing failures, and the announcement from Pfizer of a $500 million dollar investment in a state-of-the-art facility shows

its commitment to getting manufacturing right. Elsewhere, antisense oligonucleotides are in the news this month, with the announcement of a collaboration between Ultragenyx and GeneTx to develop GTX-102, currently in late preclinical development, for the treatment of Angelman syndrome; on a less positive note, FDA rejected Sarepta's antisense oligonucleotide for Duchenne muscular dystrophy patients with mutations affecting exon 53, citing safety concerns.

CLINICAL/REGULATORY

LENTIVIRAL GENE THERAPY SHOWS CLINICAL BENEFIT IN MACAQUE MODELS OF PARKINSON'S DISEASE

A pre-clinical study has demonstrated the safety and clinical efficacy of an experimental gene therapy, AXO-Lenti-PD (OXB-102), in non-human primate models of Parkinson's disease (PD). AXO-Lenti-PD is co-developed by Oxford Biomedica and Axovant. Findings from the study support an ongoing Phase 1/2 clinical trial being conducted in PD patients.

PD is a neurodegenerative disorder involving loss of neurons that release dopamine in the striatum. Oral dopamine replacement therapy is the standard treatment prescribed to patients to compensate for the loss of dopamine. However, as the disease progresses, these therapies become less effective and is associated with motor fluctuations, involuntary movements and other complications.

To address the complications associated with oral therapies, strategies aimed at providing a continuous and local restoration of dopamine to the striatum is required and gene therapy has emerged as an attractive tool for that.

ProSavin, one such lentiviral vector-based gene therapy for dopamine replacement, was evaluated in pre-clinical and clinical studies recently. Although the treatment yielded promising results in early clinical trials, data suggested that this gene therapy didn't increase dopamine production enough for maximum benefit.

In a more recent study published in *Molecular Therapy Methods & Clinical Development,* researchers at Paris-Sud University in France investigated the safety and clinical benefit of another lentiviral-based gene therapy, AXO-Lenti-PD, in non-human primate models of PD. Like ProSavin, AXO-Lenti-PD gene therapy uses lentiviral vector to deliver a genetic payload that lets cells make more dopamine. But AXO-Lenti-PD uses an optimized expression cassette to get the most dopamine production possible.

The therapy was administrated surgically directly into the brain of the MPTP macaque model of PD. The monkeys were treated with either a high or low dose of

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AXO-Lenti-PD, or with ProSavin (positive control; developed by Oxford Biomedica) or a control vector.

Data showed that, compared to animals in the control group, those given the active gene therapy displayed fewer parkinsonian symptoms at 3- and 6-months post-treatment. Animals that received the high dose of AXO-Lenti-PD had higher motor scores than the ProSavin-treated animals at the two time points tested.

Furthermore, assessment of the macaques' brains suggested that those treated with either dose of AXO-Lenti-PD produced significantly higher levels of aromatic L-amino acid decarboxylase (AADC, an enzyme that helps in the production of dopamine) than those treated with ProSavin. The highest AADC expression was found in the high-dose AXO-Lenti-PD group, suggesting that this group had the most dopamine production.

The treatment was safe and well-tolerated by the animals. Findings from the study thus clearly demonstrate that AXO-Lenti-PD is both safe and effective. The study supports the clinical evaluation of this therapy in PD patients which is now underway in England and France (SUNRISE-PD trial).

AXO-Lenti-PD is developed by Oxford Biomedica and Axovant. Early clinical date has shown that a single dose of AXO-Lenti-PD was well-tolerated and improved motor function after 6 months in two people with advanced PD.

AUTOLUS EXPERIENCES DELAYS IN ITS CAR-T PROGRAMS

A manufacturing delay at its clinical trial manufacturing site at the UK's Cell Therapy Catapult is delaying Autolus' multiple CAR-T programs. The programs, AUTO3, AUTO4 and AUTO5 are experiencing manufacturing delays which is related to a 5-month delay in the timeline for qualifying the Cell Therapy Catapult, where Autolus licensed the manufacturing site in March.

Christian Itin, Autolus' CEO stated on a second-quarter results conference call with investors: "The Cell Therapy Catapult site is a brand-new facility. And the challenge that we're seeing with the facility is a delay on the construction and qualification of the main facility. That actually basically resulted in a situation where the delay in the buildup and the construction of facility had a knock-on effect on our own ability to get our own suite that we're operating fully licensed and operational.

As per the re-scheduled plan, Autolus will start the Phase 2 portion of the AUTO3 trial of CD19x-CD22 dual-CAR-T therapy in the second quarter of 2020. The Phase 2 was due to get underway before the end of 2019. Autolus pushed back initial Phase 1 data on AUTO4, a TRBC1-targeting CAR-T, from the first quarter of 2020 into the second half of the year. AUTO5 is now due to enter the clinic in the second half of 2020.

The delays have caused a drop in Autolus' already depressed stock price, which is down around 70% from the high it hit last year.

In addition, Autolus has also announced its decision to ditch its first-generation version of AUTO2 in the face of tough competition in the BCMA space. It is dumping the current version of the BCMAxTACI-targeted CAR-T therapy in favor of a next-generation successor that is due to start testing in humans next year.

Earlier this year, Gilead had also decided to drop its anti-BCMA program, KITE-585. The decision reflects the number of companies with BCMA assets in the clinic. Bluebird bio, Celgene and Johnson & Johnson all have anti-BCMA CAR-T therapies in the clinic and a clutch of other drug developers are going after the target via other modalities.

FDA DITCHES SAREPTA'S ANTISENSE OLIGONUCLEOTIDE TREATMENT FOR DMD

Sarepta Therapeutics has announced that the FDA has rejected its New Drug Application for golodirsen, developed for treating Duchenne muscular dystrophy (DMD). The company's stock dropped as much as 13% in after-hours trading following the news.

Golodirsen, the follow-up to Exondys 51 (Sarepta's first approved treatment for DMD), is an antisense oligonucleotide used to skip the exon 53 in order to restore the reading frame of the dystrophin protein, encoded by the *DMD* gene.

DMD, one of the most common inherited genetic diseases, is a fatal genetic neuromuscular disorder affecting an estimated one in approximately every 3,500–5,000 males born worldwide.

FDA laid out the reasons for the rejection in a complete response letter to Sarepta. In February this year, the company had submitted a New Drug Application seeking accelerated approval of golodirsen injection. The FDA has now denied the drug for two reasons: the risk of infection linked to the port of infusion of the drug where the devices are placed to give doctors access to the vein and the risk of kidney toxicity as observed in animal studies.

The reasons have taken Sarepta by surprise as the company claims that the kidney toxicity was seen in animal models that got doses that were ten times higher than the dose used in clinical studies. Kidney toxicity was not seen in the study on which golodirsen's application was based. Sarepta will "immediately request a meeting with the FDA" to clarify and figure out its next steps.

Back in 2016, Sarepta managed to receive the FDA approval for Exondys 51, its first antisense oligonucleotide treatment for DMD, through much controversy.

Sarepta's New Drug Application seeking the accelerated approval for golodirsen was based on a study testing it in 25 boys with DMD amenable to exon 53 skipping. The study showed that the drug boosted the amount of dystrophin. If golodirsen works as predicted, it can be used to treat approximately 8% of the mutations known to cause DMD. In another ongoing study, the ESSENCE trial, the company

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is studying golodirsen alongside another DMD drug, casimersen.

Doug Ingram, Sarepta CEO commented:

"Over the entire course of its review, the agency did not raise any issues suggesting the non-approvability of golodirsen, including the issues that formed the basis of the Complete Response Letter. We will work with the Division to address the issues raised in the letter and, to the fullest extent possible, find an expeditious pathway forward for the approval of golodirsen. We know that the patient community is waiting."

Sarepta has received a knock back from FDA following the Agency's rejection of its New Drug Application for golodirsen in Duchenne muscular dystrophy (DMD). The concerns seem to be primarily with the safety of the product, in particular infection linked to intravenous infusion ports and nephrotoxicity seen in animal studies. Nephrotoxicity is recognized to be an issue with antisense oligonucleotides; in clinical trials of Biomarin's drisapersen, which was rejected by FDA in 2016, important toxicities included proteinuria, thrombocytopenia and injection site reactions. Treatment with golodirsen could help up to 8% of the DMD population with mutations in exon 53, but FDA's rejection may indicate a toughening in the Agency's stance since the accelerated approval of the company's first DMD treatment – Exondys 51 – in September 2016.– Richard Philipson

T-CELL BANKS COULD STORE HEALTHY T CELLS FOR FUTURE CAR-T THERAPY

Cell Vault, a US-based startup company provides T-cell banking facility where a person could choose to cryopreserve his own healthy T cells for future use in CAR-T therapies. The company has raised $1 million in an initial round of funding.

CAR-T therapies are developed from a patient's own T cells, which are genetically modified to better recognize, target and destroy tumor cells before being re-administered to the patient. Treatments like Novartis' Kymriah and Kite Pharma's Yescarta have shown promise in clinical trials, but these cell therapies are only used after other lines of treatment have failed.

Many patients might not be able to provide enough viable T cells to support a CAR-T therapy because of multiple chemotherapies and immune system-depleting treatments or due to the progression of the disease itself.

To overcome this, Cell Vault's solution is offering healthy individuals a chance to bank their cells while their cells are still functional and plentiful.

According to Cell Vault's founder, Kevin Kirk, the company's focus mainly is on marketing and driving adoption of its idea while the cryopreservation and biobanking work will be handled in partnership with Brooks Life Sciences who will produce, distribute and receive the kits, and then process them down to the cellular level.

The process works like that of cord blood banks or freezing eggs for in vitro fertilization. Blood is collected from an individual, processed into peripheral blood mononuclear cells which contains T cells, B cells, NK cells and stem cells, and cryopreserved until needed. This could be done at the time of diagnosis before beginning treatment or at any other point in a person's life. The company plans to offer T-cell banking services at different rates and packages, ranging from annual payments to an 80-year plan that costs $100 per year.

Because CAR-T therapies have only been done with T cells from patients, it's not known at this stage whether starting the process with healthier T cells could result in a stronger or easier-to-produce therapy down the road. In addition, it's not known how years of being frozen may affect T cells' potency as a final CAR-T product. Cell Vault's venture will address these concerns and according to Kirk, "for the next five, seven, 10 years, however it plays out—if I can save one person from passing away because they had access to their own cells, I'm happy."

PFIZER INVESTS $500 MILLION IN NEW GENE THERAPY FACILITY IN SANFORD, NC

Pfizer's investment in gene therapy continues as it invests half a billion dollars to construct a state-of-the-art gene therapy manufacturing facility in Sanford, North Carolina.

The new site, which is projected to add approximately 300 new jobs, will support the research and development and manufacturing of gene therapies, like its Chapel Hill and Kit Creek R&D sites in North Carolina.

By expanding its manufacturing capabilities in North Carolina, Pfizer aims to strengthen its ability to produce and supply both clinical- and commercial-scale quantities of gene therapies to people living with rare genetic diseases. Specifically, the new facility will help advance Pfizer's manufacturing capabilities in recombinant AAV vector manufacturing.

Pfizer's investment in gene therapy started in 2014 when it signed a deal with Spark Therapeutics for hemophilia gene therapy. At the same time, it also started a dedicated gene therapy research center in London, known as the Genetic Medicines Institute. The company also has several ongoing academic research agreements, including one with King's College London and another one with the University of Iowa Research Foundation. In addition, Pfizer has also entered into collaboration with Molecular Therapeutics to discover and develop next-generation rAAV vectors for cardiac disease.

In 2016 Pfizer acquired Bamboo Therapeutics to develop AAV-based gene therapies for central nervous system and neuromuscular disorders. In 2017, Pfizer entered into a global collaboration and license agreement with Sangamo for developing gene therapies for neurodegenerative diseases. Earlier this year,

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it secured an exclusive option to acquire Vivet Therapeutics, a privately held biotech based in France. The collaboration focuses on developing a gene therapy for Wilson disease.

The Kit Creek facility focuses on process development where scientists work from 2L to 250L bioreactors. The process is optimized at the Chapel Hill facility where researchers work at a 250L scale while implementing quality control under GMP standards. With the latest addition of the new Sanford facility, Pfizer intends to expand its end-to-end capabilities in gene therapy.

Mike McDermott, President of Pfizer's Global Supply commented: *"This investment will further strengthen Pfizer's leadership in gene therapy manufacturing technology. The expansion of the Sanford site is expected to create hundreds of highly skilled jobs, which would increase Sanford's high-tech manufacturing environment and is part of our overall plan to invest approximately $5 billion in US-based capital projects over the next several years".*

CITY OF HOPE INITIATES T-CELL THERAPY TRIAL FOR HPV-ASSOCIATED CANCERS

The first-in-human, Phase 1 trial will investigate Kite's T-cell receptor (TCR) therapy in patients who have human papillomavirus (HPV)-associated cancers that have relapsed or are resistant to treatment.

The study is now enrolling patients at City of Hope, one of America's best hospitals in cancer treatment. The institution is the first to open such a trial on the West Coast.

Kite's TCR therapy (KITE-439) is designed to target HPV-16, a viral strain associated with 70% of all cervical cancers worldwide as well as oropharyngeal, anal, penile and vaginal cancers. This personalized immunotherapy thus activates the immune system's ability to recognize and target specifically the tumor cells.

The trial is designed to evaluate the safety and efficacy of KITE-439 in HLA-A*02:01+ Subjects with relapsed/refractory HPV16+ cancers. The receptor is designed to target antigens expressed in the cancer cells that are infected by HPV, potentially inducing T cell activation against the cells.

During the trial, a person's own T cells are collected and genetically engineered with KITE-439. They will receive cyclophosphamide and fludarabine conditioning chemotherapy prior to receiving the T cells to allow engraftment of the engineered T cells into the body.

The trial which will be conducted in two phases, Phase 1A and Phase 1B, will investigate the safety and efficacy of the treatment, respectively. Phase 1A will evaluate the dose-limiting toxicity, meaning the treatment-related adverse events within the first 21 days following KITE-439 infusion. Phase 1B will evaluate the efficacy, defined as the incidence of complete and partial responses.

AMICUS' GENE THERAPY TRIAL OFFERS HOPE FOR BATTEN DISEASE PATIENTS

Amicus Therapeutics provides early update on its Phase 1/2 gene therapy trial developed to treat patients with CLN6 Batten disease, an inherited childhood neurodegenerative disorder.

Batten disease (also known as Neuronal Ceroid Lipofuscinoses, NCL) is a group of severe, inherited childhood neurodegenerative disorders caused by mutations in either soluble enzymes or membrane-associated structural proteins that result in lysosome dysfunction.

Over 400 mutations in 13 different genes have been described that cause the various forms of Batten disease and they are the most common cause of inherited neurodegeneration in children. The current trial targets Batten disease caused by mutation in the *CLN6* gene.

The hallmarks of the disease include accumulation of lysosomal residual bodies in neurons and extracerebral tissue and loss of neurons. These diseases share common pathological characteristics including motor problems, vision loss, seizures, and cognitive decline, culminating in premature death. Currently, no form of the disease can be treated or cured, with only palliative care to minimise discomfort.

Amicus' gene therapy program is licensed from the Abigail Wexner Research Institute (AWRI) at Nationwide Children's Hospital. Interim efficacy data obtained from the first eight children with CLN6 Batten disease treated with one-time AAV-CLN6 gene therapy showed meaningful impact on motor and language function. The treated children were evaluated for up to 24 months post-administration of the gene therapy. The Hamburg Motor and Language Score, an assessment of ambulation and speech, was used to evaluate the changes in motor activity and language in patients over the course of recovery. Data showed that the gene therapy rendered a positive impact on motor and language function and the disease was stabilized over the course of 2 years.

Treatment with AAV-CLN6 gene therapy was generally well tolerated. The study lacked control groups; therefore, Amicus compared the results with the performance of the siblings of patients treated in the trial. For example, one of the patients scored five out of six on the Hamburg Motor & Language scale at the time of treatment and was still at that level 24 months later. In contrast, the score of the sibling of that patient reduced from five to two over the same 24-month window. The Hamburg Motor & Language Score (0-6) separately measures performance of mobility (0-3) and speech (0-3). For each domain, a 3 represents the child's normal function and a 0 represents no ability to walk or speak, with each point decline representing significant impairment.

Amicus is hopeful with the results and intends to dose additional patients and advance talks with

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regulators. In parallel, Amicus will continue development of its other gene therapies, that target CLN3, CLN8 and CLN1 Batten disease.

DUOCAR-T THERAPY COULD ERADICATE HIV INFECTION

A collaborative research study has developed multi-specific anti-HIV CARs targeting different portions of the HIV envelope protein and has demonstrated the capability of these CARs in controlling HIV in a humanized mouse model.

Although monoCAR-T therapy has been tested against HIV many years back, the success of CAR-T therapies in various cancers has prompted scientists to explore it further for its use as antiretroviral drug therapy. Previously scientists had made anti-HIV CARs that used the CD4 receptor as the targeting site to kill infected cells. However, they found that these CD4-based CARs rendered the engineered T cells susceptible to HIV infection. To overcome this, a team of scientists from the Albert Einstein College of Medicine and the University of Pittsburgh together with Lentigen, a biotech focused on designing lentiviral vectors for gene and cell therapy, developed HIV-targeting CAR-T cells that could target multiple sites on the HIV envelope glycoprotein.

In the study published in Science *Translational Medicine*, researchers showed that the duoCAR-T cells could effectively kill immune cells infected with HIV in a humanized mouse model. They also suggested that the strategy might be able to reduce HIV-infected dormant immune cells that can't be targeted with traditional antiretroviral drugs.

The team developed over 40 HIV-based lentiviral vectors and found that the most effective one contained two CAR molecules that could target three binding sites on the HIV envelope. Results showed that these duoCARs eliminated up to 99% of immune cells infected with 11 different strains of HIV, including some treatment-resistant ones, and was more potent compared to conventional monoCAR-T cells.

In the spleens of the humanized mice, the therapy suppressed HIV infection by 97% after seven days of treatment. This was significantly higher than the 42 and 61% suppression rate observed by the two monoCAR-T cells.

More interestingly, the team also observed that most of the mice treated with the duoCAR-T cells had no detectable HIV DNA in their spleens, which the researchers believe could have caused by elimination of the infected cells by the CAR-T cells. This would mean that CAR-T cells could work along with other agents to reactivate latent HIV so that the reservoirs can be eliminated successfully.

Findings from the study suggest that multi-specific anti-HIV duoCAR-T cells could be an effective approach for treating patients with HIV infection. Clinical trials to test the approach in real-time patients are planned to begin in early 2020 at the University of California, San Francisco and later at the Jacobi Medical Center, which is affiliated with the Albert Einstein College of Medicine.

DUO CAR T FOR HIV

One of the challenges of suppressing HIV is its ability to rapidly evolve and escape the effects of anti-HIV agents. This led to the need to treat patients with cocktails of drugs rather than a single drug. CAR-T therapy targeted to HIV-infected cells has been pondered for some time, but there is also doubt in the scientific community that a T cell with a single CAR could eradicate HIV due to evolution of the virus in vivo. A team of scientists at the Albert Einstein College of Medicine, in collaboration with the University of Pittsburgh, were able to generate T cells that express two CARs, or what they have termed duoCARs. They showed that duoCARs were effective at destroying HIV-infected immune cells in a humanized mice model. The technology has promise, though an important question that remains is whether a CAR-T approach can eliminate reservoirs of long-lived CD4 T cells that house viral DNA but are not shedding virus. It is these reservoirs that lead to persistence of HIV infection over the course of a human's life. – Mark Curtis

ZIOPHARM'S DRUG-INDUCIBLE INTERLEUKIN GENE THERAPY OFFERS HOPE IN TREATING GLIOBLASTOMA

Results from a Phase 1 clinical trial, sponsored by Ziopharm Oncology, have shown the safety and preliminary clinical efficacy of using a regulatable interleukin-12 gene therapy as a therapeutic strategy in glioblastoma patients.

Glioblastomas are the most aggressive tumors of the central nervous system, and the least responsive to intervention. In almost all cases, tumors return within months after intervention.

Human interleukin-12 (hIL-12) has been considered as a powerful immunotherapy that can activate the immune system to attack cancer cells, but its clinical usefulness is hindered by its excessive toxic inflammatory responses.

To tackle this, Ziopharm Oncology in collaboration with Brigham and Women's Hospital and Dana-Farber Cancer Institute designed a control system that can switch on IL-12 gene therapy when in need. The combination approach has shown early promise in a small number of glioblastoma patients in a Phase 1 multi-center trial.

The trial was designed to test the safety and effectiveness of a combination approach: hIL-12 gene therapy together with an oral activator, veledimex – a drug that can control when a gene gets turned on – in 31 patients with recurrent glioblastoma.

Patients received a dose of veledimex before surgery to remove brain tumors. They then received an injection of an hIL-12 vector, which delivered an IL-12 drug, at the time of surgery. Patients continued taking veledimex for 14 days.

10-40 mg of veledimex was tested, and researchers reported dose-related increases of veledimex, IL-12, and immune activity in the blood of patients. Frequency

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and severity of adverse events, including cytokine release syndrome, correlated with the veledimex dose, reversing promptly upon discontinuation. Patients taking the 20 mg dose of veledimex had a median overall survival rate of 12.7 months.

Although the trial was not intended to show efficacy, researchers found signs of positive responses, which they believe could lay the groundwork for future testing of the therapy for brain cancer and beyond. The study was published in *Science Translational Medicine*.

The team also noticed that administering corticosteroids negatively affected the survival. Increased checkpoint signaling was observed in the biopsy of tumor samples indicating the involvement of checkpoint signaling. A Phase 1 trial is now underway to study the combined effect of IL-12 gene therapy with intravenous checkpoint inhibitors.

Dr Antonio Chiocca, corresponding author and chair of the Department of Neurosurgery at the Brigham commented:

"In a Phase 1 trial, we're always trying to find a glimmer: Is there any evidence of efficacy? These results give us that glimmer of hope. We believe it is now possible to do regulatable immunotherapy via genes. It's well-tolerated in patients with glioblastoma, with some encouraging evidence that the drug is having its intended effect."

FDA GRANTS PRIORITY REVIEW OF ALNYLAM'S NEW DRUG APPLICATION FOR GIVOSIRAN

Alnylam, a Cambridge, MA-based biopharmaceutical company specialized in developing RNA interference (RNAi)-based therapeutics has announced that the FDA has accepted the company's New Drug Application (NDA) for givosiran, for the treatment of acute hepatic porphyria (AHP), and has granted Priority Review for the NDA.

Givosiran is an investigational subcutaneously-administered RNAi therapeutic targeting aminolevulinic acid synthase 1 (ALAS1). Monthly administration of givosiran has the potential to significantly lower induced liver ALAS1 levels in a sustained manner and thereby decrease neurotoxic heme intermediates, aminolevulinic acid (ALA) and porphobilinogen (PBG), towards normal levels. By reducing accumulation of these intermediates, givosiran has the potential to prevent or reduce the occurrence of severe and life-threatening attacks, control chronic symptoms, and decrease the burden of the disease. The safety and efficacy of givosiran were evaluated in the ENVISION Phase 3 trial with positive results.

FDA's Priority Review designation is granted to medicines that they believe have the potential to provide significant improvements in the treatment, prevention or diagnosis of a serious disease. Under this status, the FDA will take action within 6 months compared to 10 months under standard review.

Additionally, the Marketing Authorisation Application (MAA) for

givosiran has been submitted to and validated by the EMA. Givosiran was previously granted an accelerated assessment by the EMA, which is awarded to medicines deemed to be of major public health interest and therapeutic innovation, and is designed to bring new treatments to patients more quickly. Accelerated assessment potentially reduces the Agency's evaluation time from 210 to 150 days.

Givosiran also previously received Breakthrough Therapy Designation from the FDA and Orphan Drug Designation in the USA, as well as Priority Medicines (PRIME) Designation from the EMA and Orphan Drug Designation in the EU.

EXPERT PICK

The announcement of Priority Review for Alnylam's RNAi therapeutic for acute hepatic porphyria could see the product approved in early 2020. Givosiran targets aminolevulinic acid synthase 1 (ALAS1); by lowering ALAS1 levels, the treatment reduces build-up of neurotoxic intermediates and prevent attacks of intense abdominal pain, neurological symptoms (muscular weakness, sensory loss or convulsions) and psychological symptoms (irritability, anxiety, auditory or visual hallucinations and mental confusion). In results released earlier this year from the company's ENVISION Phase 3 clinical trial, givosiran met the primary endpoint of reduction in the annualized rate of composite porphyria attacks relative to placebo, with statistically significant results for five of nine secondary endpoints and an encouraging safety and tolerability profile. -Richard Philipson

LICENSING AGREEMENTS & COLLABORATIONS

CELGENE COLLABORATES WITH IMMATICS FOR T-CELL THERAPIES

Celgene Corporation have entered into a strategic collaboration and option agreement with Immatics Biotechnologies to develop adoptive T-cell therapies against multiple cancers.

Immatics Biotechnologies is a clinical-stage biopharmaceutical company developing T-cell redirecting cancer immunotherapies. It is currently working on T-Cell Receptor Engineered T-cell Therapy (TCR-T) programs against solid tumors. These programs use Immatics' proprietary T-Cell Receptors (TCRs) identified by Immatics' XCEPTOR® TCR discovery and engineering platform.

If successful, Immatics will be responsible for developing and validating these programs through lead candidate stage, at which time

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Celgene may exercise opt-in rights and assume sole responsibility for further worldwide development, manufacturing and commercialization of the TCR-T-cell therapies. Immatics would have certain early stage co-development rights or co-funding rights for selected TCR-T-cell therapies arising from the collaboration.

Under the terms of the agreement, Immatics will receive an upfront payment of $75 million for three programs and may be eligible to receive up to $505 million for each licensed product in option exercise payments, development, regulatory and commercial milestone payments as well as tiered royalties on net sales.

Harpreet Singh, CEO of Immatics commented:

"By combining Immatics' world-leading discovery engines as well as our cellular manufacturing and clinical development platforms with Celgene's broad expertise in cell therapy research, development and commercialization, the companies join forces to enable the development of truly novel opportunities for patients with solid tumors who currently have no other treatment options."

ULTRAGENYX PARTNERS WITH GENETX FOR ANTISENSE TECHNOLOGY

Ultragenyx Pharmaceutical has entered into collaboration with GeneTx Biotherapeutics to develop GTX-102, GeneTx's antisense oligonucleotide for the treatment of Angelman syndrome.

Ultragenyx Pharmaceutical is a biopharmaceutical company developing novel products for serious rare and ultra-rare diseases. Under the terms of the agreement, Ultragenyx will make an upfront payment of $20 million for an exclusive option to acquire GeneTx. This option may be exercised any time prior to 30 days following FDA acceptance of the IND for GTX-102.

Ultragenyx has the option to extend the option period by paying an additional $25 million incase if it wants to see the early Phase 1/2 data before proceeding. Ultragenyx may exercise this extended option any time until the earlier of 30 months from the first dosing of a patient in a planned Phase 1/2 study or 90 days after results are available from that study.

Angelman syndrome is a serious and rare neurogenetic disorder that affects approximately 1 in 15,000 people worldwide. It is caused by loss-of-function of the maternally inherited allele of the *UBE3A* gene, encoding ubiquitin protein ligase E3A.

Studies suggest that ubiquitin protein ligase E3A plays a critical role in the normal development and function of the nervous system. While both copies of the gene are turned on in most of the body's tissues, in certain areas of the brain, however, only the maternal copy is active (due to a phenomenon known as genomic imprinting).

Silencing of the paternal *UBE3A* allele is regulated by

the *UBE3A* antisense transcript (*UBE3A-AS*), the target of GTX-102. In almost all cases of Angelman syndrome the maternal UBE3A allele is either missing or mutated, resulting in limited to no protein expression.

Antisense oligonucleotide technology holds promise in addressing Angelman syndrome. GTX-102 is designed to inhibit the expression of *UBE3A-AS*. Preclinical studies have shown that GTX-102 reduces the levels of *UBE3A-AS* and reactivates expression of the paternal *UBE3A* allele in neurons of the central nervous system. Reactivating paternal *UBE3A* expression by GTX-102 in animal models of Angelman syndrome was shown to improve some of the neurological symptoms associated with the condition.

GTX-102 is currently in late preclinical development with an investigational new drug (IND) application expected to be filed with the FDA in the first half of 2020. If IND is successful, GeneTx will initiate the trial in patients.

GeneTx Biotherapeutic is a start-up biotechnology company that was launched by the Foundation for Angelman Syndrome Therapeutics (FAST), a patient advocacy organization and the largest non-governmental funder of Angelman syndrome research. The company licensed the rights to antisense technology intellectual property from The Texas A&M University System in December 2017.

During the exclusive option period, GeneTx will provide regulatory and scientific expertise and fund all development activities, while Ultragenyx will provide staff support, including strategic guidance and clinical expertise.

Both companies will together submit the IND and manage the Phase 1/2 study, which is expected to begin next year. If Ultragenyx acquires GeneTx, Ultragenyx will then be responsible for all development and commercialization activities. If Ultragenyx decides to exercise its option, it will purchase GeneTx for an initial purchase price and contingent milestones and royalties.

BAYER TO ACQUIRE BLUEROCK THERAPEUTICS TO EXPAND CELL THERAPY PORTFOLIO

Bayer AG and BlueRock Therapeutics have entered into an agreement under which Bayer will fully acquire BlueRock Therapeutics, a US-headquartered biotechnology company which was jointly launched by Bayer and Versant Ventures in 2016.

With this latest acquisition, Bayer intends to establish itself in the cell therapy space. Earlier this year, Bayer along with Versant Ventures had announced a major investment of $215 million for the formation of Century Therapeutics to develop pluripotent stem cell (PSC)-derived cell therapies for oncology. This will complement with the development work of Blue Rock Therapeutics which is using induced PSC (iPSC) platform to develop cell therapies for diseases related to neurology, cardiology and immunology. BlueRock's

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lead program for Parkinson's disease is expected to enter the clinic by the end of the year.

Bayer currently holds 40.8% stake in BlueRock and will acquire the remaining stake for approximately USD 240 million in cash to be paid upfront at closing and an additional USD 360 million payable upon achievement of pre-defined development milestones. The investment will correspond to a total value of approximately USD 1 billion. The transaction is expected to complete during the third quarter of 2019.

After the full acquisition, BlueRock will remain as an independent company and Bayer will own full rights to BlueRock's CELL+GENE™ platform, including a broad intellectual property portfolio and associated technology platform including proprietary iPSC technology, gene engineering and cell differentiation capabilities.

Dr Emile Nuwaysir, CEO of BlueRock commented:

"We are extremely excited to be part of the world-class Bayer organization. We have built a premier cell therapy platform at BlueRock Therapeutics, with industry-leading R&D, process development and manufacturing capabilities. With the expertise and support of Bayer, we will be even better positioned to pursue the discovery, development and commercialization of revolutionary new cell therapies for patients suffering from diseases previously thought of as intractable."

FINANCE

RENOVACOR RAISES $11 MILLION IN SERIES A FINANCING TO ADVANCE GENE THERAPY FOR A RARE CARDIOVASCULAR DISEASE

Funding supports preclinical development through IND submission for its *BAG3* gene therapy, the first gene replacement product for genetic forms of dilated cardiomyopathy.

Renovacor, a preclinical-stage biopharmaceutical company developing gene therapy-based treatments for cardiovascular disease, has announced that it has raised $11 million in a Series A financing.

Proceeds from the funds will advance the preclinical development of its lead program through IND submission. The financing round was co-led by Novartis Venture Fund, Broadview Ventures, and BioAdvance, and joined by New Leaf Venture Partners and Innogest Capital.

Renovacor's lead program is a recombinant AAV-based gene therapy for treating patients with dilated cardiomyopathy (DCM) caused by mutations in the *BAG3* gene.

Renovacor's pioneering *BAG3* gene therapy is based on 10 years of research performed by its scientific founder, Dr Arthur Feldman at the Lewis Katz School of Medicine Temple University.

DCM is a heart condition caused due to ischemic heart disease. Recently Bcl2-associated athanogene 3 (*BAG3*) gene has been shown to be linked to a sub-population of DCM patients. *BAG3* gene coding for BAG3 protein is involved in several critical cellular processes, including autophagy (protein quality control) and the prevention of apoptosis. Currently DCM patients with a *BAG3* mutation are treated with standard of care for heart failure. Despite improvements in pharmacotherapy and care, the 5-year survival of a patient with DCM is only 50%. Development of a *BAG3* gene replacement therapy for patients with DCM that carry BAG3 mutations could potentially prevent progression of disease in this otherwise healthy population of young adults.

Dr Magdalene Cook, President and CEO of Renovacor commented:

"There are currently no precision medicine options for cardiovascular patients with specific genetic mutations - a deficiency that Renovacor hopes to address. By bringing the first precision therapy for a cardiovascular disease to the market, we aim to change the therapeutic paradigm that has existed in this field for more than three decades."

ALLOGENE APPOINTS RAFAEL AMANDO AS ITS NEW CMO

Allogene Therapeutics, a clinical-stage biotechnology company developing allogeneic CAR-T therapies for cancer, has announced that it has appointed Dr Rafael G Amado, as its Executive Vice President of Research and Development and Chief Medical Officer. In this new position, Dr Amado will be responsible for leading the company's clinical and research functions including the pipeline of allogeneic CAR T therapies for hematologic and solid tumors.

Dr Amado has more than 15 years of experience in the biotechnology and pharmaceutical industry sector. He joins Allogene from Adaptimmune, where he served as President of R&D, after serving as CMO. Prior to Adaptimmune, he held several roles of increasing responsibility at GSK, most recently as Senior Vice President and Head of Oncology R&D. Prior to GSK, Dr Amado served as Executive Director of Therapeutic Oncology at Amgen, faculty at the University of California, Los Angeles, most recently serving as Assistant Clinical Professor, Department of Medicine, Division of Hematology/Oncology.

Written by Dr Applonia Rose, Cell and Gene Therapy Insights

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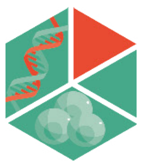
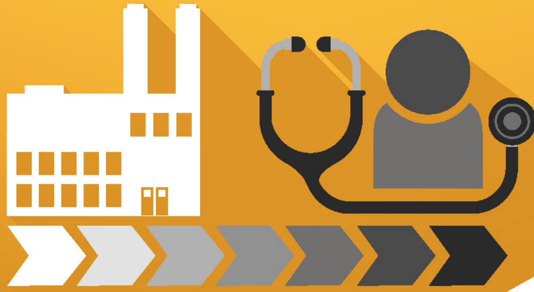
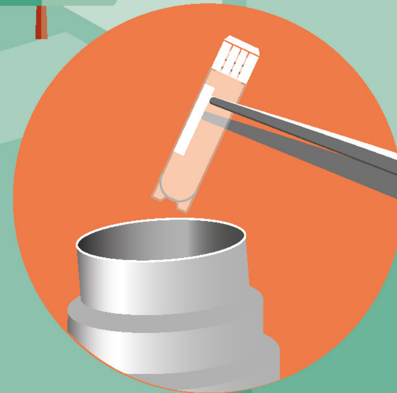


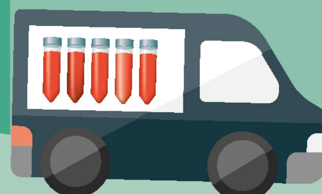
CELL & GENE THERAPY INSIGHTS

Supply Chain Channel





BIOLOGISTICS STRATEGY
FOR SCALE-UP EDITION



Biologistics Strategy for Scale-Up

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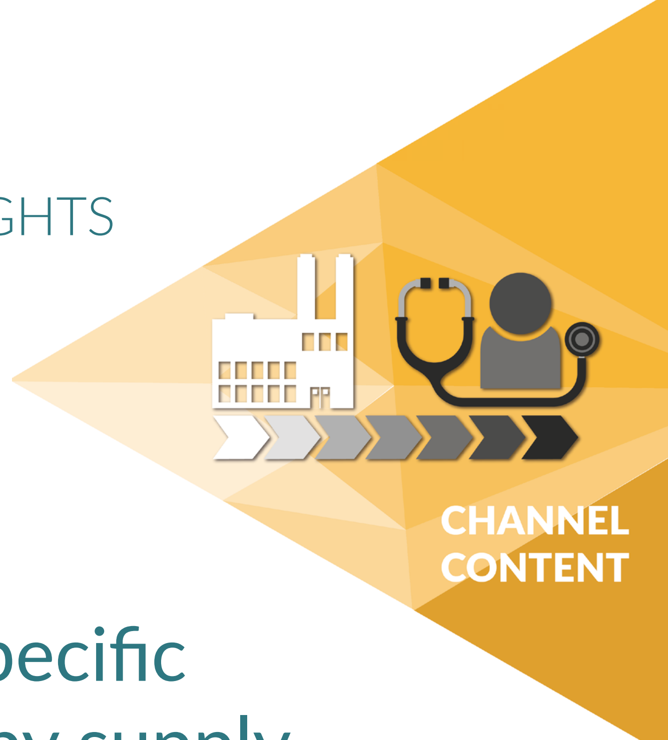

SUPPLY CHAIN FOCUS:
BIOLOGISTICS STRATEGY
FOR SCALE-UP

INTERVIEW

Preparing a patient-specific cellular immunotherapy supply chain for commercialisation

DIRK DE NAEYER is the Chief Operating Officer of Kiadis and an experienced executive in Life Sciences and Biotech, with a sustained track record of leading organizations through complex change and enabling them to deliver growth and operational performance. He possesses broad experience in Operations and R&D (supply chain, manufacturing, R&D, procurement) as a leader of global and local teams, as well as post-merger integrations.

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Q What are you working on right now?

DN: We're preparing for commercial launch of ATIR101 – our lead product, which is an adjunctive T-cell immunotherapy designed to follow stem cell transplant in AML, for the prevention of graft-versus-host diseases (GvHD). We are in Phase 3 for that product candidate with our 009 study and driving towards study completion there. We are also preparing for the next study of a recently acquired NK cell therapy, CSTD002. These are both small volume, patient-specific cell therapies.

For Kiadis as a whole, our challenge is evolving from an early-stage biotech company to a company that is ready to go to commercial. Developing and

implementing the systems, processes, structures and capabilities needed to drive this evolution both in a predictable way and at scale is really what is occupying most of our attention right now. We are shifting from a mostly technical focus on the product to our overall required supply chain capabilities.

Q What can you share with us in terms of the chief considerations relating to supply chain development and management for Kiadis' cell therapy product candidates?

DN: Our primary drivers to date have first of all been about the reliability of the supply chain. Ours is a patient-specific supply chain and we have fairly limited hold times for our biological starting materials, especially in the front end of our supply chain (patient and donor apheresis material/buffy coat). We've therefore been very much focused on defining the most reliable partners and supply chain available.

We've historically chosen a largely outsourced model because as a small company, you're up and running faster by selecting capable partners rather than seeking to reinvent the wheel and building out fully internal capabilities. But again, the reliability of our operations and processes has been really the first focus, with highly capable courier solutions to ensure timely delivery, using the right shippers, traceability from apheresis centers to the manufacturing plant and back to the hospital, and so on.

As we move on through late-stage development and into commercial launch, what were initially secondary drivers such as cost efficiency and scalability are now becoming much more important. We are expanding our CMO network, building up strategic capacity buffers to improve service levels, and investing in new processes and systems to automate workflows and improve end-to-end traceability.

Q Can you go deeper on any particular challenges facing the supply chain for ATIR101 as it progresses through the clinic, and how are you seeking to address them?

DN: Our main challenge is to create full visibility, traceability and coordination in what is a fairly complex supply chain.

In our specific case, we need to achieve this at higher volumes. It's fairly easy to do if you've got a few patients per week, much less so once we go significantly above that. That puts a whole different spin on what capabilities you truly need to have in your processes, people and structures.

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We are in the process of moving from what are very highly manual processes to much more automated, system-supported workflows.

Another challenge, faced by us and I believe all groups with patient-specific therapies, involves the site qualification and onboarding process, especially as it relates to the product-specific activities that hospitals need to do for us. It's becoming clear that as an industry, we're overwhelming hospitals and institutions with all of our specific requirements and systems. I don't think that's a sustainable model in the long-term. I could actually see a model emerging whereby hospitals increasingly start to be selective in terms of who they will work with, especially in cases where there are multiple competing products, because of the burden we are placing on the clinical sites.

We're really evaluating how we should simplify our start-up approach with experienced clinical sites, focusing qualification on what is needed for our products versus what you would generally do if you consider them a typical 'GMP service provider'. Ideally, we would evolve as an industry to having shared and agreed standards.

Q Tell us more about the specific tools and technologies you are employing to enable your supply chain strategy.

DN: Now that we're scaling-up, we are ready to start the implementation of a Cell Orchestration Platform – a system that streamlines the scheduling, logistics and coordination with hospitals and apheresis centers. This incorporates everything from patient onboarding and scheduling to booking manufacturing slots, and from planning the logistics of pickup and delivery of materials to labelling, track and trace, and will replace our current processes which are labor-intensive and therefore error prone.

I would hope that in this area sponsors/pharma companies will accept a move to a single sign-on principle. I'm not convinced deploying a custom-built solution is a competitive advantage, that's not where the difference is made. It only makes the life of the site and the apheresis center more complicated to have multiple logins to essentially the same system. Instead, let's start with taking the viewpoint of what will help our customers.

> "It's becoming clear that as an industry, we're overwhelming hospitals and institutions with all of our specific requirements and systems."

Secondly, we need to become better at good risk management. For instance, by being really smart about

what the true risk of a specific clinical site is and adapting our qualification and onboarding approach accordingly.

Lastly, we generally prefer to use proven external solutions instead of trying to define or develop customized, tailored Kiadis solutions. From the supply chain/logistics point of view, I don't think cell therapy is as unique as people claim it is. Yes, there is great complexity in product development or product characterization; yes, it's individualized, but at the end of the day, you're picking up a product that needs to go from A to B. Then you're shipping it back from B to C. It requires great coordination across multiple parties, but the solutions exist and the work can be standardized. So we now aim to reduce customized in-house solutions and adopt capable standard solutions that are out there.

Q Can you summarize the key building blocks to be put in place now which will prepare Kiadis for supply chain success at commercial scale?

DN: Obviously, it's a mix of the right processes, the right systems, and the right capabilities and people. I know that's a bit of a plain vanilla answer, but at the end of the day those three things are required.

To be more specific – and focusing again on our external supply chain versus technical manufacturing – our systems backbone will be made up of the standard ERP solution coupled with a Cell Orchestration Platform, through which we connect with apheresis centers and hospitals. Potentially coupled with an MES solution for our inhouse production.

Coupled with this is setting up the right customer service capability– including call center services, IVR solutions and field-based support for site start-up. We have a relatively small team working on this now, which we are scaling-up. We do feel it's a capability we want to strategically own, because in the end, this is how we shape the opinions of our customers on a day-to-day basis. Their mindset should be to always put patients and customers first and go the extra mile to support every patient and hospital.

> "...if I project myself forward 2 years, we will want to have automated and standardized a lot of the workflows that today are labor intensive."

In terms of how we look at partnerships, the first things to ask, 'are we the natural owner of this activity? Do we have the scale to do it well? Is it a start-up activity or something that we'll need to do on a steady, recurring basis? And is it critical to the customer experience?' To me, that shapes whether we own an activity versus whether we source it.

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From the shipping and logistics point of view, we're quite simply looking for reliable partners who can provide us with standard shipping solutions. Equally, from a field service point of view, site onboarding is something we want to do in partnership – we don't have the scale to create a field team that's going to be active in 10 to 15 different countries, especially not for something that is usually a one-time, start-up activity. That's just going to create a lot of strained resources down the road.

Q Can you distil a few learning points from your experiences to date in scaling-up Kiadis' supply chain – anything that has worked particularly well, or that you might do differently next time?

DN: I started in October of last year and our single greatest point of focus since then has been building a stronger team with people who know how to do this and are in it to make a difference and not to 'have a job'.

A key learning and a challenge for any small biotech is that you want to think about these things as early as possible. Obviously, before moving into Phase 3 you ideally want to have all this in place, but that was actually not the case at Kiadis – we're only now in the process of making such investments in processes and systems.

This challenge comes down to one fundamental question, of course – when is the right time to invest?

Finding that trade-off point is difficult for many biotech companies – having good discussions internally on how to make what are somewhat risky investments in these sorts of solutions, while at the same time not overinvesting so you're actually pulling money away from R&D. I don't think there's a standard answer to it.

Q Tell us about what the next 12–24 months holds in store both for yourself and Kiadis as a whole – what will be the major goals and priorities over this period?

DN: For Kiadis as a whole, our recent acquisition of a highly innovative NK platform gives us a unique opportunity in that we now have both T cell and NK cell therapy platforms and have become a more global company with a stronger presence in Europe and the USA. Both product platforms carry great promise for improving treatment options for leukemia patients. That's just extremely exciting.

With our lead product, ATIR, we have commercially filed in the EU already – we're hoping for approval with Phase 2 data, but in the meantime, we will obviously continue our current Phase 3, which would support filing a BLA with the US FDA.

For the NK platform, we've got some really good proof of concept data based on a previous MD Anderson clinical trial in AML transplant patients. Our goal now is to go into clinical trials as quickly as possible with CSTD002 and be in Phase 2 in 2020.

That sums up our main company priorities for the next 12 months. Beyond that, we will seek to expand our NK platform into additional indications.

From the supply chain point of view, if I project myself forward 2 years, we will want to have automated and standardized a lot of the workflows that today are labor intensive. I would like us to have established really solid partnerships with two to three key vendors and service providers. Third, we're going to be significantly expanding our manufacturing footprint to drive the growth of the R&D portfolio.

Finally, from a product development point of view, we will focus on creating much deeper process knowledge, better product characterization, as well as developing a second-generation manufacturing process for ATIR. This process will be highly automated, providing for much greater ease of manufacturing. This will be a big step forward for our manufacturing processes – we're making perfectly good product now, but I think we will be able reach the same or a better result with a lot less effort. That's always a good place to end up!

AFFILIATIONS

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Kiadis Pharma

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOLOGISTICS STRATEGY FOR SCALE-UP

WEBINAR TRANSCRIPT

Process development considerations for cryopreservation of cellular therapies

Alireza Abazari, PhD

This article discusses the biopreservation steps as part of the manufacturing process, and reviews what considerations should be part of the picture when incorporating cryopreservation. It will also review Biopreservation Best Practices recommendations for the cryopreservation step through two case studies – one using a human T-cell model and the second, actual human T-cells.

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THE ROLE OF CRYOPRESERVATION IN CELL AND GENE THERAPY MANUFACTURING

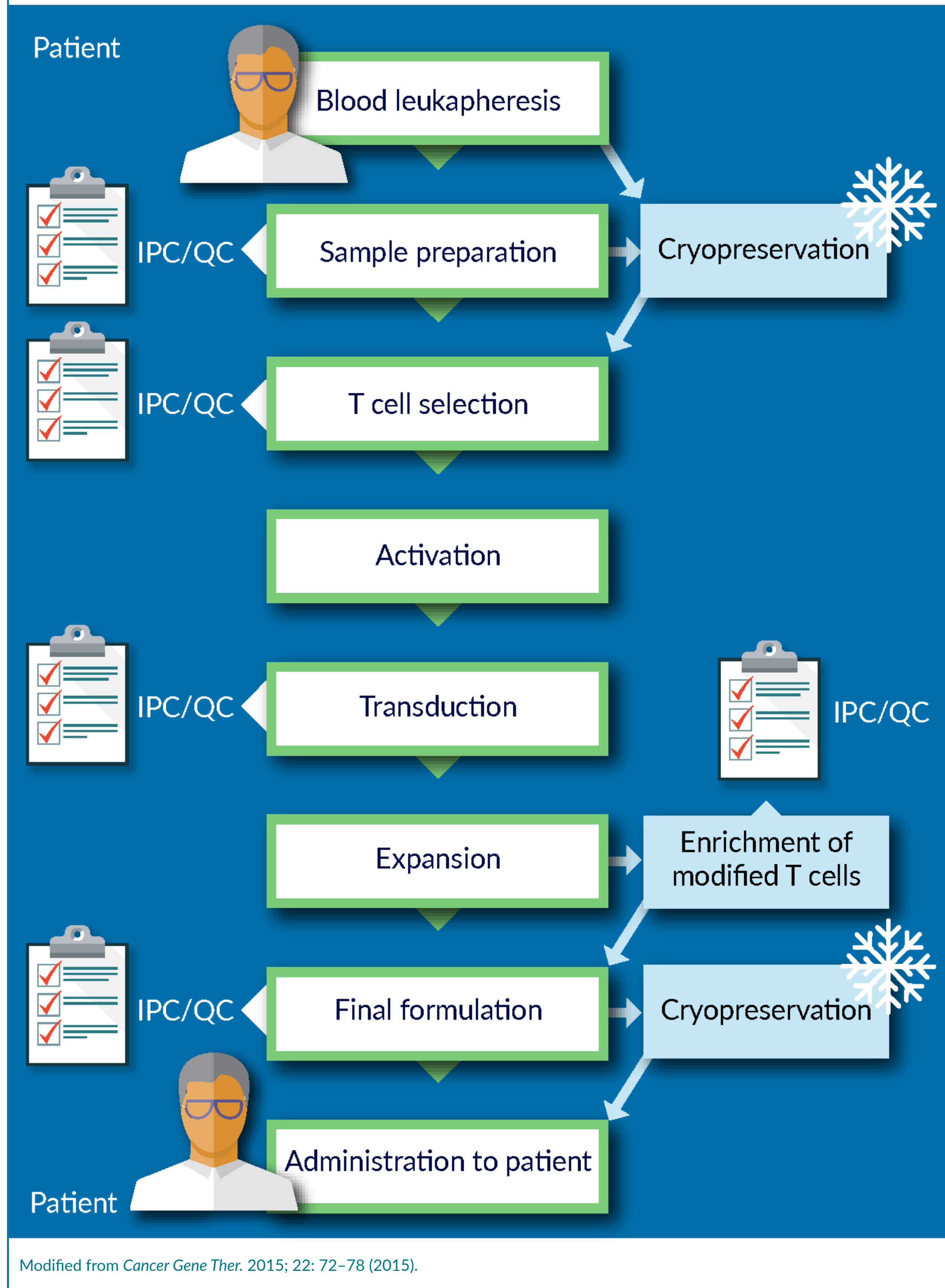
Figure 1 shows a typical immunotherapy workflow. A typical cellular product is collected from a donor or patient and goes through the manufacturing process. The starting material may, or may not, go through a cryopreservation process before manufacture. Following the manufacturing process, the final product is formulated for administration to the patient. Again, at this point the final product may, or may not, go through a cryopreservation process.

We are all familiar with the fact that biological cells deteriorate over time, even in normal conditions. When outside of normal conditions, this deterioration process accelerates quite significantly. To date, the only process that is capable of arresting this biological activity is cryopreservation.

Cryopreservation is essentially the process of lowering the temperature of the biological system

FIGURE 1

Typical immunotherapy workflow.



Modified from *Cancer Gene Ther.* 2015; 22: 72–78 (2015).

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to below -130°C, at which point the water-based biological system will be below the glass transition temperature. Molecules or motion will be arrested and the whole system will be in a state of "suspended animation".

This concept theoretically allows for indefinite storage of the system. It buys the manufacturer time to conduct all necessary assays and prepare documentation for release. It also allows for more robust, reliable transport and shipping options. Today, there are shippers available that maintain the temperature of -130°C or below for days at a time, offering a considerable degree of convenience and flexibility over non-frozen cell products.

One needs to ensure that cryopreservation is not adversely impacting the starting material entering the manufacturing process: the robustness of the manufacturing process depends in large part on the quality of the starting material, and the quality of the final product could also be significantly affected. As it represents both the first and the last step of commercial manufacturing of cellular therapies, it is crucial to minimize the impact of cryopreservation on process and product.

Traditionally, students and academic lab technicians approach cell preservation by putting the cells into a formulation commonly known as a "home-brew" – a mixture of culture media with various amounts of human or animal serum, and different concentrations of dimethyl sulfoxide (DMSO). Once in this formulation, the cells are then typically stored at -80°C overnight.

When it comes to processing a cell sample, one may require just a portion of the cells. A recovery rate of only 50% of cells is perfectly acceptable to a student, because they will only have to wait for a further day or two to allow the cell population to increase again to the number required for the experiment. However, that is not the case when one is developing a cell therapy product. The advent of tissue engineering medicine has led to more stringent quality and regulatory considerations for therapies that are considered first-line treatments for patients.

Certain best practices are recommended across the field of cell and gene therapy. Some of these best practices are based around risk mitigation – for example, using a GMP-manufactured, fully-defined media, whether it be a culture media in upstream processing for vector manufacturing, or downstream processing, or in a cryopreservation step. Essentially, using a fully-defined media versus a non-fully-defined media reduces a lot of the risk associated with process change. It increases control because you know exactly what you have in your system, making it easier to pinpoint the causes and reasons for any change.

A further example of best practice for cryopreservation is avoiding a wash and reformulation step after thawing. This may be achieved by qualifying the cryopreservation reagent as an excipient rather than as an ancillary material. One could consider having a wash and reformulation step if there is a component in the cryopreservation media that must be removed prior to administration to the patient. However, in that case, one would require equipment, trained personnel and potentially a cleanroom

facility either at or near to the patient's bedside. It would essentially mean extending the manufacturing facility beyond the central bioprocessing site to the actual point of care, which would be counterintuitive to having a centralized manufacturing model. The requirement for additional equipment and trained labor would also add significantly to cost.

RISK MITIGATION IN CRYOPRESERVATION

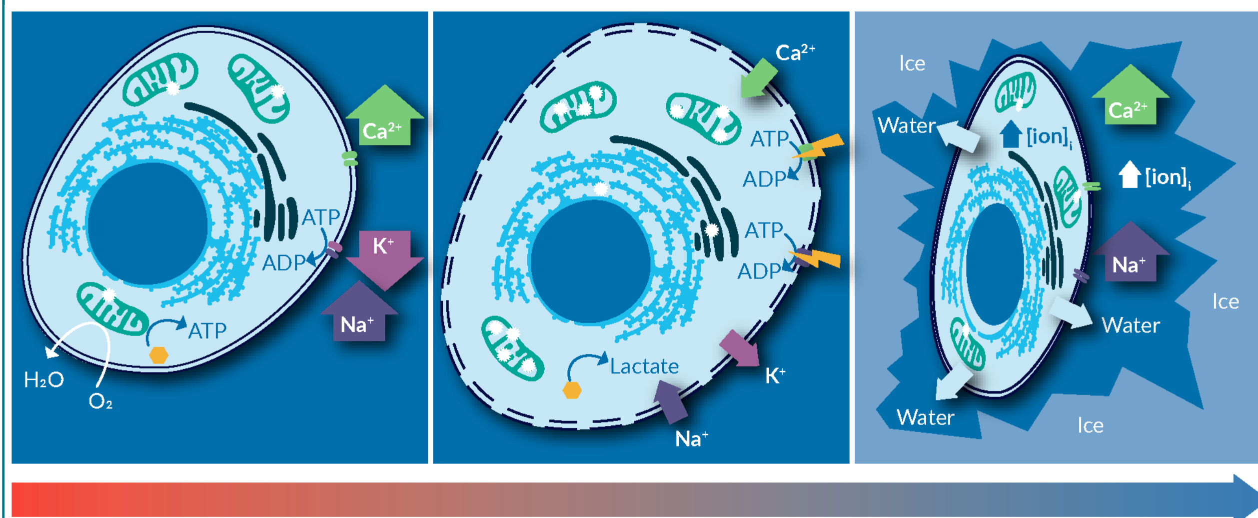
An example of risk mitigation is the use of intracellular-like media. On the left of Figure 2 is a cell that is functioning normally at normothermic temperature in a media that mimics the ionic balance of plasma. This cell essentially uses the gradient of ions across the cell membrane to conduct its own business.

The cell membrane can't use that gradient in order to import and export components: the intracellular milieu is stringently controlled because the concentration of certain ions is important for facilitating cellular signaling. In particular, sodium must be kept out to facilitate proper protein folding and transcription. However, as the temperature cools down to around 0°C, one of the first things that happens is the lipid membrane goes through a phase transition and loses its fluidity. As a result, the cell membrane becomes permeable and there is a free flow of ions in the direction of the ion gradient.

This effect is exacerbated below 0°C, where ice formation essentially concentrates the sodium in a solution around the cells. At -20°C, the concentration of sodium in this solution is 20-times the normal cell's concentration in normothermic conditions. One can imagine how toxic that can be for the cells, how it impacts the pH salinity of the intracellular milieu, and how it impacts the protein folding. This means that when the cells come out of thaw in this condition, all of the intracellular milieu is effectively a mess. Intracellular signaling is disrupted, and protein folding is all out of order. Due to this effect, a portion of the population of cells that are frozen experience so much damage that they essentially

FIGURE 2

Recommended best practices: evidence-based workflow.



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understand they cannot repair, and they initiate apoptosis – the reason cell loss is observed post-thaw.

An easy fix for this problem, one that is applicable to most cell types, is to use a solution that mimics the intracellular environment. This eliminates the gradient of ions across the cell membrane, removing all of those stresses exerted on cells during the freezing process.

However, it is to be noted that most evidence-based best practices are process dependent. The use of intracellular- versus extracellular-like media is general advice, but for example, if you have T-cells or NK cells versus stem cells, they might have different biology and different susceptibility to cold, to sodium or ionic strength in the media, or to shrinking and swelling.

Furthermore, the specific steps applied between harvest and thaw may impact the cells differently. The suggestion is therefore to identify all the steps in a process and all the parameters of each step. A few of the parameters for each potential step are listed in Figure 3. These are representative of an end-of-manufacturing process, involving pre-freeze processing, formulation, cryopreservation, storage, transport, thaw and delivery.

It is imperative to begin at a very early stage of cell therapy development to think about how the final product is going to reach the patient. Is it going to be in frozen form or in 'fresh' liquid form? The option chosen will dictate the manufacturing process and overall model: a centralized manufacturing model involving shipping a frozen product, or a more decentralized or localized manufacturing model allowing for a 'fresh' product to reach patients sufficiently quickly, given its shorter shelf-life.

CASE STUDIES

Two case studies are presented here. The first is on functional assessment

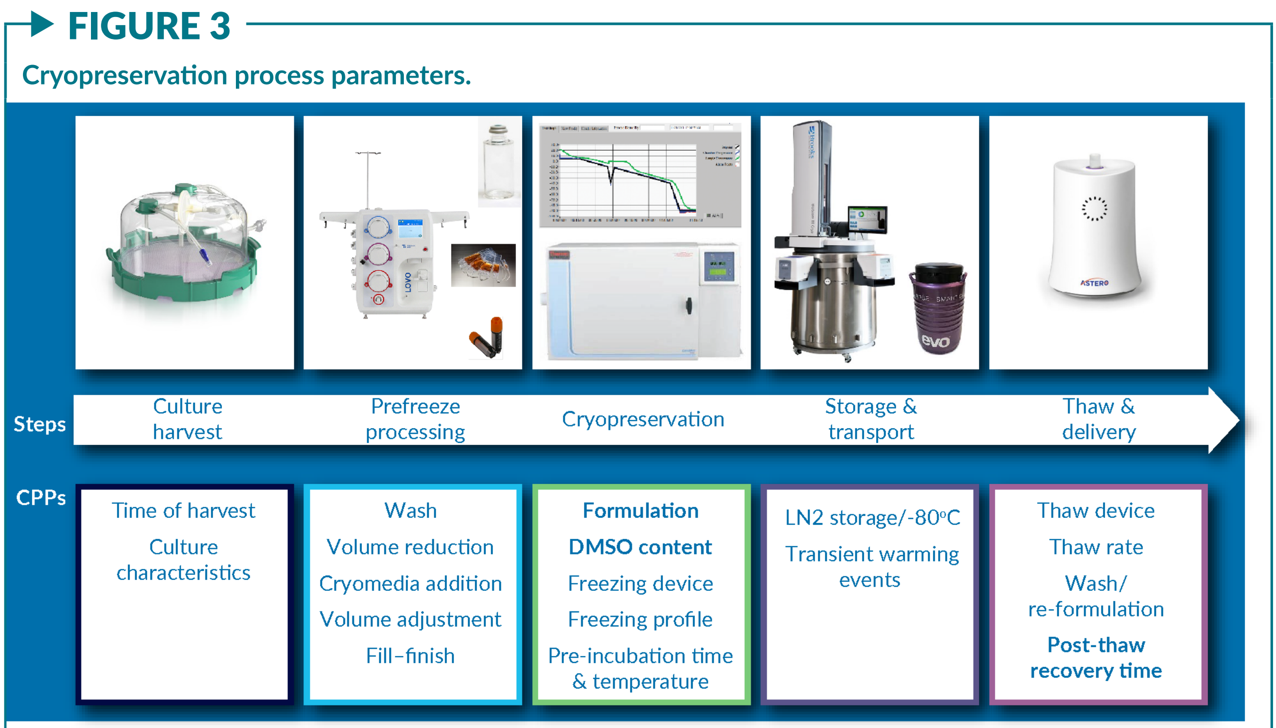


FIGURE 3

Cryopreservation process parameters.

of the impact of cryopreservation on human CD3 T-cells, while the second is on identifying critical process parameters of cryopreservation using a T-cell model (Jurkat cells).

Functional assessment of the impact of cryopreservation on human CD3 T-cells

The first case study was chosen to scrutinize the impact of intracellular- versus extracellular-like media formulation, comparing the DMSO content, and how it impacts the critical quality attributes (CQAs) of the product.

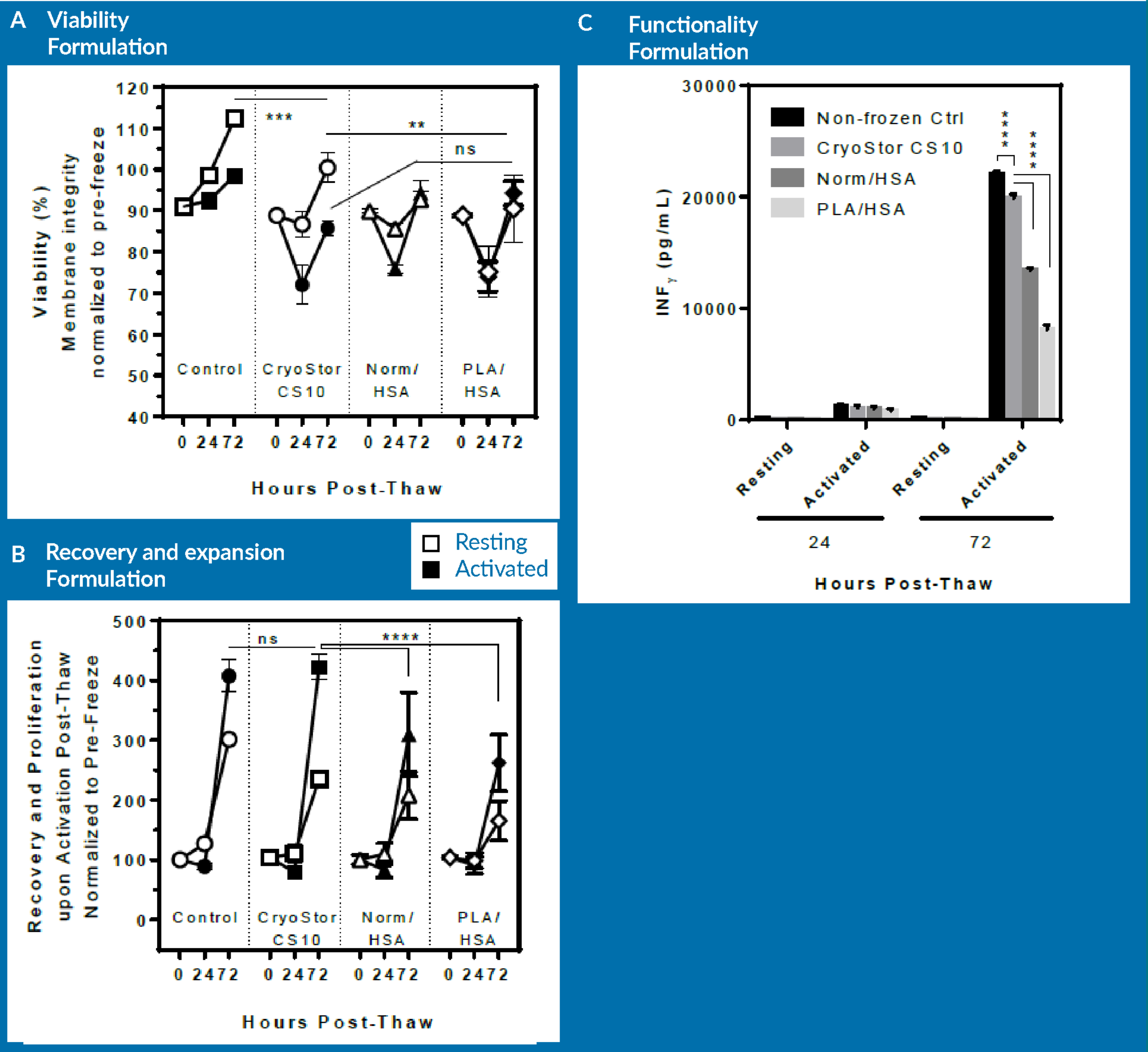
This case study also illustrates a couple of parameters regarding thawing and delivery that pertain to real world scenarios. Post-thaw stability timeframes are a topic of much conjecture: how stable is the product post-thaw, and how long does one have between product thawing and administration to the patient before the product begins to lose potency? A further common question is, 'if the cellular starting material arrives at the manufacturing site in a frozen state, what is the best option for thawing it and entering it into the manufacturing process: should one allow the thawed cells to 'rest' for a period of time before putting them through the process of activation and transduction, or should they enter this process immediately?'

Human Pan CD3 T-cells were used in this study. The primary goal was to determine the advantages and disadvantages of serum elimination, and use of intracellular media. For this purpose, four different media formulations were used: traditional home-brew formulations using Normosol R and PlasmaLyte-A, together with 5% weight per weight recombinant human serum albumin, and 10% volume per volume DMSO. All of these formulations are very commonly used across publications in the cell therapy field. (In fact, one of these formulations is currently used in an approved product). The performance of these formulations was compared with the performance of intracellular-like media, which is the basis for formulation of CryoStor media. CryoStor CS5 and CryoStor CS10 were used, both of which are devoid of serum and protein, but are instead formulated with sugars and other macromolecules at a similar weight per weight percentage. (CS5 has 5% DMSO and CS10 has 10% DMSO).

The experiment began with a clear and very clean population of human CD3 T-cells, expanded and then frozen down to different media. A liquid nitrogen-free controlled rate freezer was used for this purpose. After transfer to, and a minimum of overnight storage in, liquid nitrogen, the samples were thawed before being placed back into culture. Each sample was divided into two: one group rested in media supplemented with IL-2, another group rested in media supplemented with IL-2 plus ImmunoCult™ - an anti-CD3/CD28/CD2 activation agent from STEMCELL Technologies.

The cells were followed post-thaw for 3 days. They were assayed for viability count and interferon gamma secretion on day zero (i.e. immediately post-thaw), at 24 hours and at 72 hours post-thaw. Figure 4A shows cell viability; 4B

 DOI: 10.18609/cgti.2019.122



shows cell recovery and expansion; and 4C shows cell functionality assessed by the secretion of interferon gamma. On each panel, there are four different groups:

1. A non-frozen control;
2. Cells cryopreserved in CryoStor CS10;
3. Cells cryopreserved in Normosol HSA with 10% DMSO; and
4. Cells cryopreserved in PlasmaLyte with 10% DMSO.

There is no significant difference in terms of the viability of cells that were frozen in the different formulations. The viability of the non-frozen control group rises above 100% because the viabilities were normalized according to what went into the freezing process - the viability was around 85% and that viability improved above 85% post-thaw, hence the increase of over 100%. For the resting groups, CS10 after 3 days had a higher viability than the other two groups. However, when activated, all three

groups essentially behaved similarly, with a loss of viability on day 1 followed by increasing viability on day 3. However, there was a significant difference between non-frozen control/CryoStor CS10 and Normosol/PlasmaLyte. This can be seen in Figure 4B, which shows the expansion potential of the cells. At day 3 post-thaw, cells preserved in CryoStor CS10 were essentially no different from the non-frozen control. However, the Normosol and PlasmaLyte groups were significantly lower.

In terms of functionality, the Normosol/PlasmaLyte groups was also lower compared to CryoStor. However, CryoStor was still statistically significantly lower than non-frozen control.

In brief, the removal of serum and incorporation of intracellular-like media resulted in improved recovery and functionality of human CD3 cells. However, it is important to note that the differences could only be observed upon follow-up with functional tests. These functional tests are essentially the expansion and secretion of cytokines. If one relied solely on viability post-thaw, or even 24-hour post-thaw, one would essentially see no difference across the different groups.

For the purpose of exploring optimization of the DMSO concentration, the cells that were cryopreserved in CryoStor CS5 were examined versus those in CryoStor CS10. Again, Figure 5D shows no statistically different results between the resting groups. The activated group in CryoStor CS5 shows a small improvement in viability. However, there was a significant improvement in the recovery and expansion of the cells in the CS10 group at day 3 compared to CS5 and similarly, in terms of interferon gamma secretion.

One can appreciate that CS10 did better than CS5 in increasing the DMSO concentration, and this actually resulted in an improvement in the process. However, incorporating the results from Normosol with 10% DMSO as well as PlasmaLyte-A and 10% DMSO in the analysis for comparison, at 72 hours post-thaw, CryoStor CS5 has the same recovery and expansion potential, as well as higher functionality as assessed by interferon gamma secretion on day 3. This shows that changing the extracellular media to an intracellular-like media essentially allows one to decrease the necessary concentration of DMSO. Again, it is noticeable that viability immediately post-thaw did not provide good means for assessing the cells and comparing the different formulations.

A further point to mention here is that CryoStor CS10 resulted in improved recovery expansion and interferon gamma secretion as compared to CS5. It is a matter of risk versus benefit assessment for each group to decide whether they wish to increase the DMSO concentration, which is something that would go into the patient if you qualify your cryopreservation media as excipient, versus how potent that final dose would be. If an increase of 5% in DMSO significantly increased the potency, it would make it a lot easier to justify the use of an extra 5% DMSO in your final product. However, if that increase only slightly improved the potency result, then one might reasonably decide to reduce the DMSO concentration and forfeit the miniscule improvement in the CQAs of the product.

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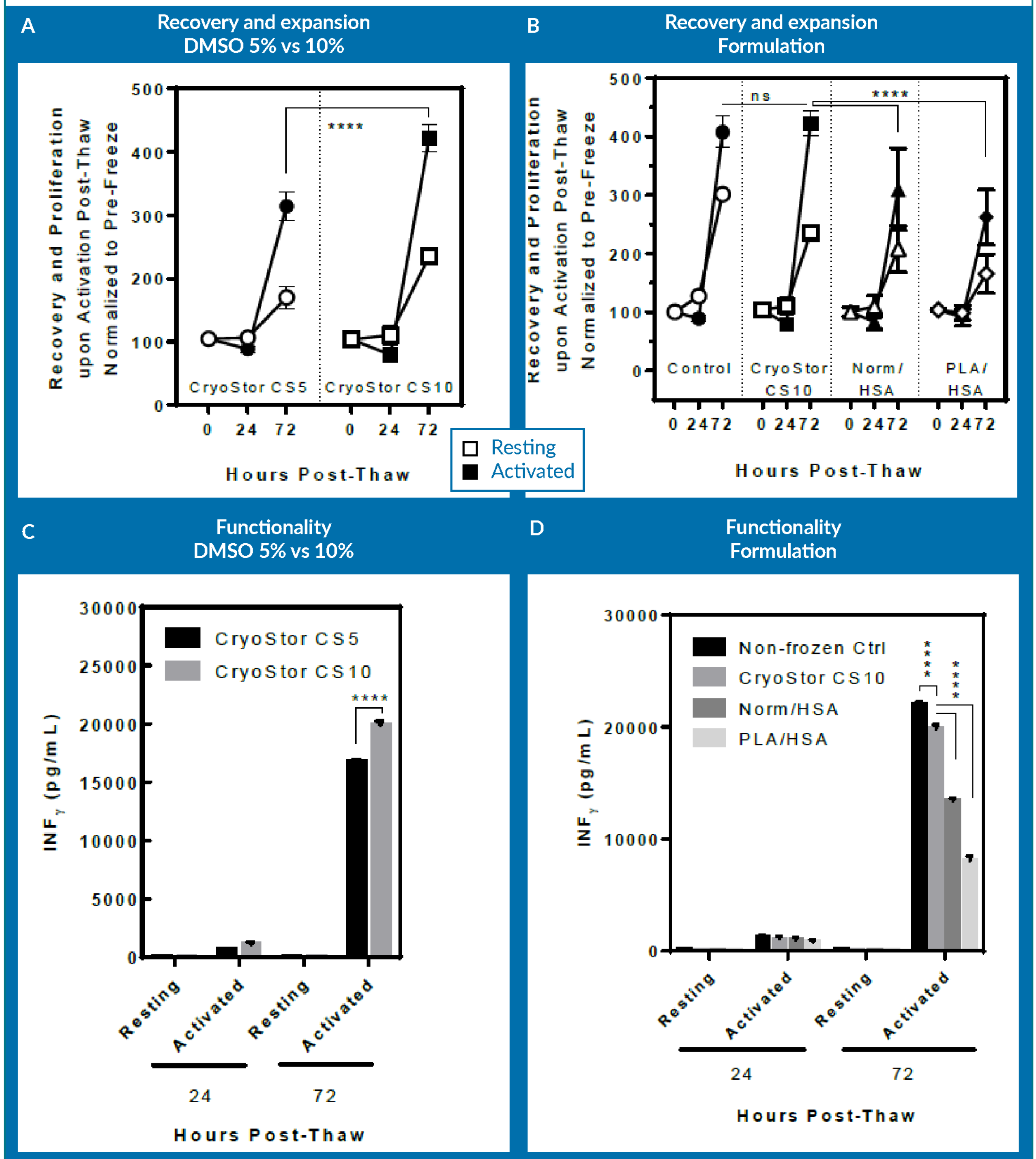
FIGURE 5

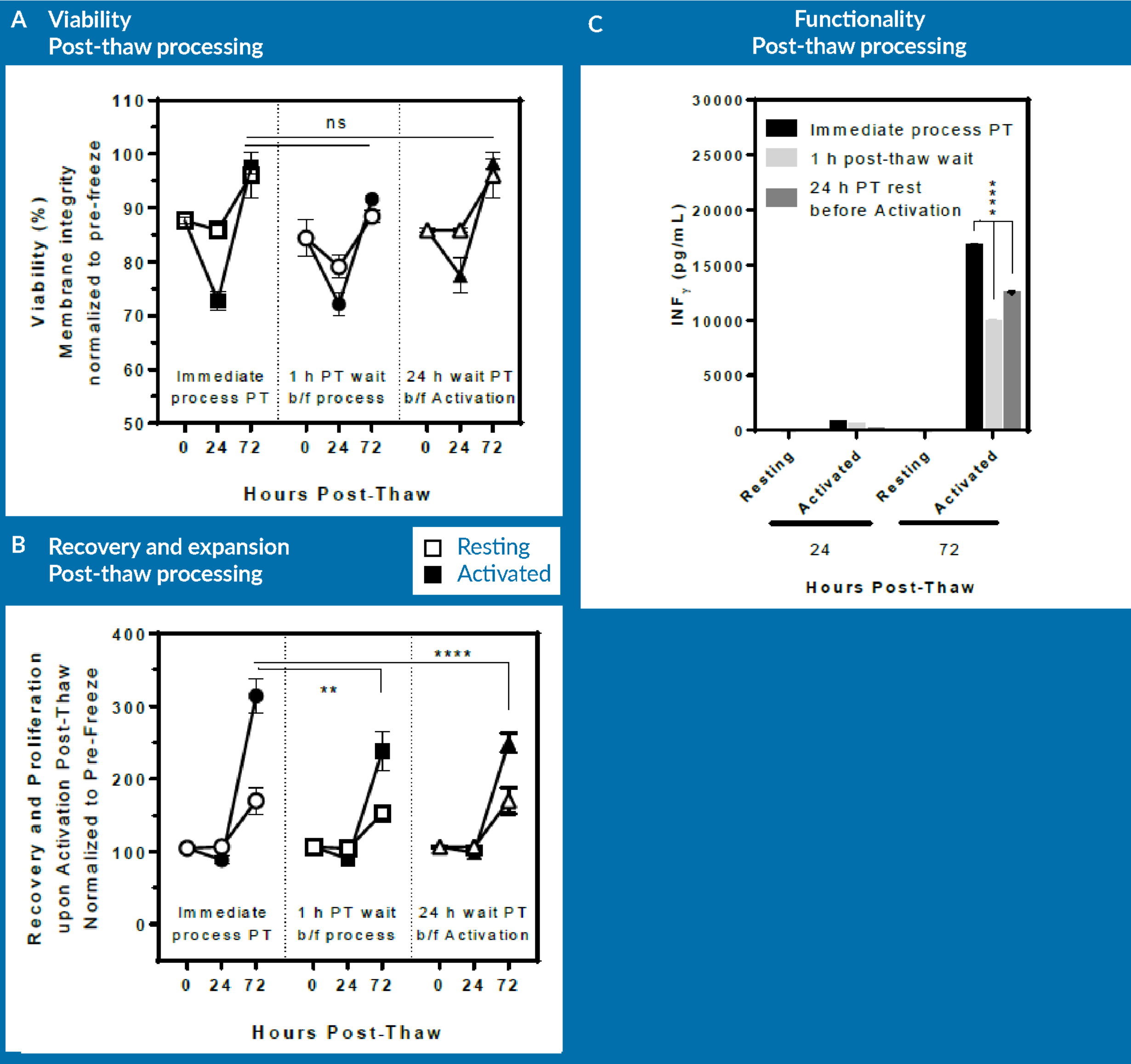
Optimizing DMSO concentration (CryoStor CS5 vs CS10).



The final goal of this study was to examine whether a delay in processing, or a delay in administering the thawed dose to patients, would have any detrimental effect on the process or product. Figure 6 has 3 panels. In each panel there are 3 groups. Figure 6A shows immediate post-thaw processing, where the cells were thawed, then immediately put into growth medium and transferred into the incubator. 6B is 1- hour post-thaw delayed samples. These are samples that were thawed

FIGURE 6

Examine delay/recovery time post-thaw as a CPP.



but then left unattended on the bench top for 1 hour at room temperature (21°C) prior to processing. 6C is for those samples immediately processed after thaw but not activated until 24 hours later. This group were included because of the observation of a loss of viability after activation in media post-thaw; the question being whether a 24-hour post-thaw rest period prior to activation and transduction would have any beneficial effect on the manufacturing process in terms of shortening its duration.

Again, there is no significant difference in viability across all groups. However, when one looks at recovery and expansion, the immediately processed sample shows a significantly higher expansion potential at day 3 post-thaw compared to both the delayed sample and also the sample allowed to rest for 24 hours prior to cell activation.

It is important to mention here that the cells that were allowed to rest for 24 hours were actually activated on 24 hours post-thaw. In order to be consistent, this group

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was allowed to continue up to 96 hours, the data for which is not included in Figure 6. However, when the 96-hour data was compared to the immediate post-thaw processing data, there was no significant statistical difference.

The lesson from this experiment is that if processing is delayed for 1 hour post-thaw, of if the there is a delay in putting cells into the patient post-thaw, one starts to see a detrimental effect on expansion potential. However, if the cells are allowed to rest in culture prior to activation 24 hours later, expansion potential following activation remains the same. Of course, one does need to consider that one is obtaining the same results having waited an extra 24 hours. The conclusion from this is that from a manufacturing standpoint, it would be best to immediately activate and transduce upon thaw: this approach leads to the same expansion potential later on while essentially saving 24 hours of bioprocessing time.

It is a similar story in terms of functionality, as assessed by interferon gamma secretion. The 24-hour rested group was statistically lower than the immediate post-thaw process group, but again, that was due to the sample being activated only for 48 hours rather than 72 hours for the other 2 groups. Samples were collected from that group at 96 hours and once again, the results were identical to those for the immediate post-thaw processing group.

In brief, immediate processing followed by immediate activation post-thaw appears to be advantageous compared to scenarios that allow recovery post-thaw. Further analysis would have to be conducted to identify the most cost-effective manufacturing approach based upon these results, or indeed, these experiments could simply be repeated in any lab and with any process to identify what is the most cost-effective approach for a given product.

To summarize the results of this first case study, some risk-based best practices may force changes to the process, including elimination of protein. Optimization of cryopreservation process including formulation can facilitate incorporation of such risk-based best practices. Incorporation of well-defined protein- and serum-free intracellular media compensated for the elimination of serum in traditional 'home-brew' cryopreservation media that contained higher DMSO content.

It is suggested that developers use more in-depth functional assessment than solely viability or recovery at immediate post-thaw to identify critical process parameters that impact the product. When conducting process validation or process changes and comparing two different results, one must employ sufficiently in-depth assessment methods to decipher the differences.

IDENTIFYING CRITICAL PROCESS PARAMETERS OF CRYOPRESERVATION USING JURKAT CELLS.

The second case study identifies some of the process parameters that are perhaps more obscure. The Jurkat T-cell model was used to study a variety of different process parameters, but for the purposes of this article, the focus is solely on

one particular aspect of the freezing profile, and a particular aspect of how packaging impacts on CQAs.

The Jurkat T-cells had been frozen in CryoStor CS5. After storage in liquid nitrogen and thawing, the cells were followed up for 48 hours, looking at viability and count post-thaw. Count or proliferation post-thaw would be the ultimate functional test for these Jurkat T-cells.

The impact of proper nucleation, which is a small but very important part of the freezing profile, was recorded. For container impact, the indirect impact of container choice on the freezing rate was assessed, as were the CQAs of the products as determined by viability and proliferation rate.

There is a very popular school classroom experiment where one freezes a water bottle. This water bottle is essentially in a super cooled condition – it is an unstable thermodynamic condition when the temperature is below freezing but the water has not yet frozen. Disruption of this equilibrium results in the system going back to the minimal energy state, which is the frozen state. The intention with this experiment was to do the same with the freezing profile. If one looks at a standard, controlled rate freezer freezing profile, one would see that at around -5°C to -10°C, a blast of nitrogen drops the temperature quite significantly, then warms it back up. That's the step designed specifically to disrupt this non-equilibrium, or pseudo-equilibrium, that exists inside the cryobags or cryovials.

The point of this is to initiate nucleation. If nucleation is allowed to occur spontaneously, without activation, one starts to see significant variability in the product. In a freezing process without active nucleation, cells start to freeze randomly at different times. However, if active nucleation is incorporated into the freezing process, all of the vials freeze uniformly at the same time. This eliminates a significant factor which can impact product viability.

A sample of cells was firstly frozen down and nucleated properly at around -10°C. A second sample was also allowed to freeze but the cells were not nucleated, meaning the equilibrium was not disrupted and nucleation was allowed to happen spontaneously or stochastically.

It is interesting to note that at 24 hours post-thaw, both groups come out with identical viabilities, or viable recoveries. If one relies solely on viability or viable recovery, there was no discernible difference between the two approaches. The difference is only visible at 24 hours post-thaw, when one begins to see a detrimental effect on the proliferation capacity of the non-nucleated group. This is a very important factor that contributes to significant variability in cell product. It is suggested that developers should always ensure that proper control is maintained over this step and that rigorous process development around this step is carried out to minimize the potentially damaging impact on viability caused by spontaneous or stochastic nucleation.

Turning to the impact of the freezing container, this particular experiment was born out of a conversation with a customer some years ago. The customer in question mentioned that physicians or nurses very much prefer to have

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cryopreserved product in a vial because after thaw, they can simply transfer the contents of a vial into a syringe and inject it into the patient. This study was designed to ascertain whether the geometry and material of the vial could have an impact upon the freezing process.

The freezing process was simulated in the largest vial, shown on the left in Figure 7. This is a vial that nominally contains about 50 ml, although it would usually contain only 30-40 ml.

This simulation demonstrates that as the temperature drops on the outside by 1°C/minute, the temperature of the inside will have a 35°C temperature gradient during the freezing process. This translates into significant variation in freezing rate.

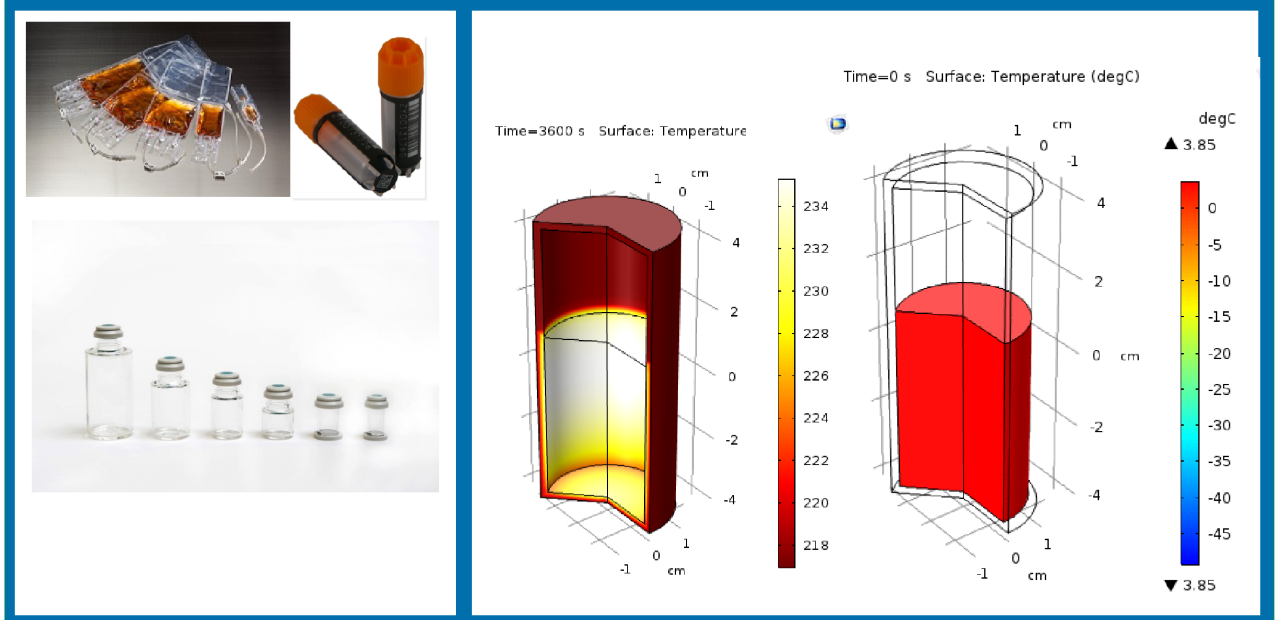
To test this, some cells were frozen at 1°C/minute, as standard, and some cells at ½°C/minute. A freezing rate of ½°C/minute or less would be approximately the freezing rate that about half of the volume of this vial would experience during this freezing profile. Changing the freezing rate of ½°C/minute transfers into a quite significant loss of proliferation capacity for the Jurkat cells: at 24 hours post-thaw, there is essentially no proliferation in the population of Jurkat cells that had frozen at ½°C/minute.

This demonstrates an important process development parameter that cell therapy developers should be aware of, as well as the capacity of the geometry and material of the storage container to significantly impact the CQAs of the product.

In summary, in-depth knowledge of cryopreservation is required to identify the critical process parameters that impact the quality attributes of the product. Robust assays and in-culture follow-up is necessary as one proceeds through process development in order to assess different approaches, media,

FIGURE 7

Container closure: impact of freezing rate.



and changes to various process parameters that may or may not impact product CQAs.

Again, it is important to stress that immediate post-thaw viability, especially after cryopreservation and particularly as measured by membrane integrity, is not representative in any way of how well that cryopreservation was conducted. It is strongly recommended hat cell therapy developers identify other assays or conduct follow-up testing themselves after cryopreservation and thaw to identify how that cryopreservation process was conducted and how it impacted the cells.

CONCLUSION

In conclusion, incorporation of Biopreservation Best Practices is a multi-faceted approach that addresses numerous commercial, quality/regulatory and process development concerns. Proper knowledge and understanding of cellular response to cold and freezing is necessary for identification of critical process parameters that impact the CQAs of the product.

FROM THE Q&A

Q How often does optimization of cryopreservation involve only substitution of the freeze media versus optimization of process development steps outside of the media?

In our experience, we have realized that optimizing the freezing media improves the results significantly. Much of the time, this meets a developer's minimum requirements. With certain cell types, you may need to look at other process parameters such as the freezing profile, which is a key element of optimizing the process. However, if you are using an optimized media, it can alleviate some of these requirements.

Q Do any of the products discussed here that were used by BioLife Solutions have FDA approval?

The FDA is concerned with medical devices and drugs and none of BioLife Solutions' products are categorized as such. The products used are instead categorized as ancillary use products or excipient products. Ancillary products assist in the production of the end product but are completely removed from that product and are not administered

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to the patient. The excipient model means that the cells and media combined are treated as one product that is to be approved by the FDA.

Q What are the pros and cons of washing the cell product post-thaw to remove the cryoprotectant?

You may have elements of the media that you wish to remove from the cells as they do not meet standards and you would not want to administer them to the patient. However, this washing step adds another manufacturing step. Washing will produce liquid form products, which tend to have shorter shelf-lives. It is important to think about transporting the product more efficiently. You will also need expertise at the patient's bedside to administer the product to the patient. This adds time and cost to the manufacturing process and is the reason why most companies are not removing the cryoprotectant post-thaw and are instead aiming for approval of it as an excipient product.

Q Are there ways to improve the stability of apheresis material between collection and processing?

There are two modes of transporting the material: fresh and cryopreserved. In the fresh form, you may seek to use an optimized media for the transport and delivery of the cells. If you are transporting fresh cells at 2°C -8°C degrees, the stress of cold can impact the quality of the product. This is why using an optimized media, designed to mitigate the cold stress on the cells, will result in increased durability of the starting material.

ASK THE SCIENTISTS

Additional questions can be addressed through **'Ask the Scientists'** on the *Cell and Gene Therapy Insights* website.

In partnership with BioLife Solutions, *Cell & Gene Therapy Insights* is giving you the chance to have your biopreservation questions answered throughout 2019.

https://insights.bio/cell-and-gene-therapy-insights/ask-the-scientists/

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOLOGISTICS STRATEGY FOR SCALE-UP

CHANNEL CONTENT

INTERVIEW

Mitigating risk in the gene therapy supply chain

CHAD PRESHER is an Associate Director of Clinical Drug supply at Biogen. In this role Chad supports the clinical supply chains for Biogen's Rare Disease Therapeutic Area. Prior to joining Biogen, Chad held commercial supply chain roles at Pfizer and Wyeth. Chad holds an MBA and a BS in Biology from the University of New Hampshire.

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Q What are you working on currently?

CP: It is my responsibility to ensure supply continuity for Biogen's clinical trials. More specifically, I manage a team of five supply chain professionals focused on Biogen's assets or the molecules in the rare disease area.

Q You have a background in big pharma clinical supply chain management – what are the major differences in dealing with gene therapy requirements?

CP: Traditional big biopharma products fall into one of three main areas: small molecule drugs, large molecule therapeutics, and

vaccines. Gene therapy is a tremendously exciting emerging area, but it does present some unique challenges. I see a couple of major differences in the context of supply chain management.

Thinking about this from a clinical trial standpoint, traditional pharmaceutical products will require many, many doses over a period of time that stretch from a few months to as long as 5–7 years, depending on the length of the trial. Over these multiple years, a significant amount of drug needs to be delivered, monitored, thought through and planned.

With gene therapies, you're talking about a 'one and done' treatment, so purely from the planning standpoint there's a fundamental difference in terms of the volume of the drug and the duration of the treatment regimen.

The second major difference to traditional pharmaceutical assets and molecules lies in the cold chain element of delivering gene therapies. The vast majority of gene therapies are shipped in ultra-cold chain conditions, so we're talking about -70 degrees Celsius or colder. It's going to be a new thing for industry to get their heads wrapped around and really understand what exactly that means – everything from shipper validation all the way through to understanding the capacity in the ultra-cold chain network.

So I think those are two huge differences: the length and duration of the trials, and the storage conditions in the shipping environment.

Q A related question – are there any general principles you can apply from your previous experience and equally, where is a novel/innovative approach required?

CP: A lot of the core fundamentals relating to supply chain execution, supply chain management and customer engagement can be leveraged and implemented. Certainly, the elements of understanding where your unconstrained demand is coming from, working that through and understanding your target inventory position at each node of the supply chain, and then the corresponding generation of a manufacturing plan that's feasible and executable, are the same.

The customer engagement parts of supply chain management are also fundamentally the same: truly understanding who your customers are, what they need, and aligning on expectations relating to the what, where and when of delivery.

> "Gene therapy is a tremendously exciting emerging area, but it does present some unique challenges."

There's always going to be supply, demand and inventory, independent of industry type or in this particular case, the modality of the drug. However,

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as I've learned more about the gene therapy space, I do think one thing that's a little bit different is long-term manufacturing capacity planning.

> "I would love to see ... some more sophisticated tools for clinical inventory, demand and production planning."

With traditional pharmaceuticals and biologicals, you have this really standard lifecycle curve where there's the clinical trial element crossing over into generating commercial demand, then there's a growth period before the product hits peak sales. That's followed by a plateau for a number of years (depending on the lifecycle of the product) before the inevitable decrease in demand as time goes on.

If you think about what that traditional product curve looks like, it's typically measured in decades – in some cases, quite a few decades. So if you look at it from a drug manufacturing capacity management standpoint, you really do need to think long-term about how much plant capacity your particular asset is going to consume in terms of drug substance and drug product. With a traditional biopharma blockbuster product, you may think about building an entire plant to give yourself multiple years of inventory production.

However, the characteristic single dose curative model of gene therapy results in the demand spike being realised much earlier in the timeline and the drop-off is going to come much sooner, too. You are therefore going to have to recoup on your R&D investment in the first couple of years before focus shifts from the rate of incidence to the rate of prevalence of the given disease: the rate of incidence drives your initial demand requirements, which is where you will see your spike, but that will then fall before plateauing at the rate of prevalence.

Q Gene therapy as a field is becoming more competitive, for instance in terms of demand for viral vectors and critical raw materials. What challenges and considerations does this present?

CP: We actually haven't encountered too many challenges yet relating to balancing the availability of the resources that are out there. What I do find interesting in this particular space is that there's been a pretty considerable amount of M&A over recent times with large biopharmas increasingly looking to buy smaller gene therapy companies. For example, Biogen recently completed the acquisition of Nightstar Therapeutics, while Novartis purchased Avexis.

So there's a degree of consolidation within the cell and gene therapy biotech sector. When I think about competing priorities in the market space,

I'm mainly interested to see what happens with these smaller companies as they become acquired by larger companies, and how the larger companies look to drive their acquired assets forward. One key question for me: is there going to be an effort for larger companies that have a much larger manufacturing footprint to internalise their gene therapy production wherever possible, as opposed to adopting the contract manufacturing model? I also wonder if there's going to be further consolidation in the CMO field.

I think it's going to be a really interesting space to keep an eye on over the next decade as more gene therapy products go through the development cycle and hopefully reach the commercialisation stage.

Q What are the key tools of your trade, and where do you see room for improvement/missing pieces of the enabling technology jigsaw?

CP: One of the things I think the industry has done a really nice job with over the past few decades from the enabling tools standpoint is getting its head wrapped around cold chain and what that means. As we've discussed, a subset relating to gene therapy is the ultra-cold chain – I think the ability to manage that elegantly has really been a wonderful win for the industry over relatively recent times.

Moving backwards from that into the manufacturing and supply chain planning space, one thing I would love to see is some more sophisticated tools for clinical inventory, demand and production planning. There are some tools out there that are really quite good in terms of their capabilities, but from a management and maintenance standpoint they can be rather cumbersome in application, making monthly demand planning and inventory planning cycles difficult to do.

At the other end of the spectrum, some folks default to using Excel. And while Excel is a great tool for a number of things, it certainly has its weaknesses as well.

In clinical planning as a whole, I'd love to see some more mature planning software that can be easily integrated with companies' ERP systems. This would lend itself well to gene therapy clinical supply chain planning in particular, because there's going to be a huge emphasis on optimising inventory given the inherently high cost of manufacture of these products. That is of course a key sub-driver for this field.

Q Finally, can you distil for us the key risk mitigation steps you would recommend as one progresses a gene therapy through clinical development?

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CP: One of the basic tools that all supply chain planners have in their tool belt is inventory, including staging inventory, so one of the first risk mitigation approaches we would take would be to optimise our inventory, as far as is possible, such that we would always have enough inventory to absorb some type of event that's outside of our control – for example, a temperature excursion, a shipment lost in transit, or a batch failure.

Another risk mitigation tool we've been utilising (and which we could certainly use more) is Failure Mode and Effects Analysis (FMEA). I think it's a pretty standard, well understood tool, but I do like the exercise of going through the process to really understand the fundamentals of your supply chain – what could go wrong and where. Once you've identified those potential breaking points in the nodes in your supply chain, you can then proactively put risk mitigation steps in place.

So I would highlight inventory from a tactical standpoint, but for a systematic approach, FMEA is a really powerful tool.

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