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SPOTLIGHT ON:
Cell therapy materials & upstream processing/Analytics

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MATERIALS & UPSTREAM PROCESSING/ANALYTICS

SPOTLIGHT

EXPERT INSIGHT

Challenges in obtaining cellular therapy starting material for patients with sickle cell disease

Yvette C Tanhehco

Hematopoietic stem cells (HSCs) collected by apheresis serve as the starting material for gene therapy for sickle cell disease (SCD). Candidates for gene therapy and autologous transplantation undergo a series of red blood cell transfusions to optimize the patients for mobilization and collection. Several challenges exist in obtaining sufficient HSCs for drug product manufacturing at every stage. This paper discusses the challenges associated with collecting HSCs for gene therapy of patients with SCD, current approaches to optimize HSC yields, and potential future areas of investigation.

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INTRODUCTION

Beta hemoglobinopathies are inherited disorders caused by mutations in the β-globin gene that lead to a reduction in the levels of β-globins or abnormal β-globins. Sickle cell disease (SCD), a type of β hemoglobinopathy, is caused by a single base substitution (A–T) in the β-globin gene that results in a single amino acid change and the production of hemoglobin S (HbS). The presence of HbS leads to sickling of red blood cells (RBCs) under deoxygenated conditions. Because sickled RBCs are more rigid and less deformable, small blood vessels may be occluded, which results in vaso-occlusive pain crises (VOCs), intravascular hemolysis, endothelial injury, end-organ damage, and anemia [1]. Patients experience significant morbidity and

mortality even with available treatment options that include RBC exchange (RCE), disease modifying drug therapy, and allogeneic stem cell transplantation.

Autologous stem cell transplantation, unlike allogeneic stem cell transplantation, is associated with several benefits including ready availability of the donor, absence of graft rejection or graft-versus-host disease, and no additional infectious disease risk [2]. The hematopoietic stem cells (HSCs) of patients with SCD would need to undergo gene modification, however, for autologous transplantation to be curative. This concept is not new since a similar strategy was adopted for the treatment of β-thalassemia. In fact, on August 17, 2022, the United States Food and Drug Administration (FDA) approved betibeglogene autotemcel (Zynteglo®, bluebird bio, Somerville, MA, USA), the first cell-based gene therapy for the treatment of adult and pediatric patients with β-thalassemia who require regular RBC transfusions [3].

Clinical trials of gene modified HSCs from patients with SCD followed by autologous transplantation have been conducted [4,5] and more are still underway (www.clinicaltrials.gov). A safety and efficacy study of lovo-cel (bluebird bio, Somerville, MA) in 35 patients showed a median total hemoglobin increase from 8.5 g/dL to ≥11 g/dL from 6 months to 36 months post-infusion. The HbAT87Q contributed at least 40% of the total hemoglobin and was distributed across a mean (±SD) of 85 ± 8% of RBCs. Among the 25 evaluable patients, all had resolution of severe VOC events in the preceding 24 months before enrollment and a reduction in hemolysis markers [4]. In a study of exa-cel, 31 patients infused with exa-cel (CRISPR Therapeutics, Zug, Switzerland; Vertex Pharmaceuticals, Boston, MA) had a mean proportion of HbF >20% by month three, mean total hemoglobin >11g/dL on and after month three, and were all free of VOCs. All eleven patients who had at least 12 months of follow-up had maintained HbF levels >20% without any VOCs. At month six of follow-up, the mean proportion of edited BCL11a alleles in the bone marrow $CD34^+$ hematopoietic stem and progenitor cells and peripheral blood mononuclear cells was 86.6 and 76%, respectively [5]. The biological license application (BLA) submissions for lovo-cel and exa-cel are expected to be complete by the end of the first quarter of 2023. Since the results of these clinical trials have been encouraging [4,5] in terms of safety and efficacy, FDA approval of these drug products is likely to occur.

Collecting sufficient HSCs for gene modification and drug product manufacturing from patients with SCD has been very challenging. Several cycles of stem cell mobilization and collection have been necessary to obtain the target number of $CD34^+$ cells/kg (usually $>10\times10^6$ $CD34^+$ cells/kg) that manufacturers require which is significantly higher than the number of HSCs typically required for allogeneic transplantation (minimum of 2×10^6 $CD34^+$ cells/kg). This large number of $CD34^+$ cells/kg in the starting material that is necessary is presumably due to significant cell losses during the manufacturing process. The difficulty in apheresis collection of HSCs lies in part with the altered RBC characteristics and blood rheology in patients with SCD [6]. Current approaches have not yielded consistent or predictable results (author's personal experience). It is unclear which patient factors play a role, if at all, and if these factors are modifiable prior to collection to optimize yields.

This paper discusses the challenges associated with collecting HSCs for gene therapy of SCD, current approaches to optimize HSC yields, and potential future areas of investigation.

RED BLOOD CELL TRANSFUSIONS

The journey for patients with SCD considering autologous transplantation with gene therapy begins with regular red cell transfusions starting approximately 3 months before the apheresis collection in order to optimize

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the patient's bone marrow microenvironment. The overall goals of RBC transfusions are to reduce the percentage of HgbS in order to suppress endogenous hematopoiesis, decrease vaso-occlusive complications, and minimize RBC transfusion during bone marrow reconstitution. Sickle RBCs could interfere with apheresis collections and lead to VOCs [7,8]. Since apheresis uses a centrifugation principle which is dependent on the RBC size, elasticity, and density, any alterations in these RBC parameters could alter their behavior in apheresis [9]. Walker *et al.* [9] found that the peripheral blood of mobilized patients with SCD have an increased population of low density RBCs which lends an explanation as to why collecting deeper into the RBC layer improves HSC yields [6]. By minimizing the amount of sickle RBCs through regular red cell transfusions, the anomalous behavior of RBCs during apheresis may be curtailed.

Patients with SCD undergo a series of RBC transfusions to decrease HgbS levels <30%. Automated RBC transfusions by apheresis (i.e., RCE) are preferred over simple RBC transfusions because the HgbS percentage can be rapidly decreased while preventing iron and volume overload [10,11]. RCE via apheresis are typically performed at set intervals (e.g., 4 weeks) with the last RCE occurring a few days (e.g., 1–3 days) prior to collection.

Apheresis is a specialized procedure that requires highly trained nurses or technologists to operate costly instruments under the supervision of trained physicians who determine procedure parameters and manage adverse events. The first challenge encountered by patients is access to RCE because not all hospitals have this service readily available. Another challenge encountered in performing apheresis procedures is determining the type of vascular access to use as there are several options available. Peripheral venous access, where two large bore needles (18-gauge or 16-gauge) are used to cannulate a vein on each arm, is suitable for patients with adequately large peripheral veins. For those without adequate peripheral veins, central venous catheters inserted into the subclavian vein, internal jugular vein, or femoral vein can be used. Implanted venous access devices (i.e., ports) and functional grafts/fistulas may also be used if frequent procedures are expected. Each have their own advantages and disadvantages and not all options will be appropriate for each patient. Regardless of the type of vascular access chosen, the line needs to be able to withstand the high pressures and flow rates present in apheresis procedures. This would require rigid catheters and large arm veins. Determining the best type of vascular access to use depends on the state of the patient's veins, duration of procedures, frequency of procedures, and patient preference.

RCE procedures utilize healthy allogeneic donor RBCs as the replacement fluid. Transfusion support guidelines for patients with SCD recommend prophylactic red cell antigen matching for Rh (C, E or C/c, E/e) and K antigens in addition to ABO/RhD matching and antigen negative blood for the alloantibodies that the patient has [10]. Because many patients with SCD are highly alloimmunized [12–14], it may be difficult to find the appropriate number of RBC units for each procedure. Planning ahead and communicating the patients' needs to blood suppliers early enough to give them ample time to obtain rare units are keys to overcoming this challenge.

Setting the right fraction of cells remaining (FCR) for the RCE procedure, which determines the post-procedure HgbS, can also be a challenge. The recommended target post-procedure HgbS is generally less than 30%, with the goal being to maintain HgbS levels at less than 30% at all times between RCE procedures. In order to achieve this goal, HgbS levels will have to be reduced to a level much lower than 30% (e.g., less than 20%) at the time of the RCE but the exact target would vary from patient to patient because daily HgbS increments vary as well. There are currently no formal guidelines on how to determine this optimal

post-procedure HgbS target or the interval of RCE procedures; thus, a trial-and-error method is frequently used.

MOBILIZATION

Hydroxyurea (HU), a standard of care medication for patients with SCD, must be discontinued at least 30 days prior to mobilization and collection as it inhibits mobilization. HU is a ribonucleotide reductase inhibitor that causes myelosuppression suggesting bone marrow toxicity and potential impairment of HSCs [15,16]. HU withdrawal was found to be associated with an increase in the number of circulating $CD34^+$ cells in patients with SCD [17].

HSCs may be collected by apheresis or by a bone marrow harvest. Bone marrow harvesting requires anesthesia and repeated procedures to obtain a sufficient cell dose for manufacturing. It is also associated with significant pain post-procedure [18]. A more convenient and comfortable alternative for patients is to collect HSCs from the peripheral blood via apheresis. Since less than 0.05% of circulating cells in the peripheral blood are HSCs, donors must be mobilized with an agent that will temporarily increase the number of HSCs in the peripheral blood that can be collected by apheresis. Mobilization agents increase the dissociation rate of HSCs from the bone marrow 'niche' spaces they are tethered to by disrupting the adhesive interactions. Granulocyte colony stimulating factor (G-CSF) has traditionally been used for HSC mobilization; however, it is contraindicated in patients with SCD because it has been found to lead to significant morbidity as a result of the hyperleukocytosis [19,20]. Severe adverse events that included vaso-occlusive episodes, acute chest syndrome, multi-organ system failure, and death have been reported [21].

Plerixafor, an inhibitor of the CXCR4 chemokine receptor, was reported to be successful at mobilizing patients with SCD when administered at a dose of 240 μg/kg [22,23]. The peak levels of $CD34^+$ cells is achieved at 3–6 h in patients with SCD as opposed to 6–12 h in healthy donors [18,24]. The quantities of plerixafor-mobilized $CD34^+$ cells varies greatly among patients with SCD for unknown reasons [25]. Leonard *et al.* found that $CD34^+$ yield correlated negatively with age and positively with baseline and pre-apheresis blood $CD34^+$ cells/ul, baseline white blood cells (WBC), and platelet counts. Specific to patients with SCD, $CD34^+$ cell yields correlated negatively with markers of disease severity such as hospitalization frequency within the preceding year and the number of medications taken for chronic pain and positively with the number of days HU was held [25]. In light of the white blood cell lowering effects of HU, it is not surprising that the longer HU is held, the better the $CD34^+$ cell yield obtained. They also found a strong positive correlation between yield/L processed and pre-apheresis $CD34^+$ cells/μl [25]. The variability and unpredictability of $CD34^+$ cell mobilization has contributed to the challenges of collecting sufficient cells for gene modification and drug product manufacturing.

The current practice is to start apheresis collections 4 h after the administration of plerixafor. Close coordination with the apheresis team is required so as not to miss this window. Serial measurements of $CD34^+$ cell numbers at various time points may be helpful in determining an optimal start time for apheresis to maximize the peak.

APHERESIS COLLECTION

Technical challenges during apheresis collection can be associated with reduced collection efficiency and $CD34^+$ cell yield so efforts must be made in overcoming these challenges.

The optimal apheresis collection parameters for patients with SCD are different from those used for HSC collections in patients without SCD. This difference can be attributed to the altered characteristics of sickle RBCs that interferes with the sedimentation of cells

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during apheresis [26]. In apheresis collections for patients without SCD, the collection preference is set to target a color between the third and fourth darkest bar on the collection preference tool of the Spectra Optia® Apheresis System (Terumo BCT, INC; Lakewood, CO). In patients with SCD, the collection preference is typically lowered to collect deeper into the RBC layer where the HSCs are believed to reside to achieve a product hematocrit of approximately 5–10% [26]. The target color of the collect line should be between the darkest and second darkest on the collection preference tool to achieve the aforementioned target product hematocrit [6]. Despite following these guidelines, however, the collection yield is still highly unpredictable and variable among patients. Obtaining a product WBC count and hematocrit after one total blood volume has been processed may be helpful in gauging the appropriateness of the parameters being used for the collection and allow sufficient time for parameters to be adjusted if the yield is lower than expected. The interface can also be highly unstable so frequent adjustments of the collection parameters may be necessary for this reason as well.

Clumping in the circuit may be another challenge encountered during apheresis collection. If clumping in the circuit is observed when using the default anticoagulant (AC) ratio of 12:1, the AC ratio may have to be lowered to 10:1 or 8:1. Aspirin has also been administered to patients prior to the collection procedure in an effort to decrease clumping in the product [27].

The inlet flow rate needs to be optimized to minimize alarms and avoid system pauses. Using a slower speed that results in no alarms is more efficient than using a faster speed that causes alarms. Increasing the inlet pump flow rate or increasing the anticoagulant (AC) infusion rate will allow the apheresis instrument to process more blood in less time but results in a greater volume of AC delivered to the patient and increases the potential for citrate toxicity [28]. When citrate toxicity occurs, the inlet flow rate is decreased or the apheresis instrument is paused to allow the patient time to metabolize citrate or to administer calcium. Pump pauses result in delays in establishing the interface. After the pumps restart, it will take time for the pumps to reach the target flow rates again, which increases the run time [28].

TRANSLATION INSIGHT

Gene therapy for SCD is on the horizon. Once this curative therapy is approved by the FDA, it will most likely be the preferred treatment modality for those who qualify regardless of genotype and symptomatology. Despite the success in clinical trials, there is still a lot we don't know in terms of optimizing the starting material. Several cycles of collection are currently needed to obtain sufficient numbers of HSCs for gene modification due to inefficient apheresis collection procedures. Further improvements in mobilization and collection strategies to decrease the number of procedures required are paramount.

The ability to collect HSCs by apheresis has undoubtedly made gene therapy and autologous transplantation more palatable for some patients. Future areas of research could focus on finding alternative mobilization agents suitable for patients with SCD. Although plerixafor has been successful in mobilizing HSCs in patients, there may be other more potent drugs with similarly minimal side effects that could serve as an alternative.

Another area of future research could focus on determining the optimal collection parameters to use. It is well established that the collection preference and anticoagulant-citrate-dextrose-solution A dosage need to be altered for patients with SCD due to the altered HSC sedimentation and hypercoagulable state associated with the disease [6]. However, these adjustments do not always result in a high yield of $CD34^+$ cells which indicates that there are other factors at play. Better characterization of the cellular

content of the products that are currently collected may provide some guidance. Determining patient factors that may affect the collection efficiency should also be an area of investigation.

Finally, there are subtle differences in how patients with SCD are prepared for mobilization and collection that may affect the outcomes of the collection but haven't been rigorously studied in a clinical trial. For example, Lagresle-Peyrou *et al.* [22] reported hyperhydrating their patients with 60 ml/kg/day of a 0.9% saline solution and providing oxygen therapy at 2 L/min as recommended by French guidelines [29] for VOC prophylaxis. Uchida *et al.* [27] reported giving their patients 325 mg aspirin before apheresis. These are not standard recommended practices and it would be interesting to know if simple management strategies like these would improve CD34⁺ cell yields.

In summary, apheresis collections for cellular therapy starting materials for patients with SCD are associated with a number of challenges. More effective strategies for optimizing mobilization and collection are needed to increase CD34⁺ cell yields and minimize the number of collection cycles required. A review of individual and aggregate data that includes collection parameters, cell yields, and patient factors may be useful in identifying areas for improvement.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MATERIALS &
UPSTREAM PROCESSING/ANALYTICS

SPOTLIGHT

COMMENTARY

Overcoming challenges to CAR-T cell therapies in India

Annu Uppal, Ranjan Chakrabarti, Narendra Chirmule, Shashwati Basak & Fouad Atouf

Chimeric antigen receptor (CAR)-T cell therapies are promising treatments for previously untreatable cancers. However, many hurdles still need to be cleared before these products become widely accessible, especially to patients in low- and middle-income countries. One significant challenge that prospective cell therapy manufacturers face in emerging markets is access to raw materials, such as donor cells and viral vectors. This problem is not simply a lack of domestic sources for raw materials but, more importantly, the inability to consistently validate their quality. To address how best to approach these issues, USP-India organized a panel discussion on challenges faced by Indian CAR-T cell therapy developers at Global Bio-India 2021. Topics discussed include local manufacturing capacity, access to critical raw materials, requirements for analytical methods, release assays, and control procedures. This article summarizes some of the critical issues and recommendations raised by experts from academia, industry, and government.

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Cancer is already one of the leading causes of death worldwide, and its role in early mortality is expected to grow significantly over the next two decades [1,2]. Much of the projected increase will occur in large emerging economies, such as India, which have the twin threats of rapid urbanization and an aging population [3]. Cancer is already a major public health problem in India, resulting in over 850000 deaths in 2020 [4,5]. Every year, over 1 million Indians are diagnosed with cancer, and that number is expected to double by 2040 [1].

The Indian government is prioritizing lifestyle changes and more accessible primary-care services as the most cost-effective way to address the coming wave of cancer diagnoses. Not surprisingly, much of the policymaking is centered on reducing tobacco use and air pollution; and mitigating the spread of human papillomavirus (HPV), hepatitis B virus (HBV), and *Helicobacter pylori* [6]. The government is also working on expanding access to early oral, cervical, and breast cancer screening [7]. Focusing on preventative measures will no doubt save countless lives [8]. But the government will also need to support options for the growing number of Indians that will be diagnosed with cancers not treatable with surgery, radiation, or chemotherapy. Recent advances in cell therapies, especially the development of chimeric antigen receptor (CAR)-T cell therapies, give these patients new hope.

A CAR-T cell therapy is made by genetically modifying a patient's T cells to recognize and attack cancer cells that express specific antigens on their surface. This approach has proved very promising in treating several types of blood cancer, such as Acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL), which are difficult to treat with other methods [9]. Several of these therapies are already approved by regulators in the USA and Europe (EU), and there are currently thousands of novel treatments in various stages of development, from pre-clinical to Phase 3 clinical trials [10,11].

All this progress notwithstanding, CAR-T cell therapies are still relatively new, and their development in India is much more limited. Only one cell-based therapy product is approved in India, and only a few companies are in the early stages of developing CAR-T cell therapies [12,13] major sticking point is that Indian CAR-T cell therapy developers need access to patients, but only a few academic institutions and hospitals work on cell therapies. There is also a lack of sufficient stem cell transplant centers that actively coordinate with the Indian Stem Cell Transplant Registry [14]. But even if CAR-T cell therapy development in India were on par with that in the USA and EU, there would still be a significant problem with access because these therapies are expensive. There is a valid concern that CAR-T cell therapies will be inaccessible to most Indians if the domestic cell therapy industry can't keep its costs low [15].

India must therefore address all the same challenges being faced by cell and gene therapy developers worldwide, such as building a regulatory framework that maintains the quality of the products and training the labor force needed to sustain domestic manufacturing; but in a way that ensures access to the average Indian. To get a better understanding of how to address this problem, the Department of Biotechnology (DBT), the Government of India, and the United States Pharmacopeia (USAP) co-hosted a panel discussion on 2 March 2021 titled "Ensuring Quality: Overcoming CAR-T cell CMC challenges" as part of the Global Bio-India annual conference [16].

The panel brought together international and local industry, academia, and government experts to discuss the potential for developing and delivering cell therapies in India. Several strategies were discussed to address current challenges with viral vector manufacturing, handling of cellular starting material, and maintaining sterility and potency.

LENTIVIRUS

To make a CAR-T cell therapy, a manufacturer needs a consistent supply of quality lentivirus vectors (LV) to turn patients' cells into cancer killers. But the infrastructure to support global demand for viral vectors has not kept pace with demand, and there is currently a lack of commercial-scale production for this crucial starting material [17].There are less than 100 contract manufacturing sites capable of producing any viral vector,

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let alone just LVs, scattered across 15 countries [18,19] Most of those facilities are in the USA and EU, with only a handful of sites in Asia and none within India [19].

Part of the problem is that viral vector production needs to be carried out under a much higher level of biosafety than other facilities because of the infectious nature of the product. A non-infectious biologic, such as a therapeutic antibody or a peptide, can be made in a biosafety level 1 (BSL1) facility, but a viral vector needs to be produced within the confines of a biosafety level 2 (BSL2) site. To the casual observer, the two facilities may appear similar as they have much of the same equipment. However, BSL2 requires a greater containment level, including pressurized rooms and retention spaces, to prevent any virus from escaping. These constraints mean that viral vector facilities take more time and money to build and cost more to operate than those used to produce monoclonal antibodies [18].

The result of insufficient manufacturing capacity, inefficient manufacturing processes, and requirements for specialized facilities is, not too surprisingly, a shortage of viral vectors. The wave of gene-modified cell therapies forming on the horizon will only exacerbate this shortage. Yet cell and gene therapy manufacturers are moving forward at a breakneck pace, with over 1200 cell and gene therapies in clinical trials worldwide, with more than half of them for CAR-T cell therapies. The industry will need dozens more facilities for manufacturing the LVs that will be required if even a fraction of these treatments are approved [20].

The situation in India is even more acute because of the absence of domestic manufacturing for LVs. A CAR-T cell therapy developer in India has to rely on imported LVs from a foreign contract manufacturer. Bringing LVs into India is complicated by the opaqueness of the regulatory process for importing viral vectors [21]. Also, complete reliance on foreign suppliers creates supply chain vulnerabilities. For example, once regulators have approved a manufacturing process, switching to a new raw material supplier is no simple task. If a supply chain is disrupted, a CAR-T cell therapy manufacturer has to find a new supplier and get regulatory approval for that change. But with no local suppliers to choose from, this can be especially burdensome for an Indian CAR-T cell therapy developer, given the logistics involved in coordinating contracts, validation, inspections, import, etc.

Improving the current situation will require substantial investments in building and maintaining cGMP-compliant manufacturing facilities and workforce development. There is some movement in this direction. The Indian government currently provides funding and training to companies working on cell and gene therapies and is actively requesting proposals to spur the domestic development of affordable CAR-T cell therapies. For example, the National Biopharma Mission (NBM)-Biotechnology Industry Research Assistance Council (BIRAC) recently funded Immuno-Adoptive Cell Therapy (ImmunoAct), a company spun out of the Indian Institute of Technology in Bombay (IIT-B), to conduct Phase 1 and 2 clinical trials with CAR-T cell therapies at Mumbai's Tata Memorial Centre [22]. If successful, ImmunoACT will be able to provide a CAR-T cell therapy at one-tenth the cost of comparable treatments such as Gilead's Yescarta® (axicabtagene ciloleucel) or Novartis' Kymriah® (tisagenlecleucel). BIRAC is also supporting efforts by Intas Pharmaceuticals to develop a CAR-T cell therapy for ALL and B-cell lymphoma; as well as Syngene's effort to build the first GMP-grade viral vector manufacturing facility in India [23].

Yet the future of cell therapies in India will likely require bolder initiatives focused on creating shared resources and facility hubs devoted to developing cell and gene therapies. This approach to supporting local manufacturing, or 'glocalization', can significantly lower costs by preventing waste

from replicated efforts and reducing the economic pressure on any individual stakeholder [24]. The ideas encompassed by glocalization aim to develop a reliable supply of low-cost, advanced medicines in countries that traditionally lack the infrastructure, resources, and skilled labor to produce and distribute drugs cost-effectively. But for glocalization to succeed, government agencies, non-governmental organizations, and local entrepreneurs must work together to create a comprehensive quality management system for CAR-T cell therapy development [25].

T CELLS

CAR-T cell therapies are often initiated in collaboration with academic laboratories or clinical settings because this is one of the most reliable ways to ensure access to patients' T cells. However, in India, there are not enough universities and hospitals with the proper infrastructure or expertise to carry out this function at the scale needed to support a national industry. Cell collection is a major concern for cell therapy manufacturers since improper sample handling carries a significant risk of contamination that can result in serious side effects and death in patients that receive impure products [17]. However, there are still no global standardized methods for cell collection for making CAR-T cell therapies. Part of the challenge is a lack of harmonization between regulatory authorities in different countries. In India, the collection and manipulation of T cells fall under the purview of the Review Committee on Genetic Manipulation (RCGM), but this committee was created to regulate genetically modified organisms (GMOs), such as plants and microbes, and is not ideal for capturing information concerning autologous cell therapies.

More must be done to ensure that the necessary starting materials are readily available to researchers and manufacturers in India [26]. To start, there needs to be a concerted effort to develop standards that support domestic cell collection centers. This should include training in assessing cell subset composition, phenotype, and quality so that cellular starting materials can be used consistently to make potent final products [27–29]. Much of the needed training could be developed through collaborations with standards-setting organizations, such as USP, which has already created some practical guidelines and best practices for cell collection [30–32].

STERILITY & POTENCY

The lack of standards combined with minimal alignment on methods for characterizing cell-based products is a significant barrier for Indian developers and CAR-T cell therapy manufacturers worldwide [26]. Regardless of location, all cell and gene therapies have tight time constraints that differ radically from other treatments. As a 'living' therapy, CAR-T cell therapies are highly perishable and require very tight storage controls and cold chain management. Also, most patients who are good candidates for these therapies are very ill and need to receive treatment within weeks, if not days. However, the current framework for ensuring that products are sterile before they are administered to patients is built for an entirely different timeline. Most sterility testing methods are culture-based and require 2–3 weeks for results. The current compromise is to release the therapy for patient use before sterility is confirmed using an approved method if the manufacturer can demonstrate that the product is likely sterile. To do this, manufacturers use an assortment of rapid microbial tests, but these methods must be bridged to conventional approaches and approved on a case-by-case basis.

Another area where standards are lacking is in defining potency. The potency of a cell therapy depends upon a combination of molecular, cellular, and biological activities. Therefore, measuring and validating potency requires a variety of several assays

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that measure different parameters. The panel of assays should also include at least one quantitative assay that measures a biological activity closely tied to its proposed mechanism of action (MOA). However, very few standards or reference materials are available for cell therapy potency assays, which makes validating these assays difficult [33].

The development of standards for sterility and potency will not be easy, given the range of test methods used to characterize the different product types for various indications [34]. But manufacturers do not need to start from scratch. Standards-setting organizations like USP have already initiated programs to develop relevant best practices and standards for advanced therapies, focusing on raw materials, potency, and safety (Table 1) [35,36]. These efforts are ongoing, and there is still much work to be done before the industry reaches any significant level of harmonization, but it represents an excellent start.

CONCLUSION

There were many perspectives on how best to address the significant hurdles ahead. However, all the panelists agreed that any solution to guarantee the accessibility and affordability of CAR-T cell therapies in India must be based on much stronger collaborative efforts between academia, hospitals, industry, and standards-setting organizations to build up local manufacturing capabilities. A glocalized approach to manufacturing cell and gene therapies has the potential to create equitable access for patients in emerging market nations, including India. But to be effective, this approach requires standards to ensure consistency and quality across multiple manufacturing sites, often spread out over long distances. Indian manufacturers are in a unique position to engage on this issue and help the industry reach a consensus on quality expectations that are balanced against the need to make these treatments available to everyone.

TABLE 1

US Pharmacopeia (USP) standards related to cell and gene therapy.

Application	Resource
Raw and ancillary products	<1043> Ancillary materials for cell, gene and tissue engineered products <1046> Cellular and tissue-based products <1047> Gene therapy products <89> Enzymes used as ancillary materials in pharmaceutical manufacturing <90> Fetal bovine serum quality attributes and functionality tests <92> Growth factors and cytokines used in cell therapy manufacturing <1024> Bovine serum
Potency/bioassay	<111> Design and analysis of biological assays <1030> Biological assay chapters–overview and glossary <1032> Design and development of biological assays <1033> Validation of biological assays <1034> Analysis of biological assays
Safety	<1116> Microbiological control and monitoring of aseptic processing environments <1211> Sterility assurance <71> Sterility tests <61> Microbiological examination of nonsterile products: microbial enumeration tests <62> Microbiological examination of nonsterile products: tests for specified microorganism <63> Mycoplasma tests <85> Bacterial endotoxins test <1229.3> Monitoring of bioburden <1237> Virology test methods

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MATERIALS &
UPSTREAM PROCESSING/ANALYTICS

SPOTLIGHT

INTERVIEW

Challenges in cryogenic storage containers for cell & gene therapy

Róisin McGuigan, Editor, BioInsights, speaks to **Sean Werner,** Chief Technology Officer, Cell Processing, BioLife Solutions & **Alex Sargent,** Director, Process Development, Charles River Laboratories

Sean Werner & **Alex Sargent** (pictured from left to right)

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Current options for cryogenic storage containers in cell and gene therapy are limited in their functionality as the industry continues to move towards increased scale and automation. In this episode, Sean Werner and Alex Sargent address specific challenges with current optionality, while also considering what future innovations in this area might look like.

Q How have we landed on the current landscape of options for cryogenic storage containers used in the cell and gene therapy space?

SW: I think that there are a few paths as opposed to just one. On one hand, some of the packaging we use originated from the blood industry and what the stem cell transplanters were using. This involved blood bags and storage bags that worked well for colder cryogenic processes. On the other hand, academic groups that developed some early cell processes were used to screw cap vials used in a biosafety cabinet to control things from an aseptic technology perspective, with not as much sterile fill as you would see in large molecule pharma. As time went by, several unique packages have been developed, moving from glass vials to cyclic olefin copolymer (COC) vials and ready-to-use sterile closed vials. There have also been improvements in bag plastic so they have a lower fracture rate and perform better in liquid nitrogen.

AS: It is often a challenge in cell and gene therapy that many of the options we have available come from research groups and academia. At that point, there is not necessarily a lot of forethought given to how you would fit that into an industrial approach to manufacturing, or in this case, cryogenic containers for cell and gene therapy.

Q Where do the current offerings fall short as the cell and gene therapy industry continues to grow?

AS: There are a number of options, all of them with their own advantages and disadvantages. One of the areas where current containers fall short is scalability. Do you have containers and systems where you can scale up or scale out? We are considering thousands or even tens of thousands of containers in order to meet growing patient demands. We typically see large-scale operations in vials, and we are hopefully moving away from screw top vials towards hermetically sealed and closed vialing systems. The limitation with these is usually around volume constraints, as they typically hold 1–10 mL, although some can go up to 50 mL. Another popular option is cryobags – these provide more flexibility in terms of volume but are more fragile in terms of stability and robustness, especially during the shipping process.

SW: On the small volume side, there are quite a few options that serve the industry fairly well. If you think about scaling up at a small volume, if needed we can move to isolator fill systems. Some of those already exist for the options that are out there. There are filling options that work well in a biosafety cabinet for smaller scale. The small volume options are good and do not require as much improvement.

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With larger volumes, there is still a gap. Bags work, but they take a lot of individual manual manipulation to get them in the right form that you need to freeze. There are additional components, like cassettes and racking systems, that you must put into the large dry shippers that are expensive to move around the world. An industry-wide scale for these therapies is a significant ask for the logistics providers for the industry to support. The main gaps exist around how to establish better, larger volume storage containers.

"Automated inspection processes, like looking for particulates and checking for closure integrity, are additional challenges that add to the cost and time of the manufacturing process."

– **Sean Werner**

AS: A few weeks ago, we had a client with a batch size of roughly one hundred bags. They had an elegant process from start to finish, including fill/finish, but at the end of that process, they had an assembly line of laboratory technicians manually fitting the overwrap on the bags for hours. This shows that the large volume systems do have those limitations Sean was talking about.

SW: That is not ideal for taking labor and costs out of a process!

Q What will be the negative impacts of continuing with these solutions, as opposed to developing containers that are more specific to industry needs?

SW: From my perspective, the labor and handling that goes into those systems is a huge addition to the cost and the time of developing, manufacturing, and shipping these products. If we are truly going to transition into an industrialized process for these therapies, we cannot be thinking about somebody individually wrapping and pushing air out of these bags to make that a reality. Another challenge is the recovery from bags, which collapse as you drain them. If you have an extremely expensive product and you need to get as much as possible out of that container, it can be challenging. Automated inspection processes, like looking for particulates and checking for closure integrity, are additional challenges that add to the cost and time of the manufacturing process.

AS: Cell and gene therapies are the most expensive in the world, and a real challenge to industrializing these types of medicines is bringing down that cost for our patients. Another part of that is quality control (QC) options. Not just how patient samples or drugs are being stored and shipped to different sites around the world, but also your QC release aisles or products, can be a significant driver in cost. The logistics of shipping and

handling these precious therapies are difficult, especially when the cost and the stakes are so high. One could imagine somebody they love having the potential to receive a life-saving cell therapy only to find that during the shipping process, the bag had been damaged to render it unusable. Containers that can overcome this are critical when we think about how important these therapies are for our patients.

SW: In stem cell transplant labs, clinicians have lots of experience of treating patients with extremely valuable products, without a second chance. Transplant clinicians have told me that when they have had breakages in the labs, they cover the patient with additional antibiotics and dose anyway. That is how important this stuff is, so this is something that we need to figure out how to get past.

Q What should the cryogenic container of the future look like? And what would you pick out as the most important considerations or features?

AS: I want out of a cryogenic container what I want out of my pickup truck – something that is rugged, tough, dependable, and adaptable. Cell and gene therapies are so complex and diverse. You need a container solution with a wide degree of adaptability in terms of volumes and the ease with which you retrieve and administer the sample. It also needs to be reliable, and like my pickup truck it needs to get you where you need to go.

SW: That is exactly it – we need cryogenic containers to be reliable, robust, and reproducible. We need something that is not going to fail, and that we can rely on. We want to enable a simple, repeatable process in which we do not have to worry about it being very easy to get out of spec from the fill process. We need a consistent form factor that allows you to get the same kind of freezing profiles from small volume to large volume, does not require continuous optimization, and is going to take variability out of the process. We have to move to something more like what we have for smaller volumes – a rigid container that works well, can fit into an automated process, and give you the same results every time.

AS: In my experience, containers are often something can be neglected or an afterthought, as this industry is thinking about the process and the therapies. But it is critical to have the right container, formulation, and fill/finish option in place for your final product. We cannot neglect that when thinking about cell and gene therapies.

Q What additional considerations need to be addressed if truly allogeneic therapies, with tens of thousands of doses, are found to be more universally successful?

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"...there is a need for containers that are reliable and rugged, because when you have tens or hundreds of thousands of doses being shipped all over the world, you need that stability and protection for these therapies."

– **Alex Sargent**

AS: Allogeneic therapies are on the rise. I am a big proponent of allogeneic therapies to help drive down costs and overcome other limitations seen with autologous cell or gene therapies. The challenge is that tens of thousands – and one day in the future possibly even millions – of doses are needed. For that, you need a container that is scalable, and can be implemented into an automated platform and solution. There needs to be adaptability in terms of volume because these therapies might not be given at extremely small volumes. Large doses at a higher volume range beyond what vials can cover now may be necessitated. Finally, there is a need for containers that are reliable and rugged, because when you have tens or hundreds of thousands of doses being shipped all over the world, you need that stability and protection for these therapies.

SW: One of the additional pieces that I have heard folks starting to talk about more is the logistics side. In addition to making sure you have sufficient protection for a container as you ship, you need to know how to ship at large scale. It seems impossible to do this in the current dry shipper type of configuration. We must find ways where high-density packaging is possible. If you are going to have these stored at a central location, then maybe you have smaller versions of shippers that go out to the clinic as needed, but that will no longer be possible with millions of doses going around the world. Figuring out how to do high density storage of these products on location at different places or in regional hubs is key. A container that can support a variety of different logistics opportunities still needs to be developed. During the pandemic, we were not ready to ship the volume of vaccines that were shipped. It is time for us to start looking at how to deliver these therapies, keeping in mind that the container is a part of this.

AS: An allogeneic therapy may be in transit for multiple days. Having a container that can hold that temperature in the right shipping conditions over multiple days is going to be critical to making that therapy more accessible to patients, to get it to where it needs to go.

SW: Everybody in the industry is so excited about how far we have come already and is looking forward to where we are going. The fact that we are talking about storage and logistics shows that we are undergoing a transition from concept to the industrialization phase. This is exciting for me, and for our company.

BIOGRAPHIES

SEAN WERNER is the Chief Technology Officer – Cell Processing at BioLife Solutions, a leading provider of bioproduction tools and services to the cell and gene therapy and broader biopharma markets. BioLife acquired Sexton Biotechnologies in 2021 where Sean was President of the company known for providing processing and handling solutions for the CGT industry. Sean received his PhD from Purdue University in Biology followed by post-doctoral positions at the Indiana University School of Medicine and Eli Lilly. Sean has previous experience filling various roles in the global regulatory and general management functions supporting medical devices, autologous cell therapy, and single use disposable development programs. In his 15 years working in the life science industry, he has guided pre-clinical and clinical testing and submission strategies leading to global commercialization of multiple medical devices and bioprocessing tools.

ALEX SARGENT is currently the Director of Process Development at Charles River Laboratories. He obtained his PhD from Case Western Reserve University in Cleveland Ohio, where he studied the challenges and promises of stem cell biology, neuroimmunology, and Cleveland sports teams. After a brief stint in academia at the Cleveland Clinic, he left Cleveland to pursue his industry career in cell therapy. During his many years in this industry, he has had the honor of working at several esteemed enterprises on new technologies and approaches for cell therapy scale up, automation, and gene editing. He is especially passionate about the challenge of curing cancer, working on CAR-T and CAR-NK cell therapy process and analytical development from discovery, through regulatory submission, manufacturing, and pivotal clinical trials. He wakes up each day excited to help advance cell and gene therapy to treat and cure disease, with the steadfast goal of improving human lives.

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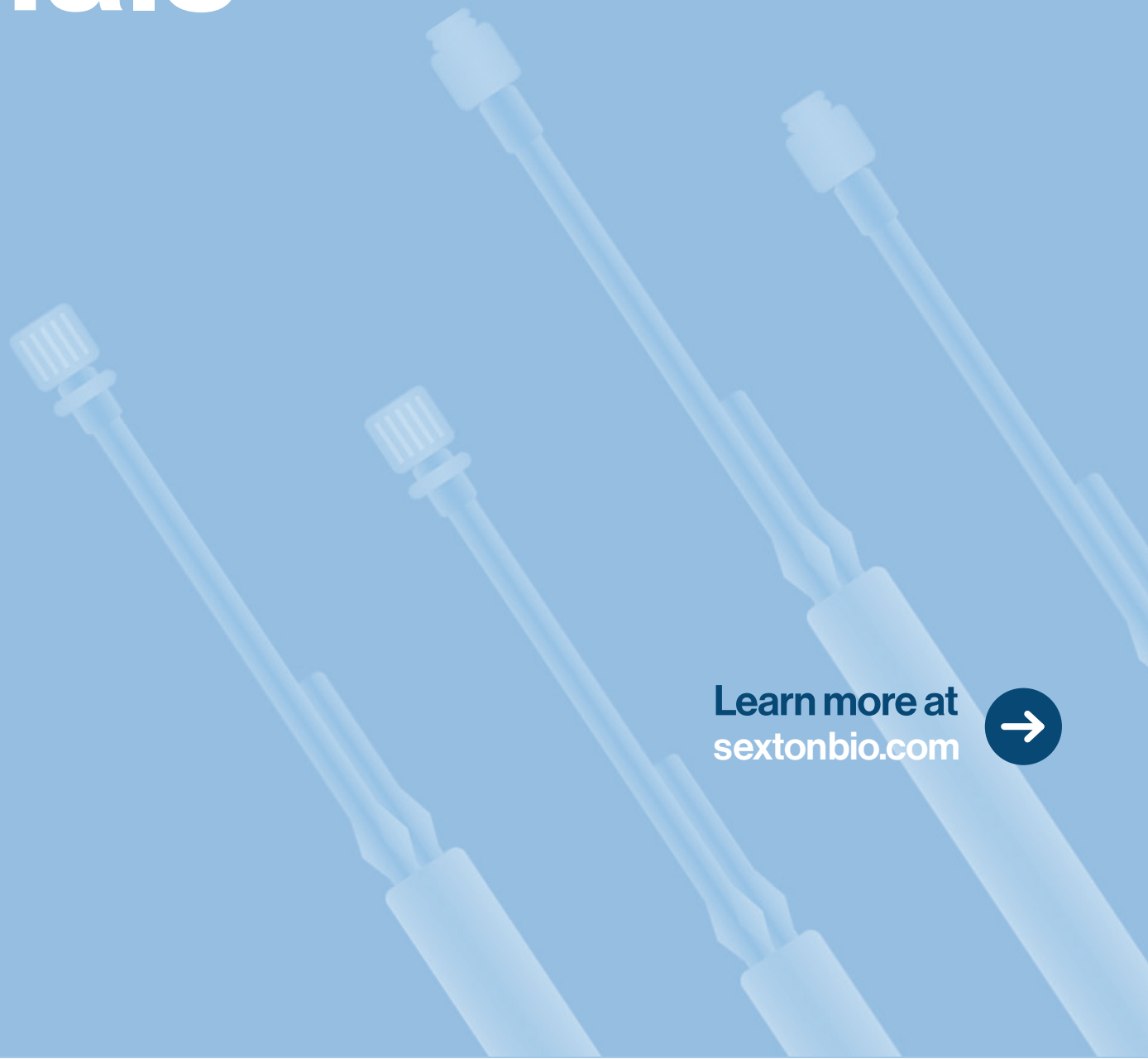

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INNOVATOR INSIGHT

Implementing a closed cell therapy manufacturing process through strategic collaboration

Øystein Åmellem, Xavier de Mollerat du Jeu & Brian Shy

The need for standardization of cell therapy manufacturing continues to be a critical driver of technological advancement and investments. Implementation of a scalable, integrated workflow can minimize open processes and reduce manual touchpoints, ultimately reducing manufacturing failures. New innovations in closed, modular, cell therapy-specific instrumentation enable the development of robust and reproducible, end-to-end manufacturing processes for commercial applications. In this article, we discuss solutions for standardizing manufacturing processes, and explain how strategic collaborations can help streamline the transition to the commercialization pipeline.

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GLOBAL CAR-T CLINICAL TRIAL ENVIRONMENT

The number of chimeric antigen receptor (CAR)-T cell therapy clinical trials has seen a dramatic global increase over the past decade, more than tripling in number. In 2016, approximately two-thirds of all active trials were academic studies. Today, these trials are almost evenly divided 50:50 between industry and academia.

The industry is now undertaking a journey to industrialize and globalize approved CAR-T drugs. An identified gap in the globalization of CAR-T cell therapy is linked to the cost structures in this field. However, tremendous investments are being made on both the scientific and the industrial sides

to bridge this. The therapeutic reach of cell therapies is also expanding, as demonstrated by approvals of already commercialized therapies for second-line treatment, indicating cell therapies are moving toward becoming a standard treatment approach for larger patient populations.

Challenges for the industry remain, which will continue to drive the need for further new technologies and improved manufacturing processes. Simplification of the complex manufacturing process and reduction in manufacturing failure rates are motivating factors driving the development, refinement, and innovation of products and instruments specifically designed for the unique manufacturing requirements of cell therapies.

AUTOMATING THE CELL THERAPY WORKFLOW

Thermo Fisher Scientific is addressing several of the current manufacturing challenges through integration and automation across the cell therapy manufacturing workflow using scalable and modular instrument platforms. All of the cell therapy manufacturing instruments in the portfolio are designed to be used either standalone, or digitally and physically integrated into an automated workflow. These instruments are specifically designed for process development and commercial manufacturing, and the closed single-use kits enable process speed and precision, while maintaining sterility. Compliant software can also be employed to further optimize and automate the process.

The integration of these platform instruments is driven by the DeltaV™ system from Emerson, which can be used to control data management. DeltaV™ is a widely used software package in bioprocessing that allows the centralization of commands and instruments, so that each small unit operation can be processed in a fully automated way. Each module can be connected to the next, with an overall goal of creating a closed, automated, reproducible, and scalable process.

The first level is control of each unit, the second level is crosstalk between different units of the workflow, and the third and final level is for the control of the entire workflow. DeltaV™ has the potential for users to control an entire plant and ensure that there are recorded batch records for each manufacturing process. This helps enable product safety management and also reduces the complexity of documentation, which has a high cost and time burden in this industry.

STRATEGIC PARTNERING TO ENABLE CELL & GENE THERAPY COMMERCIALIZATION

Thermo Fisher Scientific's collaboration program is designed to help facilitate optimization and integration of cell therapy manufacturing solutions, tailored to customer requirements. The customer can leverage Thermo Fisher's innovative solutions and process optimization knowledge and integrate them into scalable manufacturing processes. Through the collaboration mechanism, customers have access to a dedicated team of process development scientists and engineers, dedicated equipment, resources, and lab space to help support the development of protocols specific to customer needs. This is met with continued support to help ensure product quality and supply continuity. Workflow automation is built in close collaboration with customers, offering early access to instruments to ensure needs are met and improvements can be made in real-time.

Thermo Fisher Scientific's Cell Therapy Collaboration Centers are meant to facilitate the development and optimization of integrated processes that may involve as many as 150 different Thermo Fisher Scientific products, including instruments, reagents, media, and consumables. There currently are collaboration centers globally situated in Carlsbad, Princeton, Oslo, and Singapore. The goal is to provide process integration support to local researchers. Current Thermo Fisher capabilities in the cell therapy space span a

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broad scope of processes that include viral and non-viral gene-editing approaches for the manufacture of autologous CAR-T cells, natural killer (NK) cell therapy manufacturing workflows, and induced pluripotent stem cell (iPSC)-derived T cells for cell therapy applications.

Figure 1 depicts an example of a CAR-T manufacturing workflow, broken down to show various instruments associated with different unit operations across the entire process.

COLLABORATION CASE STUDY: NON-VIRAL CELL & GENE THERAPY MANUFACTURING AT THE UNIVERSITY OF CALIFORNIA SAN FRANCISCO (UCSF)

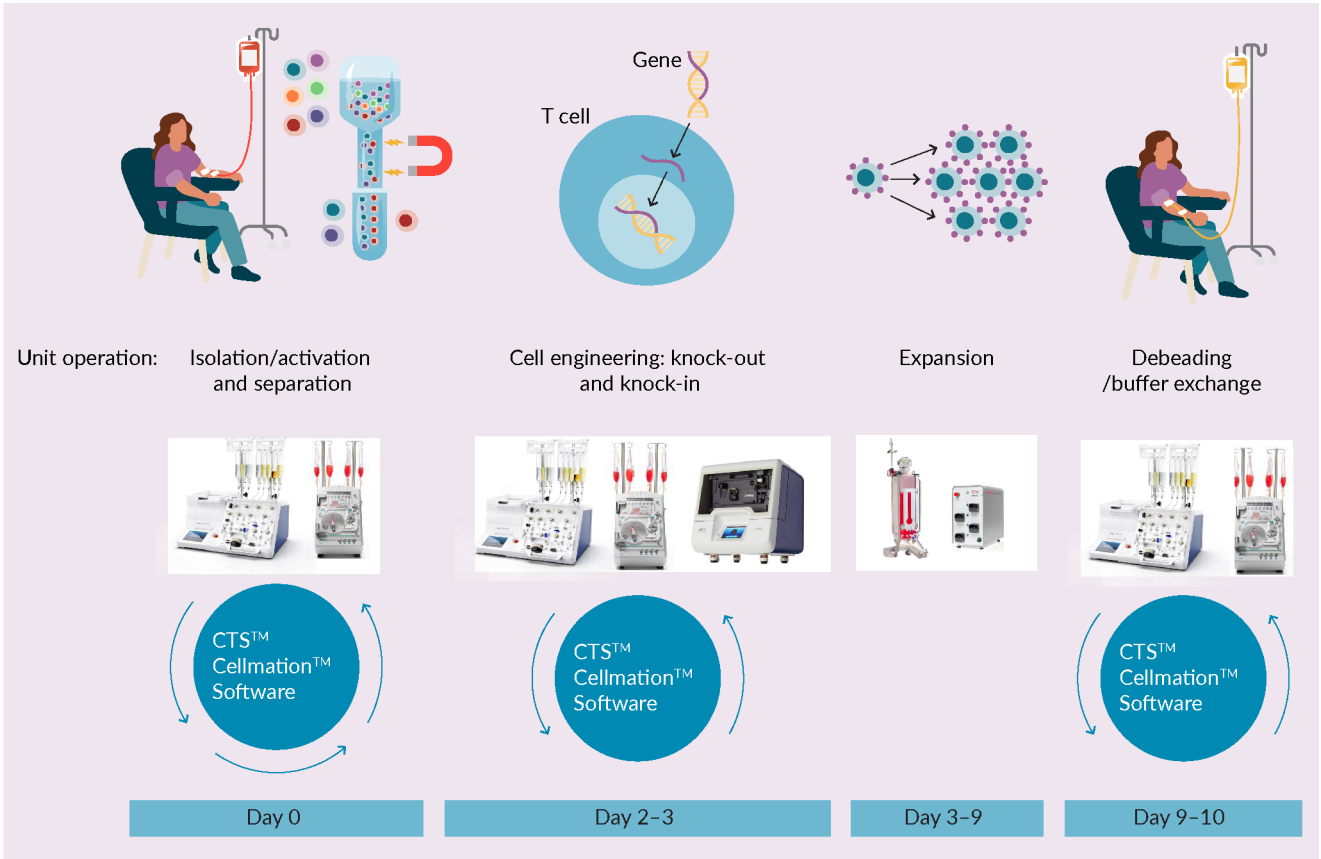
UCSF's Experimental Cell Therapy Group focuses on taking good ideas for cell and gene therapies from the wider UCSF biomedical research community and transitioning them to viable clinical products with support for preclinical development, regulatory filings, and manufacturing.

Current projects supported by the UCSF Experimental Cell Therapy Group include a historical focus on regulatory T cell (Treg) therapies, with many programs now in Phase 1/2 trials. However, projects are increasingly expanding to include other viral and non-viral engineered T cell therapies.

CRISPR-based non-viral approaches allow the targeting of a specific region of the genome for gene disruption, correction, or insertion. This has proven to be a powerful approach for many types of therapies. UCSF has used this method to reprogram the specificity of T cells enabling, for example, the insertion of a synthetic receptor such as a CAR or other antigen receptor to target cancer

FIGURE 1

Example workflow for cell therapy manufacturing.



cells. This approach of applying the normal regulatory mechanisms to achieve a defined level of T cell receptor expression has been demonstrated to improve the function of CAR-T cell products [1].

Challenges with viral CAR-T cell manufacturing are well-documented and revolve around the cost, complexity, and lead times of virus manufacture. Many academic programs, including UCSF, have struggled to secure manufacturing slots for all the patients they would like to treat with current generation commercial CAR-T products. This was the reasoning behind developing a fully non-viral knock-in strategy for a B-cell maturation antigen (BCMA)-CAR product in collaboration with the UCSF Myeloma Program [2].

One of the challenges with using naked DNA for transgene insertion is that the DNA can be toxic to cells, especially at the high concentrations needed to achieve efficient knock-in. The UCSF team found that long single-stranded DNAs are less toxic to cells, and the inclusion of Cas9 target sites (CTSs) can improve the delivery of these long ssDNAs by enabling binding of the co-electroporated Cas9 ribonucleoproteins (RNP) which helps shuttle these templates to the nucleus of cells. This provides higher knock-in efficiencies and higher yields, allowing for clinically relevant doses.

This concept has been taken forward to a good manufacturing practice-compatible manufacturing process at a clinical scale. With a 10-day expansion period, high yields suitable for clinical production are achieved. As receptors are not constitutively activated with a viral product, a robust immunophenotype is achieved, consistent with a memory stem cell immunophenotype. Cells are functional both *in vitro* and *in vivo* in terms of their capacity to kill myeloma cells.

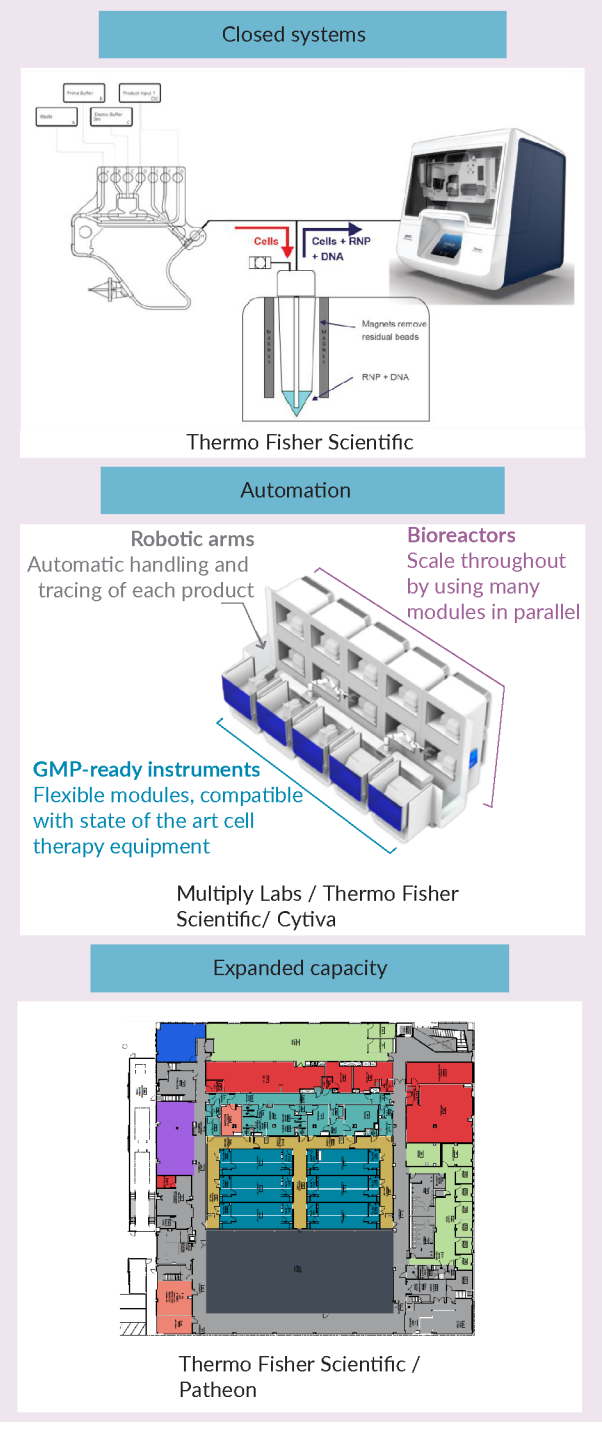
A key benefit of this system is its modularity. It is easy to switch the BCMA CAR-T to another TCR in order to target different indications. It is hoped that this process can be expanded to as many patients and indications as possible to improve access to CAR-T cell therapy.

A COLLABORATIVE FUTURE

Moving forward, UCSF's collaborations have several aims relating to both the expansion and streamlining of cell therapy manufacturing (Figure 2).

FIGURE 2

CSF Experimental Cell Therapy Group collaborations [3].



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The first aim is to move toward closed systems, limiting work in the biosafety cabinet in order to increase product throughput. A second, related goal is automation. Here, a further collaboration with Thermo Fisher Scientific and Multiply Labs utilizes robotic arms to interface with equipment in the same way that a person would. This can potentially allow multiple products to be made in the same room without the need for highly-trained staff. The third aim is to expand capacity to include more cleanrooms. Through collaboration with Thermo Fisher Scientific/Patheon, a large capacity increase is being made possible at UCSF.

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Q&A

To accelerate speed to market, and facilitate adoption and integration of tools and technologies into existing workflows or develop new processes, it is critical for tools providers and therapeutic developers to closely work together. The nature of cell therapy manufacturing precludes traditional product and instrument use, and greatly benefits from collaborative technology transfer, open communication, strong technical and product specific support, and strict regulatory and quality standards.

Xavier de Mollerat du Jeu, Senior Director of Research and Development, Thermo Fisher Scientific, speaks to (pictured left to right) **Brian Shy**, Director of the UCSF Experimental Cell Therapy Group about their ongoing collaboration

Q I love your model of taking the great science that is happening at UCSF and moving it into the manufacturing space. There is no point in developing the best therapies if they are not accessible to patients. I am excited about our collaboration – what motivated you to work with Thermo Fisher Scientific?

BS: We are focused on the manufacturing side and moving towards closed, automated approaches. We think that this is the key to being able to scale to all the patients

whom we would like to treat. You and I had some interactions in the past through the larger collaborations between UCSF and Thermo Fisher, and we spoke about your work to connect these different instruments and reagents to achieve exactly the types of things that we want to achieve here at UCSF. There were a lot of opportunities to collaborate on those approaches.

Q What does this collaboration look like in terms of day-to-day activities?

BS: Firstly, I am always impressed by the number of people on the Thermo Fisher team! I have especially enjoyed working closely with the scientific teams in our collaborations. We can speak scientist-to-scientist and explore a lot of the important issues that come up in manufacturing that we do not have the capacity to explore within our own scientific teams. In terms of the day-to-day activities, we have bi-weekly meetings where everyone gets together to talk about their work. In addition to that, we routinely send people back and forth between our organizations for training purposes.

Q What insights have you gained by working with us at Thermo Fisher Scientific?

BS: Our academic programs are relatively small, so we do not have the capacity to ask every question we would like to about each piece of equipment, reagent, or process. In those situations, we would often just rely on the manufacturer's recommendation without knowing where that recommendation is coming from. We have gained many insights through these collaborations in terms of justifying our choices to use product or piece of equipment X, Y, or Z. An example of this is how various bead to cell ratios can affect purity, activation, or knock-in efficiency. With Thermo Fisher, we get to see the data driving certain decisions and we can think about the ways we could potentially modify an approach or technology choice in order to achieve the outcome we are pursuing. That has been powerful for us.

XMJ: That is a great point. We have spent around 8 years developing these products and there is a lot of data from this work that we do not typically share in public forums – we often only show the final conclusions to the customer. But through collaborations like ours, the wealth of information that we gain through the development process is accessible to the customer, which really allows us to support you in a unique way.

BIOGRAPHIES

ØYSTEIN ÅMELLEM'S professional experience is derived from different leadership positions in R&D, Product Management and Business for over 22 years in Thermo Fisher Scientific, he is responsible for development and commercialization of products and services for the cell therapy market. His scientific training is in the field of molecular cell biology and he received a PhD from the University of Oslo. Øystein's academic career focused on the study of physiological

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& molecular mechanisms of tumor cell growth and the development of novel therapies for the treatment of solid tumors in collaboration with Norsk Hydro.

XAVIER DE MOLLERAT DU JEU, PhD is the Senior Director of Research and Development in the Cell and Gene Therapy business unit at Thermo Fisher Scientific, developing new products and solutions for cell therapy manufacturing. Xavier studied molecular biology and plant physiology at the University of Montpellier II in France and received his PhD in human genetics in 2003 from Clemson University in South Carolina. His thesis work involved identifying the gene(s) responsible for Split Hand/Split Foot Malformation 3 (SHFM 3). His post-doctoral fellowship research was in the laboratory of Dr Michael G Rosenfeld at UCSD, where he studied the roles of microRNAs in pituitary gland development. He joined Invitrogen (Life Technologies, Thermo Fisher Scientific) in 2005.

BRIAN SHY is Director of the UCSF Experimental Cell Therapy Group and GMP facility. He trained at UCSF in Cell Therapy and Transfusion Medicine, and completed his post-doc with Alex Marson exploring therapeutic applications of CRISPR T cell engineering. His research focuses on developing tools, technologies, and applications for cell therapy manufacturing in the academic setting.

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Closed cell therapy manufacturing solutions

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We offer a cell therapy manufacturing workflow that serves as a foundation for digital integration of your cell therapy process and data management, while enabling the interaction of production and control layers to manage all aspects of clinical manufacturing. When used in concert with our comprehensive portfolio of Gibco™ Cell Therapy Systems (CTS™) consumables, reagents, and instruments, you can transition to the clinic with ease.

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MATERIALS &
UPSTREAM PROCESSING/ANALYTICS

SPOTLIGHT

Distributed manufacturing models for ATMPs: can they work: do we understand the pitfalls?

Mark W Lowdell
Professor of Cell & Tissue Therapy, University College London, UK
and
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"Digitization of batch manufacturing records is now available for ATMPs and has become affordable even for hospital-based facilities. This is an essential step for distributed manufacture of licensed ATMPs..."

VIEWPOINT

Cell & Gene Therapy Insights 2023; 9(2), 159–164

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The field of advanced therapy medicines, advanced therapy medicinal products (ATMPs) to use European terminology, arose from the long history of hematopoietic stem cell transplants (HSCT) and adoptive immunotherapies invented by academics. The explosion of

interest in these therapies began with Rosenberg's earliest trials of IL-2 stimulated 'lymphokine activated killer cells [1] and his use of tumor-infiltrating lymphocytes [2]. The proof of the ability of allogeneic lymphocytes came 5 years later, with Kolb's demonstration of resolution of relapse of chronic myelogenous leukemia after T cell depleted allogeneic HSCT by infusion of small numbers of lymphocytes from the original HSCT donor [3].

In both of these examples and in hundreds of other similar trials, the cells used for treatment were manufactured near to the patient, sometimes a matter of meters away in the same facility. In Europe, it was not until the publication of the Medicines Directive in 2001 (2001–83-EC) that human cells were first included as medicines which required manufacture to the standards of Good Manufacturing Practice (GMP) within licensed facilities. The subsequent ATMP regulation in 2007 and the revised medicines directive in 2009 (2009-120-EC), plus parallel regulations around the world, cemented this position and, arguably, allowed for the commercialization of advanced therapy medicinal products (ATMPs).

However, the unique manufacturing challenges of both autologous and allogeneic ATMPs and the need for biological starting material from patients and/or donors in most cases, means that hospitals and academic centers often remain essential partners in the commercial delivery of these medicines. Autologous chimeric antigen receptor (CAR-T) cell medicines have been the 'poster child' of the field, with rapid progress to marketing authorizations and biologic license application as licensed medicines. However, these require contracts with hospitals to provide the starting materials, and often, with hospital 'stem cell labs' for the initial processing of the patient cells to a cryopreserved mononuclear cell suspension for shipping to the centralized manufacturing site for the final CAR-T drug product.

The costs and complexities of centralized manufacture of ATMPs, especially autologous, has led to interest in establishment of decentralized manufacturing models aimed at reducing costs and easing the supply chain problems. Recently, the United Kingdom's Medicines and Healthcare Products Regulatory Agency (MHRA) has released a consultation on point of care manufacturing [4] which highlights the enthusiasm for regulatory changes to facilitate the most ambitious aspect of distributed manufacture.

Until last year, I was the Director and Qualified Person of the Center for Cell, Gene & Tissue Therapeutics (CCGTT) at Royal Free Hospital & University College London (UCL), London. This GMP facility was established in 2001 and licensed to produce ATMPs for clinical trials and compassionate use. CCGTT has supported manufacture for academic and commercial clinical trials and has allowed UCL to be the fifth largest centre for CAR-T trials in the world. It has never sought a commercial manufacturing authorization for marketed products. CCGTT is typical of the hospital-based GMP facility envisaged for distributed manufacture.

In 2013, I spoke about distributed manufacture at an ISCT conference and showed in Figure 1.

This envisaged two types of ATMP: those which are so patient-specific that they could never achieve a marketing authorization, such as allogeneic mesenchymal cells for treatment of acute graft-versus-host disease (GvHD); and those which could go through full clinical trials and proof of efficacy leading to marketing authorization application (MAA). I have always emphasized the importance of the protection of products with MAA from competition from similar products made under the European Union Hospital Exemption Scheme or UK Specials, where proof of efficacy is not required.

Here I will share my opinions and concerns about the models for distributed manufacture of ATMPs. For the sake of brevity, I will assume that the challenges of distributed supply of starting materials is solved, although that too remains a significant challenge. I will

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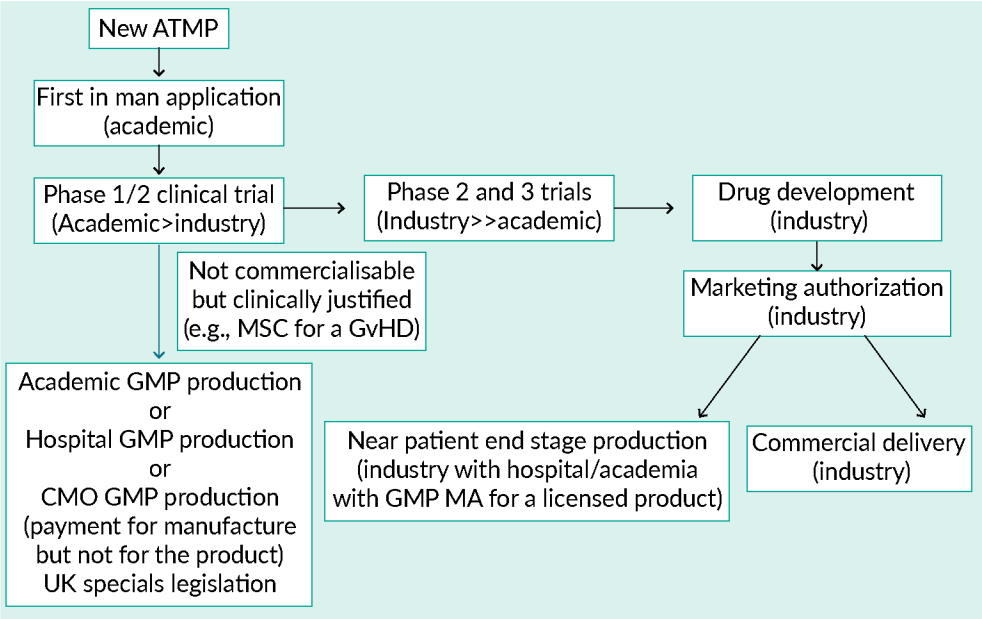
FIGURE 1

Possible development and supply pathways for ATMPs.



ATMP: Advanced therapy medicinal products; CMO: Contract manufacturing organization; GMP: Good manufacturing process; GvHD: Graft-versus-host disease; MSC: Mesenchymal stromal cells.

also restrict the subject to ATMPs that will have obtained marketing authorization, since these are the ATMPs which have proven efficacy and, hence, the greatest burden of CMC control.

These are my opinions and not, necessarily, those of any of the companies for whom I work or advise.

MODELS OF DISTRIBUTED MANUFACTURE

Hub & spoke where the spoke is owned by the holder of the marketing authorization

This is the easiest model to imagine and to operate. Here the manufacturing process is wholly owned by the pharma company and the spoke sites are controlled under a comparable or identical quality management system. There will be different manufacturing authorizations for each site but the processes can be identical and owned by the company. Although this is the most simple of the models, it is not without challenges. The fact that distributed manufacture is needed implies that the drug is licensed for use in many countries and thus, more than one regulatory domain. There are many examples of manufacturing reagents that are acceptable in one country, but which are not acceptable in a second. Most recently, I have come across a human albumen solution, which we use for ATMP manufacture, and the same supplier distributes different versions for European Union (EU) and United States (USA). Neither can be bought in both territories so, inevitably, the drug product will be made with different reagents in two countries. This means two supply lines, two validations, two procurement lines, etc, etc. None of this is difficult but it all adds cost. This seems a trivial issue but even in my limited experience, we have multiple reagents that we use in ATMP trials in the EU and the United Kingdom (UK),

which US Food and Drug Administration remains unwilling to allow to be used for the same products made for the USA market.

Hub & spoke where the spoke is owned by a third-party company contracted to the holder of the marketing authorization

This is the typical scenario when a company uses a commercial contract development and manufacturing organization (CDMO) to manufacture their product. It has the limitations described above, but with the added complexity of use of a third-party quality management system and staff who are employed by the third party for manufacture. Each site of manufacture of a licensed medicine has to be named in the marketing authorization applications/biologic license application (MAA/BLA) and comparability of production has to be demonstrated. This includes all in-process quality control (QC) and release assays. In the European Union, each batch must be qualified person (QP) released, but the QP in this scenario is employed by the CDMO and the legal responsibility for the product rests with the holder of the manufacturing authorization. The contractual obligations of the third-party CDMO will require very high definition since the quality of the product falls under the CDMO, yet the reputation of the product remains with the holder of the MAA. This model already works for biotechnology products such as recombinant proteins, but those are batch manufactured and a failed batch can be remanufactured. The same is rarely true of autologous products and the reputational risk profile for the holder of the MAA is thus far greater.

Fully distributed manufacture where the product is manufactured at the hospital site:

This is certainly the most challenging model. Many academic hospitals across the world have embedded good manufacturing practice (GMP) facilities which manufacture ATMPs for clinical trials. The regulatory oversight required differs across geographical regions, however, it is fair to say that the level of regulatory control of facilities making ATMPs for clinical trial is leagues away from that imposed on commercial sites manufacturing licensed products with MAA/BLA. Every aspect of the GMP process is affected and the number of quality assurance (QA) and QC staff required is, in my experience, ten-fold greater as a minimum. These staff must be employed and managed even if the facility is not manufacturing the drug product, and the concept of dual manufacture of investigational ATMPs alongside licensed ones is a very great challenge for inspectorates. Alongside the challenges of manufacture to commercial standards are the parallel challenges of running a QC laboratory to the same standard; validated assays and equipment, back-up equipment and staff, secure storage of reagents and data, etc, etc, etc. I do not believe that the level of activity in any hospital for the manufacture of a specific licensed ATMP can justify the cost of obtaining and maintaining a commercial manufacturing authorization (MA). This has been recognized in the consultation document issued by UK Medicines and Healthcare Products Regulatory Agency (MHRA), where the commercial MA is held by the 'hub' and extended to each 'spoke'. The details of how this can work and the levels of compliance needed at each spoke remain to be determined but, as ever, the Devil will be in the detail.

One of the highest risks associated with licensed drug manufacture is label control and it is difficult to imagine how this can be managed adequately with this level of distributed manufacture.

In the real world, there is also the challenge of how the contract between the holder of the MAA and the hospital is maintained. The hospital must agree to maintain the GMP facility and staff to meet the demands

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of the MAA holder, but the hospital's first duty is to treat patients and resources are inevitably skewed to that purpose. Essential equipment breakdowns in hospital GMP suites are never treated as the priority they would be in a CDMO or pharmaceutical company. This puts patient-specific ATMP manufacture at risk and presents very high reputational risk for the holder of the MAA. If batches fail and patients die through lack of treatment, it is reputational suicide for a pharmaceutical company to sue the hospital responsible. In fact, in my experience, it is very unlikely that the hospital would accept that level of liability for a commercial medicine.

Fully distributed manufacture where the product is manufactured at the patient bedside - 'black box' manufacture':

This is the dream of many ATMP developers and many more engineers. Closed system, automated manufacture on the basis of quality By design, with no QC release assays, and a 'virtual' QP appears to address all of the challenges I've presented above. This may come to pass but the 'black box' will be very large, and will still have to be managed under a complex quality system covering the QA of stock control, monitoring of stores, training of staff who load and unload the device, management of data trails, reporting of adverse events. The hospital will need to employ coordinators who can ensure that the patient-derived starting material is procured at the right time that the 'black box' and staff are available to manufacture.

In this scenario, under whose manufacturing authorization is the drug made and released? Who is liable for drug failures when such an event could be due to reagent control, staff error, equipment error, or simply the quality of the starting material that couldn't be tested prior to manufacture in the 'black box' model?

CONCLUSIONS

Technologies are advancing in our field, which give some hope to models of distributed manufacture with the greater availability of closed manufacturing systems and semi-automation of processes. QC testing remains a challenge, not least, design and delivery of suitable potency assays. Digitization of batch manufacturing records is now available for ATMPs and has become affordable even for hospital-based facilities. This is an essential step for distributed manufacture of licensed ATMPs and, in some cases, can truly facilitate automated analysis of QC data and reduce the QP role to 'release-by-exception', which could allow distributed product release.

The manufacture and delivery of autologous ATMPs is challenging and will always be expensive. Distributed manufacture is inevitable but is not the panacea that it is often presented as being. Hybrid models of those described above are being developed and championed, but each will face the same issues I have highlighted and will need real-world business models to determine the actual savings that could be achieved. In 25 years of ATMP manufacture, I have learned that contractual issues are often the hardest to resolve, followed by staff retention, and facility management/maintenance. All of these only get greater as the manufacture gets more distributed.

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FASTFACTS

Increase quality & standardization of cellular starting material

Matt Hemstreet, Director Global Marketing Cell Therapeutics at Terumo Blood and Cell Technologies

Cell and gene therapy manufacturing and the commercialization of cell therapies comes with real challenges for cell collection centers and manufacturers. Ensuring the best possible starting material is critical to providing the best outcome and avoiding downstream manufacturing failures.

INCREASE CONSISTENCY: WHAT ARE THE PARAMETERS THAT YOU CAN influence?

Variability comes from everywhere

1) Variability in donors: all donors are different

All donors are different

- Consider blood-related malignancies or diseases in cell therapy candidates
- Look at the timing of preparatory regimen
- Mobilization or related therapies
- Vascular access

2) Variability in collections: how are your cell collections performed?

Different SOPs and operator training/expertise cause deviations in collected product

- Yield, concentration, volume, anticoagulant collections (AC), etc.
- Monitor consistency across sites to ensure process scalability

3) Variability through settings and operational practices:

Device can be optimized for:

- Cell type
- Patient physiology
- Minimization of off-target contamination
- Targeting the desired yield, concentration, and volume goals
- Alarm and procedure management

ANALYZE THE COLLECTION PROCESS: IDENTIFY THE CAUSES OF VARIABILITIES

The numbers don't lie

Spectra Optia® Apheresis System from Terumo Blood and Cell Technologies is a widely used system for cell collections.

Our data shows:

- System configuration parameters (such as language, time, and date format settings)
- Entered patient data (sex, height, weight, hematocrit, and platelet and WBC counts for MNC procedures) and any updates made during the run
- Procedural targets (such as inlet, AC Ratio, AC infusion rate, and end-run targets)
- Technical settings data such as pump speeds and flow rates, valve positions, accumulated bag volumes, outputs from the centrifuge and the many sensors, detectors, and internal computers as the procedure progresses **(see Figures 1–3)**
- Attainment of the goals for yield, concentration, and volume
- Alarm and procedure management

Figure 1. "Inlet pressure was too low" alarms.

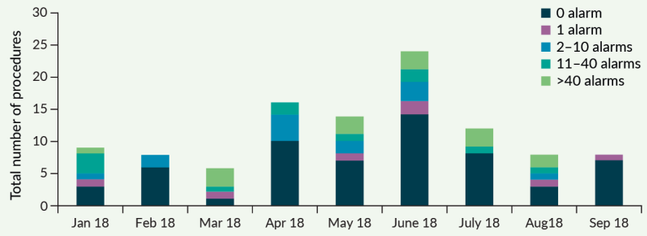


Figure 2. Inlet pressure alarms versus percentage of collections spent re-establishing the interface.

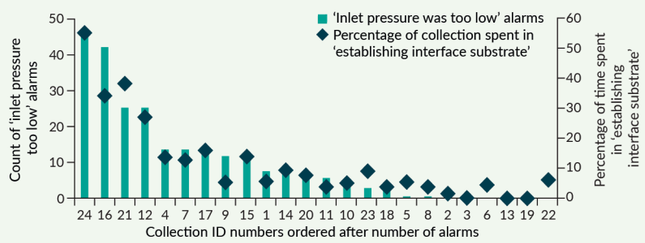
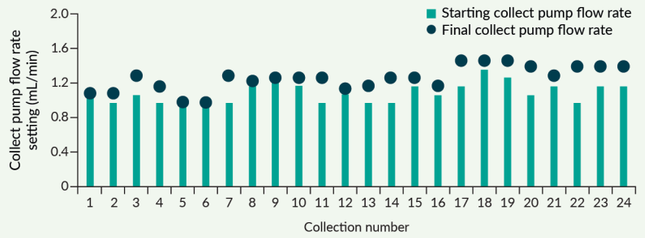


Figure 3. Collect pump flow rate comparison.



CONNECT WITH COLLECTION SITES: ENGAGE & APPLY STANDARD PROTOCOLS

What we can do

Terumo Blood and Cell Technologies has over 40 years of blood and cell processing experience worldwide, offering comprehensive solutions to improve the quality of the cellular starting material. Our services drive safe and predictable procedures for cell and gene therapy manufacturers.

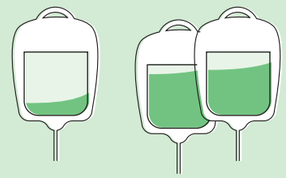
Our services

Spectra Optia cell collection data analysis:

- Comprehensive analysis from pre-procedure data through final therapy results
- Optimization suggestions
- Collection site comparison and benchmarking
- Design protocol for standard collections

Spectra Optia prediction algorithm:

- One of the most significant inefficiencies in the cell collection process is collecting more or less patient blood than required



- Predictive algorithm analyses avoid over or under collections

CELL & GENE THERAPY INSIGHTS

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In partnership with: TERUMO BLOOD AND CELL TECHNOLOGIES

CELL & GENE THERAPY INSIGHTS

CELL THERAPY MATERIALS &
UPSTREAM PROCESSING/ANALYTICS

SPOTLIGHT

INTERVIEW

Ensuring success in working with apheresis centers

David McCall, Commissioning Editor, BioInsights,
talks to **Suzanne Kamps,** Associate Director,
Apheresis Operations, Adaptimmune

SUZANNE KAMPS, BSN RN, currently serves as the Associate Director of Apheresis Operations at Adaptimmune Therapeutics. Suzanne provides expert oversight guiding the procurement of starting material for Adaptimmune's clinical trials. In her previous role at the Children's Hospital of Philadelphia, she was a Safety Quality Specialist for Apheresis, responsible for onboarding clinical and commercial cell therapies at their Apheresis Center. In addition, she maintained compliance with regulatory standards to ensure uninterrupted accreditation of the Apheresis Center. She is a Registered Nurse with over 10 years of experience treating Oncology and Apheresis patients.

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Q What are you working on right now?

SK: I am building on and expanding apheresis operations at Adaptimmune, a T cell therapy company focused on solid tumors. This includes developing standards

for apheresis and maintaining our apheresis manuals (and expanding upon them, where appropriate). I am also engaged with the training at clinical sites during our site onboarding process, and I am working on a project to try to characterize our starting material with our scientific departments. Last but certainly not least, I am involved in a rolling Biologics License Application (BLA) submission, which we are conducting this year. For this, I am utilizing my experience and expertise to assist writing of the starting material-related component of the BLA submission document. This work is running alongside preparations for building out what will be our commercial infrastructure.

Q You are in a great position to be able to refine Adaptimmune's approach to working with apheresis centers, given your previous experience on the other side of the fence at the Children's Hospital of Pennsylvania (CHOP). Can you firstly reflect on the concerns, considerations, and frustrations you experienced when working in the hospital setting with apheresis for cell-based therapies?

SK: The strain on apheresis centers has been increasing due to the growing number and variety of cell-based therapies. This expansion in the field is great for patients but leads to many other considerations and requirements for apheresis centers, and the variability that occurs across the cell-based therapy industry can be time-consuming to navigate. Participating in the audits and onboarding for each different product drains time and resources for the apheresis centers, and at the end of the day, that ultimately affects their ability to care for patients and perform the quality assurance (QA) necessary to provide high-quality starting material. At CHOP, I had the privilege of working with a very experienced cell therapy team who did all they could to ease those burdens for the apheresis center. However, the center was still required to dedicate time for review of, and training on, all of the varying apheresis manuals. This is something that I hope will change in the coming years, with standardization across industry becoming more and more of a priority. For me, any chances I get to advocate for apheresis centers are opportunities I want to take. I want to ask, 'what can we do as industry to lessen that strain on apheresis centers?'.

Q Now you are on the industry side, what are some of the key challenges you face there? What were your related main priorities when you took the role at Adaptimmune?

SK: To be honest, the main challenge I initially faced was navigating a matrixed biotech environment, as it varies so greatly from the hospital setting. In doing so, I became aware of all the work that is being done during the patient journey as a whole, from

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"Standardization is key to reducing the burden on hospitals and thus allowing more patients to receive treatment: the more time that hospitals have to collect cells, the higher the potential for patients to be treated."

apheresis collection through to infusion. The hospital setting sees the apheresis collection and the infusion stages, but from a hospital perspective, what goes on in between can be unclear for roles outside of the prescribing physicians. Being able to see the work, the research and dedication, that occur between those two stages has been eye-opening for me.

When I started, my main priority was the starting material and its impact on various different departments within the company. I've focused on education of variabilities in starting material, quality measures that are done at a hospital, and all the regulations that apheresis centers must follow, so that our teams at Adaptimmune can better understand how we can – and also where we cannot – engender positive change in the collection of starting material for our specific products.

Q The need for standardization is an overriding theme in this particular area – what should be some of the specific priorities in this regard?

SK: Standardization is key to reducing the burden on hospitals and thus allowing more patients to receive treatment: the more time that hospitals have to collect cells, the higher the potential for patients to be treated. Having read many apheresis manuals in my previous role, I believe that the standardization priorities should be documentation, auditing, and the requirements for collection.

In terms of the auditing of apheresis centers by industry, I strongly believe that we should capitalize on widely recognized standards to determine the quality of an apheresis center. There are different standards and accreditations, but I would specifically highlight FACT (Foundation for the Accreditation of Cellular Therapy) accreditation. Apheresis centers work diligently to obtain and maintain FACT accreditation, and the FACT standards are heavily focused on the quality of the starting material. Therefore, if you are working with an apheresis center that is FACT-accredited, you should feel confident that the apheresis center has achieved a high level of quality – that they can collect a quality product. You should adjust your auditing to remove any redundancies between what your audit covers and what is already addressed in the FACT standards. Focus instead on your company's specific requirements.

There are a number of additional forums being developed with key industry leaders right now for standardization. Some cover auditing while others cover the apheresis collection and

documentation. Again, those three key areas stand out to me as the most important in the effort to reduce the burden on apheresis centers, particularly in terms of addressing the variability challenge. After all, the more variability you have, the greater the likelihood of error.

Q With Adaptimmune building towards a potentially landmark approval for the field with afami-cel, can you identify some ways in which your role has changed and is changing as preparations for commercialization intensify?

SK: I have been heavily involved in creating and building out the infrastructure that we are going to need for a successful commercial launch. More specifically, I am currently working on developing our auditing and onboarding process for apheresis centers, keeping in mind everything I just previously said. Other aspects I anticipate being involved in include assisting in the drafting of quality agreements, creating our commercial apheresis manual, and then onboarding a team to go into the field to support our sites.

Q What are some of the important 'take-homes' from this experience for earlier stage developers? What should be done early on to address issues that will arise later?

SK: I may sound biased here, but I would suggest that when a company is building their team for any type of cell-based therapy, they should consider hiring or consulting professionals who have direct experience in caring for patients in the cell and gene therapy space. The background knowledge and understanding that these individuals bring are incredible attributes that fill the knowledge gap between what industry believes and what actually goes on with direct patient care at the apheresis and infusion stages. Hiring people that have that experience at an early stage can definitely boost your company's relationship with the clinical sites you will utilize and rely upon to be successful later.

Q Can you distil for us your key words of advice regarding how to approach development of an apheresis manual?

SK: Overall, I would say there is no need to complicate operations required on the side of the apheresis sites, as this can impede their participation with these products. You want to try to be as flexible as possible and rely on the site's expertise. This

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includes being as flexible as you can be with your manufacturing, particularly in terms of patient scheduling. These patients are going through multiple treatments and being flexible on when you can collect and deliver the treatments to these patients is beneficial. Furthermore, offering an option to collect apheresis earlier in the patient's cancer journey, when their cells are healthier, increases the chance of manufacturing success.

"...offering an option to collect apheresis earlier in the patient's cancer journey, when their cells are healthier, increases the chance of manufacturing success."

Involving the apheresis center early in development means you can get a lot of those conversations out of the way early, leading to a much smoother onboarding process down the line. Also, do not get too specific unless you have solid data to back up what you are putting in your manual. Guidance in the manual is great because some apheresis centers might not be as experienced with these types of products as others, but think about what you require an apheresis center to do, and weigh carefully that balance of control over the starting material with the actual practical feasibility from the apheresis center's perspective. Failure to do this may cost you the participation of an apheresis center in your clinical trial and ultimately, your commercialization activities.

Q Finally, can you sum up some important priorities and goals that you have for your work over the foreseeable future?

SK: As we are moving closer to the completion of our rolling BLA submission, my focus is to ensure we are comprehensive in everything that we are doing from an apheresis perspective. Furthermore, clearly communicating our message about our specific treatment and allowing apheresis centers the autonomy to excel in collecting a quality starting material are real priorities for me. I want to continue to advocate for apheresis centers, and to reduce the wait-time for their participation with cell-based therapies.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELL THERAPY MATERIALS & UPSTREAM PROCESSING/ANALYTICS

SPOTLIGHT

INTERVIEW

Bridging the knowledge gap in donor cellular starting material quality & variability

David McCall, Commissioning Editor, BioInsights, talks to **Yiran Zhou,** Director of Process Development and Manufacturing, Cell Therapy, Sorrento Therapeutics

YIRAN ZHOU, PhD, majoring in Cell Biology and Biochemistry, currently serves as director of process development and cGMP manufacture in cell therapy at Sorrento Therapeutics. She has twenty years' experience in cell biology with nearly eight years' experience in cancer immunotherapy field. In her role, she leads process development and clinical product manufacturing activities, mostly focusing on allogeneic CAR-T therapy, including the strategy development of both upstream and downstream processing with the coverage from cellular starting material selection, cell activation, engineering, expansion to cell harvesting and fill/finish.

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Q What are your working on right now?

YZ: I'm currently working on the process development for large-scale, non-viral, allogeneic CAR-T cell therapy across the spectrum of several targets at Sorrento

Therapeutics. I also lead the same team to conduct current good manufacturing practice (cGMP) manufacture of the product for which we ourselves developed the manufacturing process.

The team's work scope covers the end-to-end process of cell production, from the very beginning (donor selection) to T cell activation, expansion, and downstream cell harvesting/purification. We also perform final product formulation as well as fill-finish and cryopreservation. For each specific target, a unique, streamlined process needs to be designed to meet the quality and yield requirement.

I'm lucky to have become involved in Sorrento Therapeutics' novel non-viral allogeneic CAR-T platform at a relatively early stage and to have grown together along with the maturation of our process and product. I'm excited to have the opportunity to continue learning and working with the whole cell therapy field.

Q Can you give us some more background on Sorrento Therapeutics' R&D platform and pipeline, and also the raw and starting materials requirements and considerations for the company's product candidates?

YZ: Sorrento's research and development is focused on driving innovation in cancer treatment, infectious diseases such as COVID-19, non-opioid pain management, and autoimmune diseases. Sorrento Therapeutics is an antibody-centered company. Sorrento's proprietary G-MAB library, containing more than 10 quadrillion distinct antibody sequences, is one of the largest fully human antibody libraries in the biopharmaceutical industry. So far, Sorrento has successfully identified antibodies against over 100 clinically relevant oncogenic targets, including PD-1, PD-L1, CD38, CD123, CD47, VEGFR2, and CCR2. Those antibodies can be used on their own or incorporated into cancer-targeting approaches, including CAR–T cell therapy, Antibody-Drug Conjugates, oncolytic virus therapies, etc.

Our company selects product candidates aligned with the core company business development model. Based on the medical needs, target efficacy, and safety profiles, candidates are identified with the greatest potential to succeed in the clinic.

In terms of the critical raw starting materials for our non-viral engineered allogeneic T cell therapy product, those include cellular starting material, gene editing protein(s), and nucleic acid constructs. They all are required to be good manufacturing practice (GMP) grade. Specifically, for cellular starting material, the US Food and Drug Administration has stringent requirements regarding donor safety. Besides the donor eligibility criteria outlined in 21 CFR Part 1271 Subpart C, additional requirements include but are not limited to EBV and human herpesvirus HHV 6/7/8 negative status, as determined by qPCR. EBV and HHV7 are widely found in 80–90% of the population, which can make the availability of eligible donors a big challenge.

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"There are two aspects to the quality of cellular starting materials: one is safety; the other relates to the properties associated with final product quality and potency. In the safety aspect, donors are screened to be free from communicable disease risks."

Q What is your view of the current analytical toolkit relating to cellular starting materials quality – where is it sufficient, and where is more innovation needed to ensure analytics are robust enough to cater for the increasing expectations of regulators in this area?

YZ: There are two aspects to the quality of cellular starting materials: one is safety; the other relates to the properties associated with final product quality and potency.

In the safety aspect, donors are screened to be free from communicable disease risks. The screen virus panel includes Human Immunodeficiency Virus, Hepatitis A, Hepatitis B, Hepatitis C, Cytomegalovirus, Epstein-Barr virus (EBV), Human T-Lymphotropic Virus, Herpesvirus 6/7/8, etc. The viruses are tested for the antigen or antibody presence in blood or the nucleic acid level by the qPCR method.

In our case, we have encountered more than once that donors who tested EBV-negative in the pre-screening about 14 days prior to collection were tested EBV-positive on the collection day. Although we cannot completely exclude the possibility that within a couple of weeks a donor becomes infected with the virus or the latent virus became active, it is possible that the testing method of qPCR may deliver inconsistent results when the copy number is at the edge of the assay detection limit. A more reliable and sensitive testing method might be required to prevent this from happening.

Another thing to keep in mind is the testing articles of cellular starting material and the final product are not composed of the same cellular types, which could lead to different results.

Besides regulatory safety concerns, the analysis of cellular starting materials related to critical material attributes (CMA) is important as well. This part is less addressed due to the incomplete understanding of the linkage between the starting material properties and final product potency. More analysis could be conducted for gene expression, functionality, phenotypic subsets, background level of cytokine secretion, etc. Corresponding advanced technologies will facilitate the analysis with greater accuracy and sensitivity.

Q Can you share some examples of how and where novel raw materials are reducing the requirements for product testing downstream in practice?

YZ: A good example for that is using non-viral gene editing material instead of viruses like retrovirus or lentivirus for transgene expression. The raw material release for virus may require longer timelines and higher costs than material for non-viral genetic engineering. The transgene-encoded non-viral raw material requires much more straightforward testing than that required for viral vectors. Non-viral gene editing components can also be shared by different targets, so quality control (QC) testing cost and time is greatly reduced, whereas virus material is target-specific and each lot of the raw material requires QC release testing. Additionally, a no-virus approach means patients don't need the 15-year follow up for replication-competent retrovirus or replication-competent lentivirus testing.

Another example is using serum-free media for cell culture, which will minimize the safety risks associated with human or animal sera and potentially cut down the necessity for certain downstream testing.

Q What for you are the key considerations for cryopreservation of cellular starting materials?

YZ: The key consideration for cryopreservation of cellular starting materials is retention of viability and functionality of the desired cell population.

Both fresh and cryopreserved cGMP-compliant leukopaks (LPs) are available commercially. Fresh LPs impose manufacturing time constraints, since after receipt, a non-stop manufacturing cycle must be executed without delay until the final product is made. Cryopreserved cellular starting materials, on the other hand, provide great flexibility and convenience for manufacture. While fresh LPs do avoid the stress on the cells during the freezing and thawing cycles, cryopreserved LPs can nonetheless serve as an acceptable alternative to fresh LPs. Studies have demonstrated that cryopreserved LPs preserve cell viability and cellular functionality. The ratio of CD4 and CD8 cells remains unchanged. Some slight changes in T cell subset population distribution (effector, memory T, regulatory T) were reported, but could be a result of the compromised expression of certain cell surface markers post-thaw. It has been reported that T cell functionality is comparable between fresh and cryopreserved cells.

Not only LPs, but also human peripheral blood mononuclear cells (PBMCs) can be cryopreserved for future use following isolation from fresh LPs. Since the PBMC isolation is a short process, it is still convenient to do so. Likewise, studies have shown that cryopreserved PBMCs retain cell viability and functionality after long-term storage.

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"Over time, using QbD, data acquisition and analysis integrating prior scientific knowledge can build a greater and more in-depth understanding to identify the critical process parameters (CPPs) and the CMAs."

However, as expected, a fresh cell sample yields more cells than a cryopreserved cell sample for the separation step that follows.

Developers should prioritize cell types of interest and ensure the cellular starting material preserves both viability and function of the desired cell types. Additionally, between frozen LP or PBMC, the choice should be made to align with the configurations of downstream procedures and application – for example, we need to evaluate which type of starting material could better meet the purity or yield requirement in the following cell separation step, as well as further consequences for final product quality. What can be considered together with this is newly emerged isolation technologies, which may enable a more streamlined workflow with less steps, and a time-saving advantage if choosing one starting material over another.

Q Looking to the future, what for you are the key next steps for the field in growing its currently limited understanding of the impact of donor materials on allogeneic cell therapies? For example, how to account for donor variability?

YZ: Donor variability may impact both process consistency, manufacturing success, and ultimately, clinical outcomes in allogeneic cell therapies.

Donor cell properties can impact any step during the manufacture, including cell isolation, activation, expansion, and even procedures further downstream. The inherent variability in biological living systems may limit the implementation of quality by design (QbD) methods. However, QbD is still a useful and necessary tool. Over time, using QbD, data acquisition and analysis integrating prior scientific knowledge can build a greater and more in-depth understanding to identify the critical process parameters (CPPs) and the CMAs. Another useful tool is process analytical technology (PAT), which provides continuous in-process analysis of process variables. This enables the flexible control and timely adjustment of the operating parameters, assuring that critical quality attributes (CQAs) are met in the end. A robust process should be able to accommodate the variability of donor materials to produce a standardized and defined end product, given that appropriate CMAs have been established in the first place. Well-defined CQAs could greatly help to reduce donor variability-induced clinical outcome variation.

Besides that, alloreactivity is a major factor that impacts clinical outcome for allogeneic CAR-T cell therapy. Gene editing to knock out T cell receptor (TCR) is a popular method to avoid graft-vs-host disease (GvHD). Other methods, like human leukocyte antigen (HLA) matching, may mitigate the risk of GvHD or Graft rejection to improve the therapeutic outcome. Complete matching of donor-recipient HLA type is limited by low compatible donor availability. Other methods to diminish CAR-T cell alloreactivity include using stem cell-derived CAR-T cells or targeting other genes (e.g., CD52 etc.) to escape host rejection. Many of these avenues are being investigated in current clinical trials. The accumulated resultant clinical data will enrich our knowledge of the optimal solution to this critical matter.

The reasons accounting for donor variability are not fully characterized. To list some, the donor selection parameters that may impact process, product, and clinical outcome include: donor's age, BMI, HLA type, genetics, and disease history. For LPs, parameters include non-T cell population, CD4:CD8 T cell ratio, T cell phenotype composition, and even the leukopak collection process itself. During production, the dynamic interaction of donor cells and transgene expression also varies, which results in an impact on CQAs. All of the above may play a role in contributing to outcome variability.

As a process or a product becomes more defined over time, it may be possible to collect more relevant data to characterize the CMAs. To identify the real linkage of a certain cellular characteristic to final product quality, extensive donor characterization needs to be made. That includes evaluation of donor age, gender, medical history, health status, etc. as well as analysis of cellular properties such as gene expression, functionality, phenotypic subsets, background level of cytokine secretion, etc.

Q Lastly, can you pick out one or two key goals and priorities, both for yourself in your own role and for Sorrento as a whole, over the course of 2023?

YZ: At Sorrento, development of the non-viral allogeneic CAR-T platform of new targets will continue to broaden the coverage for unmet medical need in cancer patients. Meanwhile, strategies may be adjusted based on our current clinical data. We hope within this year that one or more of them will be able to achieve the milestone of Investigational New Drug filing.

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FASTFACTS

Exosome production process development in stirred-tank bioreactors

Stefan Schlößer, Eppendorf SE, Scientific Communications Manager. (*Experiments conducted by* **Jorge L Escobar Ivirico** & **Ma Sha**)

Exosomes derived from mesenchymal stem cells (MSC) are a new therapeutic alternative used in regenerative medicine. Increasing the mass production of exosomes in a controlled environment is the next step toward therapeutic use of these novel drug carriers. This poster highlights stirred-tank bioreactors as a valuable tool in the process development of current and future therapeutic applications.

Exosomes are small vesicles involved in cell-to-cell communication. They are released by numerous cell types such as MSCs, various immune cells, and tumor cells. Challenges of exosome production for therapeutic use include achieving optimum culture conditions for sufficient cell/exosome yields, the re-creation of the cells' physiological environment, maintenance, and reproducibility.

METHOD

A small-scale experiment to isolate MSC-derived exosomes was conducted by Eppendorf application specialists using the BioBLU® Single-Use Bioreactor with a pitched blade impeller. Growth parameters were controlled using SciVario® twin bioprocess controller.

First, MSCs were seeded into a flask with suitable medium containing microcarriers, followed by incubation under static conditions, enabling attachment of the cells to the scaffold surface. At the same time, the bioreactor was filled with fresh medium preconditioned to a suitable pH, dissolved oxygen content of 40%, and a temperature of 37°C.

Figure 1. FACS analysis of the culture demonstrates the presence of MSCs and absence of other cell types.

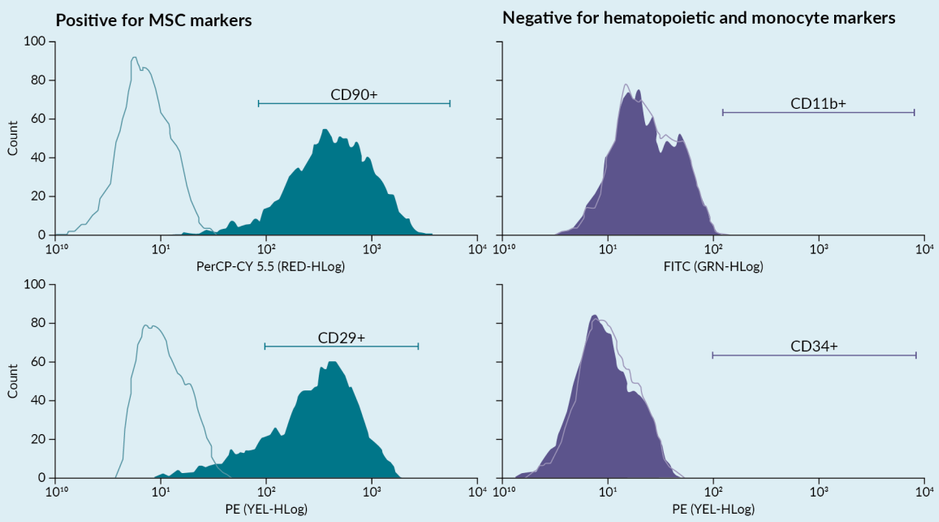
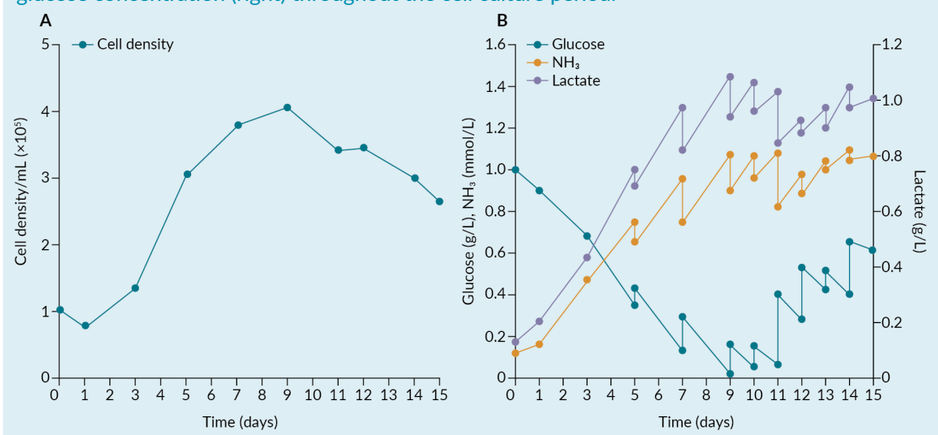


Figure 2. Graphs showing cell density (left) and production of cell culture by-products as well as glucose concentration (right) throughout the cell culture period.



After preparation, the cell-coated microcarriers were transferred into the bioreactor to a final volume of 1 L, and cells were expanded for 15 days. Starting from day 5 of the culture, 10% of the medium was exchanged every 2 days, and nutrients such as glucose were fed into the culture at specific time points.

RESULTS

Figure 1 shows the purity analysis of the MSC culture, which was measured using fluorescence-activated cell sorting (FACS). Cells were positive for MSC-specific markers CD90 and CD29 and negative for the hematopoietic cell marker CD34 and monocyte marker CD11b, demonstrating the purity of the culture.

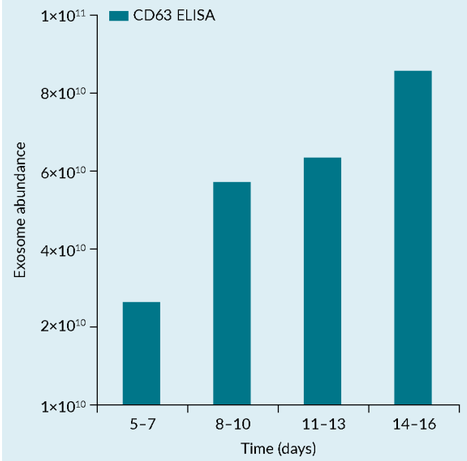
During the culture period, cells showed a typical growth curve with cell densities peaking at 4.1×10^5 cells/mL on day 9 (**Figure 2A**). Regular medium exchange assured cell culture byproducts, such as lactate and ammonia (NH_3), were kept at non-toxic levels (**Figure 2B**).

During the culture period, samples were taken to isolate and quantify MSC-derived exosomes from the culture supernatant. Exosome abundance was quantified by performing an ELISA against the exosome marker CD63. Results are illustrated in **Figure 3**, showing a steady increase in exosome abundance during the culture period.

CONCLUSION

The culture growth parameter control of the SciVario twin bioprocess controller, together with regular medium exchange, created a suitable environment enabling efficient cell growth and exosome production. These preliminary results demonstrate the feasibility of stirred-tank bioreactors in stem cell-derived exosome production and can serve as a starting point for further culture and scale-up process development.

Figure 3. Exosome abundance quantified by CD63 ELISA.



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Supply Chain Channel: Cryopreservation & Cold Chain

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SUPPLY CHAIN CHANNEL: CRYOPRESERVATION & COLD CHAIN

March 2023

Volume 9, Issue 2

EXPERT INSIGHT

Taking lessons from nature to improve cell therapy cryopreservation

Nishaka William, Mackenzie Coatham & Jason P Acker

FASTFACTS

Benefits of upstream seed train intensification & high-density cell banking

Patrick Shu Miao

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN EDITION:
CRYOPRESERVATION & COLD CHAIN

EXPERT INSIGHT

Taking lessons from nature to improve cell therapy cryopreservation

Nishaka William, Mackenzie Coatham & Jason P Acker

Cryopreservation is crucial to the delivery of cell-based therapies, breaking logistical bottlenecks that would otherwise compromise both the manufacturing and accessibility of the intervention. Conventional methods of cryopreservation leveraged by the cell therapy industry remain, for the most part, unchanged from those developed in the 1950s shortly following discovery of the first cryoprotective agents (CPAs). These methods suffice but, in many ways, have been proven suboptimal. While this may not be significant cause for concern in most basic science research settings, it certainly is the case clinically as even minor alterations in the post-thaw efficacy of a therapeutic product could impact patient outcomes. An overarching tenet in cryobiology that is guiding recent developments to improve the standards of cryopreservation involves marrying substitutes for dimethyl sulfoxide (DMSO), the historically 'gold-standard' CPA, with additives that protect against ancillary sources of cryoinjury. It is primarily mechanisms through which natural organisms tolerate environmental stressors that are serving as a template for promising developments in cryobiology that could prove invaluable when integrated into cell therapy cryopreservation protocols. The present article aims to provide an overview of these strategies and contextualize their potential to improve the current standards of cryopreservation.

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DMSO-FREE CRYOPRESERVATION

The motivation behind reducing & replacing DMSO

Dimethyl sulfoxide (DMSO), considered the 'gold-standard' cryoprotective agent (CPA), is used to cryopreserve nearly all cells of therapeutic interest (other than red blood cells), yet it has been found to be deleterious to both the product and the patient. DMSO is highly cytotoxic during extended exposure, and upon infusion, can induce minor adverse reactions in up to 50% of patients, as well as rarer, yet substantially more serious cardiovascular, neurological, or respiratory issues [1]. Generally, the maximum allowable dosage of DMSO upon infusion is 1 g/kg body weight (BW) or 10 mL product/kg BW; however, adverse reactions are known to occur at much lower dosages of approximately 0.4 g/kg BW [2]. In cases where cell therapies must be administered to localized sites rather than systemically, there is the additional concern that DMSO could cause damage to the surrounding tissue [3]. Thus, while DMSO is an effective CPA (barring its significant cytotoxic effects) that can yield high cell viability in an optimized cryopreservation protocol, these challenges have seeded a decades-long effort to reduce or replace DMSO.

The notion of reducing DMSO is by no means novel and was in fact pursued shortly following its discovery in 1959 [4]. A concentration of 10% was regularly utilized in cryopreservation applications until more recently when incorporation of sugars and/or polymers in place of DMSO facilitated a reduction in its concentration. For example, it is conventional now in many centers that hematopoietic stem cells (HPCs) be cryopreserved using formulations consisting of 5% DMSO supplemented with 5% HSA (human serum albumin) and 6% HES (hydroxyethyl starch) [5]. Kymriah™ and Yescarta™ (the first two FDA-approved chimeric antigen receptor T cell therapies) are cryopreserved using 7.5 and 5% DMSO, respectively, with similar sugars and or macromolecules supplemented in the cryopreservation solutions [6]. Rather than DMSO reduction, it is the idea of replacing DMSO that has most recently been popularized in the field of cryobiology, leading to the development of at least fifteen commercially available DMSO-free cryopreservation solutions [7]. As the compositions of these solutions are proprietary, it remains challenging to provide insightful commentary on them. However, there are some recently published studies, such as that of Kasushal *et al,* 2022 or Pasley *et al,* 2017 indicating that many of these solutions can perform equivalently to DMSO-containing solutions [8,9]. The reviews of Ekpo *et al,* 2022 and Awan *et al,* 2020 offer comprehensive overviews of the DMSO-free cryopreservation efforts implemented to date, which we refer the reader to for a more in-depth appreciation of the scope and outcomes of these approaches [7,10]. Nevertheless, an inability to fully assess these commercial solutions should by no means discount their perceived efficacy, but in lieu of this commentary, we will instead discuss a major source of inspiration guiding the development of DMSO-free solutions: natural deep eutectic systems (NADES).

Natural artificial deep eutectic systems facilitating the shift towards DMSO-free cryopreservation

Research into NADES in the context of cryopreservation was in-part prompted by initial failed attempts at DMSO-free cryopreservation, indicating that no single molecule discovered to-date can effectively recapitulate the putative mechanism of action of DMSO (i.e. rapid permeation, membrane stabilization, and osmotic stabilization) [11,12]. NADES, defined as a mixture of two or more compounds whose melting point is lower than that of any of its individual components (intermolecular hydrogen bonds are the guiding force behind this characteristic) are a unique, yet common element of nature. Since the first description of NADES in 2003, they have been

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investigated as an eco-friendly alternative to conventional organic solvents used in chemistry due to their relative sustainability, low cost, low toxicity, and biodegradability: characteristics also valuable for putative CPAs [13]. Certain compounds found in NADES, such as glycerol and trehalose, have been used in specific cryopreservation applications for several decades, but the association of these compounds with NADES has only more recently been established [14]. Thus, not only will the study of NADES bring compounds not commonly used as CPAs to the forefront, but it will also shed light on how to best utilize the CPAs we already know of to elicit the synergism that is characteristic of NADES.

Dr Alison Hubel's group at the University of Minnesota has spearheaded some of the more promising applications of NADES in cryobiology, focusing on the development of multicomponent DMSO-free solutions consisting of sugars, polyhydric alcohols, and amino acids [15–18]. Hubel's group has shown that when combined with sucrose and isoleucine, glycerol concentrations of ~10% can allow for post-thaw recovery levels in T cells and mesenchymal stem cells (MSCs) that are no different those obtained when cryopreserved in DMSO [17,19]. It is a compelling finding, as the use of glycerol was largely discontinued following the discovery that DMSO is a more effective CPA for cells other than red blood cells [20,21]. Therefore, it implies that when combined with sucrose and isoleucine at specific molar ratios, the efficacy of glycerol is no less than that of DMSO. Disaccharide sugars, by virtue of their higher polar contact area with lipid bilayers, act as stabilizers to resist cryopreservation-induced cell disruption. On the other hand, polyhydric alcohols, such as glycerol, are similar to DMSO in the protection they impart, offering osmotic stabilization to the cell and thermodynamic stabilization to intracellular components [19,22]. Albeit promising, the use of glycerol at similar concentrations to DMSO (~10%) poses its own limitations; namely, that it generally has a lower membrane permeability coefficient relative to DMSO, which would further complicate workflows during CPA addition and possible removal [23,24].

The choice of amino acid is likely to be paramount in facilitating the use of reduced glycerol concentrations. Hubel's group leveraged isoleucine, which is not known to offer significant cryoprotection in isolation but serves to stabilize the sugar in the multicomponent solution such as to avoid precipitation. Dr Lei Zhang's group from Tianjin University in China has recently shown that the amino acids L-carnitine, -betaine, and -alanine, at concentrations less than 6%, can allow for the successful cryopreservation of select cancer cells during ultrarapid freezing protocols (i.e. liquid nitrogen plunge) when used in their zwitterionic forms [25–27]. Assuming the rapid freezing rates do not cause vitrification (which was stated not to be the case in the aforementioned studies), a variety of mechanical stressors are introduced that are insignificant during the conventional slow cooling rates (less than 2°C min) required when using standard CPAs such as DMSO [28]. It is known that the freezing point suppression and osmoregulatory capacity of these neutral amino acids does not significantly exceed that of conventionally used CPAs. Thus, there are likely other mechanisms of protection these amino acids impart that is distinct from that of standard CPAs [27]. It is invaluable that additional research be done to determine the specifics of these mechanisms and replicate these findings in cells that are relevant to the cell therapy industry.

ICE RECRYSTALLIZATION INHIBITION AS AN ANCILLARY APPROACH TO FACILITATE DMSO-FREE CRYOPRESERVATION

The relevance of ice recrystallization in cryopreservation

In addition to the synthesis of colligative agents whose function is not unlike that of DMSO, many freeze-tolerant organisms in nature synthesize antifreeze proteins (AFPs)

to actively curb injury that arises in response to ice recrystallization: the growth of large ice crystals at the expense of small, more thermodynamically stable ice crystals. Recrystallization is a phenomenon characterized extensively in metallurgical [29–31], food science [32] and cryobiological [33] literature which, in the context of cryopreservation, is known to damage cells frozen in suspension [34]. Through increasing solution viscosity, conventional CPAs indirectly reduce recrystallization rates. However, as it is not an 'active' mechanism of recrystallization inhibition, the extent of reduction is far less than when compounds that directly inhibit recrystallization are utilized [34]. Considering recrystallization is in-part alleviated using CPAs such as DMSO, the introduction of compounds that directly inhibit recrystallization is known to reduce the required concentrations of DMSO. Although such compounds are unable to completely recapitulate the functionality of DMSO, they can facilitate attainment of DMSO-free cryopreservation while also targeting a mechanism of damage that manifests irrespective of the DMSO concentration utilized. Any of the DMSO-free solutions developed to-date which are void of agents that directly inhibit recrystallization can also significantly benefit from the addition of these compounds.

While recrystallization can take place during the freezing and thawing phases of a cryopreservation protocol, it most commonly manifests in response to intermittent periods of rewarming during the handling of frozen products (e.g. when bags or vials of frozen cells are temporarily removed from a freezer). Generally, the greater the temperature fluctuation, the more extensive the recrystallization as there is more kinetic energy available for this process to take place at higher temperatures. Several recent studies have shown direct links between transient warming and functional impairment in stem cells and peripheral blood mononuclear cells (PBMCs), with recrystallization most certainly serving as the preeminent mechanism of damage [35–38]. However, because recrystallization is both secondary to the major sources of cryoinjury (i.e. those directly targeted by DMSO) and there were historically no potent, easily accessible compounds to ameliorate recrystallization, it is not actively targeted in routine cryopreservation procedures. This paradigm is changing as there now exists a diverse range of biocompatible compounds / materials developed to inhibit ice recrystallization which are also amenable to large-scale production.

Small molecule ice recrystallization inhibitors

Predominant among these technologies has been small-molecule ice recrystallization inhibitors (smIRIs) developed by Dr Robert Ben's group at the University of Ottawa [39,40]. These compounds are unique amongst all other recrystallization inhibition-active (RI-active) compounds in that they do not bind to the ice crystal surface, and therefore lack dynamic ice shaping properties that can lead to the formation of highly spicular ice crystals that can exacerbate cell death. Oftentimes this phenomenon is correlated with the concentration of an ice recrystallization inhibitor (IRI) and can limit the use of an IRI to its maximal capacity in cases where deleterious dynamic ice shaping occurs well-before solubility or cytotoxicity limits. This ultimately proved, among other things, to be one of the limiting factors associated with the direct use of AFPs in cryopreservation applications, and thus the lack of dynamic ice shaping in smIRIs is exceedingly noteworthy [41]. However, despite the low cytotoxicity and dynamic ice shaping characteristics allowing higher concentrations of these compounds to theoretically be used, they have relatively low solubility limits due to their amphiphilic properties. This has been highlighted as a major research and development avenue for the subsequent generations of these compounds, with some promising results already evident [42]. Despite there being opportunities for

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functional improvement, these compounds in their present iterations have facilitated the retention of post-thaw functionality in cryopreserved red blood cells, platelets, HPCs, induced pluripotent stem cells (iPSCs), chimeric antigen receptor (CAR) T cells, and natural killer (NK) cells [43–45].

Synthetic polymers & nanomaterials with ice recrystallization inhibition activity

Synthetic polymers represent another major class of IRI initially characterized in the 1980s that rival the smIRIs produced by Ben's group in potency [46]. In contrast to smIRIs, the mechanism of ice recrystallization inhibition very much recapitulates that of AFPs, so deleterious dynamic ice shaping is not absent. However, as the degree of dynamic ice shaping is much less than that seen in AFPs, this proves not to be a cardinal concern. In some ways they are more attractive than smIRIs as recent advances in synthetic polymer chemistry have made it possible to carefully tune polymer architecture with relative ease through precise alterations in chain length / orientation or through the addition of functional groups [47]. This has facilitated our ability to study the mechanisms underlying the RI-activity of this class of compounds, as well as ways to improve RI activity. Much attention towards this end has historically been placed on polyvinyl alcohol (PVA) as it is the most potent commercially available RI-active synthetic polymer, with recent ground-breaking studies generating PVA variants that have ten times the RI activity of standard variants due to a simple increase in backbone flexibility [48]. A notable observation consistent amongst attempts to define mechanisms underlying the RI activity of synthetic polymers is a distinct positive correlation between chain length and RI activity [49]. Albeit a seemingly simple observation, it presents an opportunity to leverage the now-flourishing field of supramolecular chemistry to overcome size limits in polymer synthesis and generate increasingly potent RI-active polymers [50]. Some of the more promising studies on this topic have shown institution of RI activity following induction of self-assembly in cases where the individual components of the assembly lacked RI activity [51–53]. As this area of research is exceptionally novel, the application of these self-assembling polymers to cryopreservation, apart from a few exceptions, has not been thoroughly investigated [54]. However, even in the case of PVA (and other RI-active polymers such as poly-L-histidine or poly-L-hydroxyproline), the applications to cryopreservation have been few despite its RI activity having been identified nearly 30 years ago.

Polymeric nanoparticles formed using supramolecular triggers represent only a small portion of the nanomaterials that are now available to inhibit ice recrystallization. The histology dye, safranine o chloride, self-assembles into aggregates that contain regularly spaced amino and methyl substituents similar to that of the ice-binding surface of AFPs, leading to the notable discovery of its RI activity in 2015, despite the regular use of this dye since the turn of the 20th century [55]. In a similar vein, graphene oxide and nanocellulose composites, commonly used in a diverse range of non-biological applications, have also recently proven to have potent RI activity [56–59]. The motivation to study the RI activity of these and other nanomaterials not commonly used in biopreservation was in some ways prompted by the greater mechanistic understanding through which AFPs and other, more well-established RI-active compounds function [60,61]. Therefore, as our understanding of these phenomena continues to grow, it is certainly possible that more RI-active compounds found in nature will be identified.

TRANSLATIONAL INSIGHT

The well-established status and routine application of cryobiology causes many to oftentimes take for granted the ability to store

living biological material for extended periods of time. However, considering nature has never been called on to adapt to -196°C it is impressive that we can sustain life in an environment that precludes life. Such feats are a rarity in science, and thus this grants acclaim to the prominent cryobiologists' that laid the foundation for the standard practices we implement to this day, but also emphasizes the challenge of extrapolating natural phenomena for this unnatural condition we impose during cryopreservation. Therefore, the multidisciplinary innovation required for the bioinspired technologies / approaches described in this article should not go understated, particularly considering the past six decades of advances in this field have rendered the remaining challenges increasingly refractory.

Leveraging differential evolution algorithms or quantitative structure-activity relationship models (QSAR) used in classical drug-screening/discovery workflows, remains uncommon in cryobiology, but may become increasingly relevant as we strive to implement novel CPAs (i.e. both DMSO replacements and IRIs) for the cryopreservation of cell therapies [62]. These methods would allow for the development of 'optimal' CPA solutions, but perhaps less intuitively (and more specifically), it would also facilitate the tailoring of CPA solutions and cryopreservation protocols for a given cell type. Variability in certain cell-specific characteristics (e.g. volume, permeability, water content, etc.), is known to also exist between the precursors to cell therapies and the final therapeutic product and could alter the ability of a cell to tolerate a given cryopreservation protocol, making one-size-fits-all cryopreservation protocols oftentimes suboptimal [63]. This variability requires that the types of CPAs, the concentrations of CPAs, and the cooling rates be optimized for every given cell type. As these variables are heavily associated with one another, the process of tailoring a cryopreservation protocol to a given cell type becomes increasingly complex when dealing with a multicomponent solution. For example, differing permeabilities of the cell-permeating components in one cell type could manifest to a different extent in another. In a broader yet more theoretical sense, differences in chemical interactions between individual components of a multicomponent system and the biological interactions that result, could exacerbate variability between individual cell types relative to when a single component is used (provided that each individual component has a different mechanism of action). Given these different points, the use of high-throughput methods to optimize storage in this 'new age' of DMSO-free cryopreservation is warranted.

The topics mentioned throughout this article by no means encompass the entirety of the advances in cryobiology that could be applicable to the cryopreservation of cell therapies. Other notable active areas of research include the use of cell encapsulation [64], the intracellular delivery of impermeant, non-toxic CPAs [65], and biochemical pathway modulation [66] to facilitate post-thaw recovery. Each referenced article offers a compendium of these respective topics which we refer the reader to for comprehensive overview. It is worth making note of the latter however as it is a notion that is becoming particularly prominent in the field of cryobiology. Biochemical cryoinjury represents a novel perspective of cryoinjury as cryopreservation was historically viewed as a purely biophysical problem. It is largely the developments in molecular biology and -omics technologies that have engendered this perspective through shedding insight on deleterious biochemical changes that manifest in response to cryopreservation [66–68]. The nature-inspired methods discussed in this article will undoubtedly minimize biophysical stress, and eventually, upon further improvement, could allow for its effective cessation (Figure 1). At this point, any deleterious forms of biochemical stress that continue to manifest could be identified and targeted in an effort to further improve the post-thaw function and potency of cell therapies.

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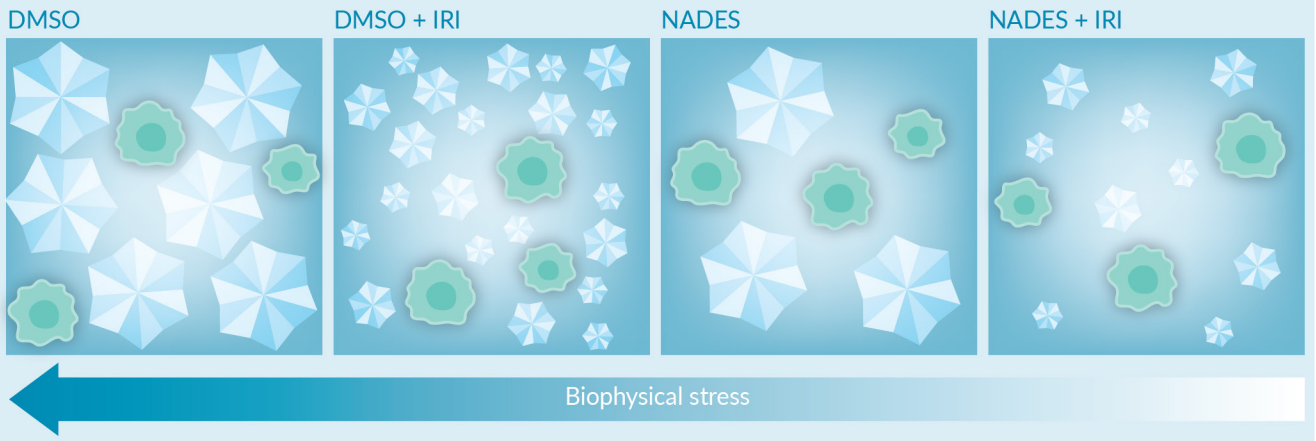
FIGURE 1

The size of the unfrozen fraction and ice recrystallization together impact the biophysical stress imposed during a suboptimal cryopreservation protocol.



Ice recrystallization inhibitors (IRIs) will decrease the average size of ice crystals, whereas cryosolutions designed based off natural deep eutectic systems (NADES) would decrease the size of the unfrozen fraction at equimolar concentrations to dimethyl sulfoxide (DMSO)-only solutions. DMSO allows for high viability, but in a suboptimal cryopreservation protocol (where cells are either exposed to transient periods of rewarming or the cooling/warming procedures are suboptimal), conditions that minimize recrystallization or increase the size of the unfrozen fraction (i.e. the ratio of unfrozen water to ice at any given temperature) would minimize the biophysical stress imposed on the cell.

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FASTFACTS

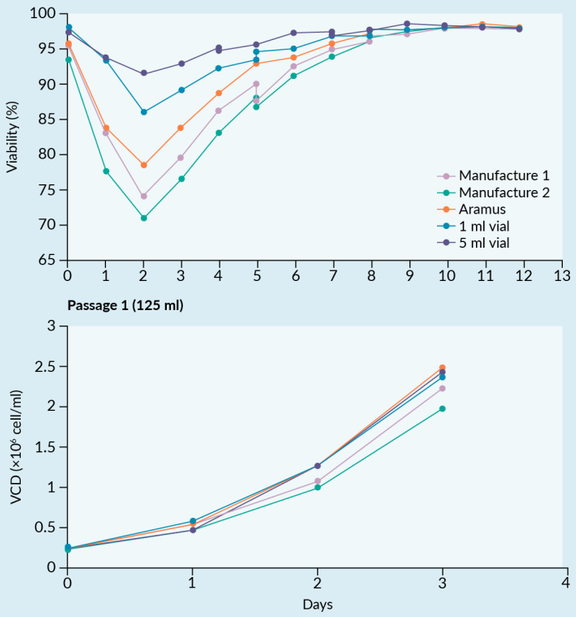
Benefits of upstream seed train intensification & high-density cell banking

Patrick Shu Miao

High-density cell banking (HDCB) involves freezing high concentrations of cells in large single-use bags. It increases productivity and reduces manufacturing costs by eliminating intermediate steps. HDCB also reduces the risk of contamination and the number of consumables needed, while enabling rapid cell recovery and high viability.

Aramus™ single-use fluoropolymer bags excel at HDCB, allowing cells to be frozen and stored at substantial volumes with a high cell concentration. After thawing, these large-volume, high-density cell cultures allow seeding direct into a bioreactor, thereby eliminating intermediate steps of the traditional seed train. The seed train process can be redesigned from vials and flasks to Aramus single-use fluoropolymer bags. The Aramus bag assembly is a closed system, which reduces the risk of contamination in the cell culture process, has lower surface energy, allowing recovery of every drop of valuable drug product, and has lower particulates per volume versus other bags. Additionally, Aramus ultra-pure low E&L profile has only one contributory element, resulting in reduced CAPA(s) due to unknown contaminants.

Figure 1. Cell viability over a 13-day CHO cell culture period (top) and cell density during the first passage of the same culture (bottom).



ARAMUS SINGLE-LAYER FLUOROPOLYMER ASSEMBLY FEATURES:

- Universal material chemical compatibility—resistant to solvents like DMSO, aggressive and highly concentrated chemicals
- Widest temperature operating range—fluoropolymer material maintains flexibility at -196°C
- Gamma-stable fluoropolymer film
- Built to be tough and 100% pressure tested, eliminating leaking welds and port fitments that can cause significant loss

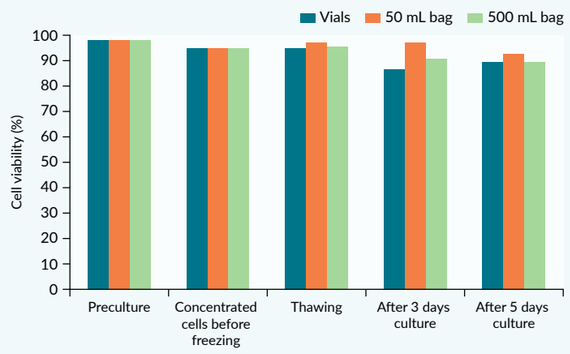
RECOVERY OF A HIGH-DENSITY CHINESE HAMSTER OVARIAN (CHO) CELL CULTURE

The recovery of a high-density CHO cell culture, post cryopreservation, was compared to alternative cryobags and 1 and 5 mL cryovial controls. The cells were frozen in liquid nitrogen for 2 weeks, then thawed and cultured for two passages up to 13 days. The results are shown in **Figure 1**. The Aramus bags demonstrated the quickest recovery among the different cryobags used and returned to a cell viability of >95%. In addition, the cells recovered from the Aramus bags showed a comparable viable cell density (VCD) to the vials and faster growth compared to alternative cryobags.

CELL VIABILITY AFTER THE FREEZE & THAW PROCESS

Viability of cells was measured before freezing and after thawing of the cells. Cultures were performed using glass vials or 50mL and 500mL cryobags. The results are described in **Figure 2**. Compared to glass vials, the fluoropolymer bags demonstrate comparable cell viability in the preculture as well as after thawing and culturing for 5 days.

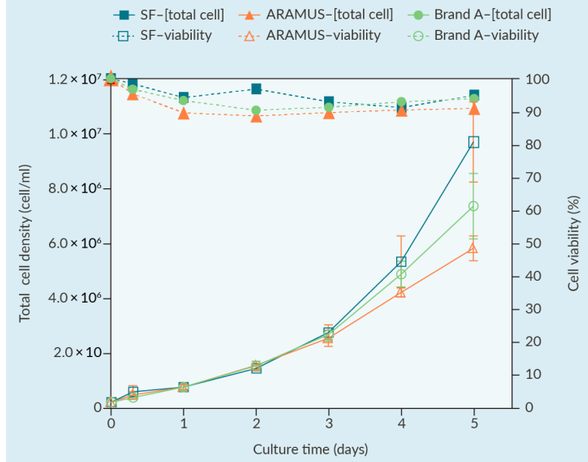
Figure 2. Cell viability during the various stages of the cell culture process (pre- and post-thawing).



CULTIVATION OF HEK293 CELLS

HEK293 cells were cultured for 5 days, and recovery was determined by measuring the total cell density and viability. Cell cultures were performed and compared between shake flasks (SF), Aramus- and brand A bags.

Figure 3. Cell density and cell viability in shake flask culture or cell culture performed using single-use bags.



The results are shown in **Figure 3** and demonstrate that the Aramus bags are suitable for cultivation of HEK293 cells.

CONCLUSION

Intensified HDCB using Aramus single-use cryobags can shorten cell culture times by weeks and reduce contamination risks by eliminating multiple manual cell transfer steps. It provides a wider window to start post-thaw seeding into the bioreactor, providing flexibility for scale-up production. Overall, the HCDB process leads to improved productivity, lower overall cost of goods, and better sustainability using fewer consumables.

Cell and Gene Therapy Insights 2023; 9(2), 147; DOI: 10.18609/cgti.2023.020

In partnership with: Entegris

MARCH 2023 Volume 9, Issue 2

CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

WEBINAR DIGEST

Enhancing non-viral gene editing, processing, & expansion of T & NK cells

Sung (Sung-Uk) Lee, PhD Scientist, Thermo Fisher Scientific **& Deepak Kumar,** PhD Scientist, Thermo Fisher Scientific

A key focus in cell therapy manufacturing is the development of closed, automated manufacturing processes to help reduce costs and increase the speed of getting treatments to patients. The Gibco™ CTS™ Rotea™ Counterflow Centrifugation system and the Gibco CTS Xenon™ Electroporation System are powerful modular tools in the quest towards creating a closed cell therapy manufacturing process by providing exceptional performance and helping to reduce contamination in a cell therapy manufacturing workflow. This poster provides a summary of how Thermo Fisher Scientific technologies have been proven for effective use in chimeric antigen receptor (CAR)-T cell workflow optimization and natural killer (NK) cell engineering.

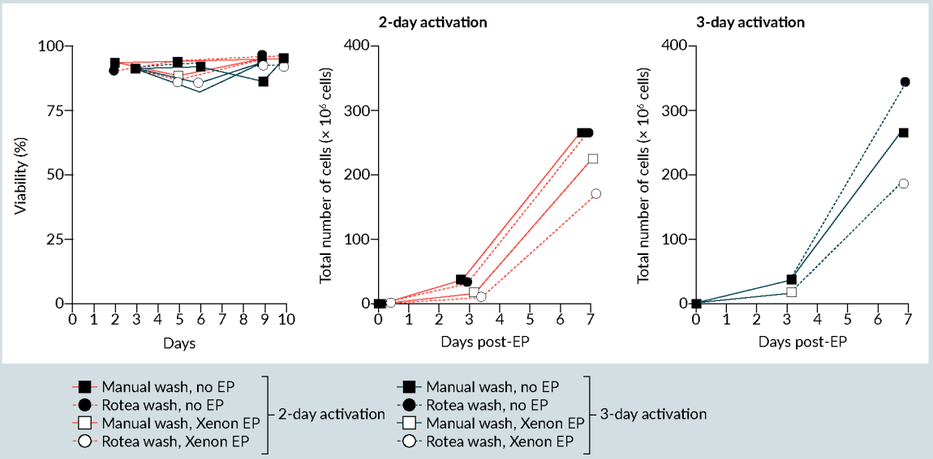
Cell & Gene Therapy 2023; 9(2), 137 • DOI: 10.18609/cgti.2023.017

OPTIMIZING CAR-T WORKFLOWS

The CTS Rotea System applies a proven counterflow centrifugation method for a broad range of cell processing applications including CAR-T therapy. The CTS Rotea system can be programmed to perform effective washout of media and buffer components and is designed to handle a wide range of input volumes from 50 mL–20 L. Wash buffer can be washed through the fluidized cell bed, enabling 95% removal of original medium components with minimal cell loss and minimal disruption to cell viability. The single-use kit enables an easy transition to commercial manufacturing and GMP compliance with industry standards.

Thermo Fisher Scientific conducted a range of experiments to observe the cell viability and growth of cells processed using the CTS Rotea system versus manual washing, with and without CTS Xenon electroporation. CTS Rotea system outperformed manual buffer exchange demonstrating automation as a time saving measure while preserving process quality **(Figure 1)**. Good viability of >80% was observed for all conditions compared to no electroporation controls. Cells from the 2-day activation protocol showed a slightly improved growth over those from the 3-day activation protocol, but overall, growth scores showed a similar trend in both groups.

Figure 1. Cell viability and cell growth up to 7 days post-electroporation using a CTS Xenon Electroporation system



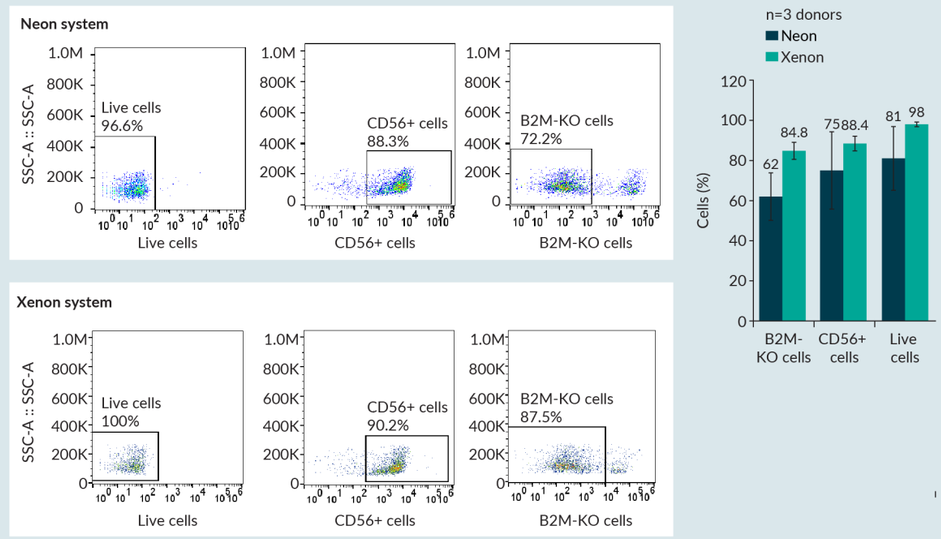
ENGINEERING NK CELLS

Engineering of NK cells is challenging using conventional methods, due to their limited efficiency, inconsistencies, and needs for high viral titer. A robust and precise toolkit is urgently needed for NK cell engineering and expansion.

Gibco CTS NK-Xpander™ Medium is designed to meet the needs of cell therapy developers by enabling expansion of human NK cells without the need for feeder cells. With this medium, cells have been proven to expand and maintain CD56 and CD16 expression as well as maintain robust cytotoxic capability.

Thermo Fisher Scientific investigated the use of electroporation to genetically engineer PBMC derived human NK cell. Results in **Figure 2** shows that, the CTS Xenon system achieved approximately 85% B2M knockout across three donors and demonstrated greater knockout efficiency than the Invitrogen™ Neon™ Transfection system.

Figure 2. Comparative analysis of gene editing efficiency in expanded NK cells using different electroporation systems.



In summary, the Thermo Fisher Scientific Cell Therapy Systems (CTS) portfolio of flexible, modular centrifugation and electroporation systems, including the CTS Rotea system and the CTS Xenon system, can be easily adapted into existing cell therapy manufacturing workflows. Find out more at **www.thermofisher.com/CTxManufacturing**.

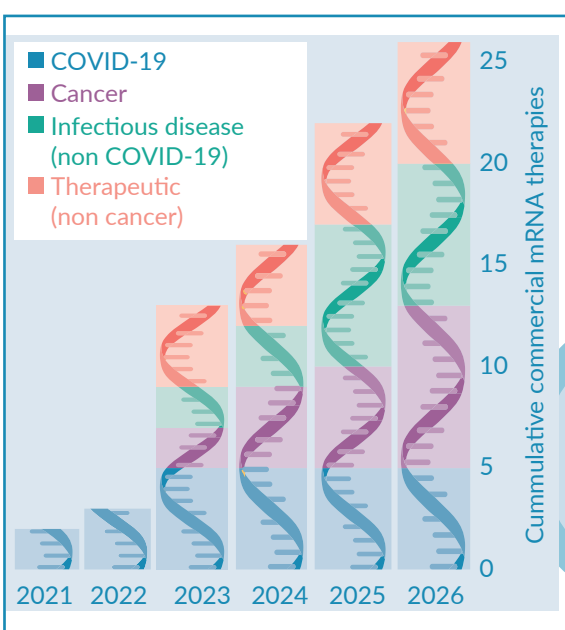
Watch the webinar here

Read the full transcript here

In partnership with: ThermoFisher SCIENTIFIC

mRNA manufacturing and analytics

With the recent surge in use of mRNA as a vaccine and therapeutic modality, optimizing and understanding the development and manufacturing of mRNA for biotherapeutics has never been of greater importance.



COVID-19 had a massive impact on on mRNA vaccines

Current value of the global mRNA synthesis and manufacturing services market

US$ 53 billion

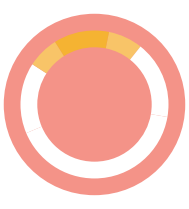
Use this infographic to guide you through the upstream and downstream steps in mRNA manufacture, along with the associated analytics

DNA TEMPLATE PREPARATION

Template design & plasmid production

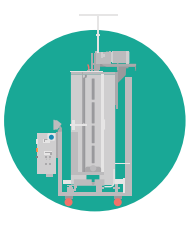
~4 weeks

TARGET GENE DISCOVERY. Target genes are discovered using techniques such as next-generation sequencing.



PLASMID CREATION. Once a gene of interest has been identified, the target sequence can be integrated into a plasmid.

12–16 h

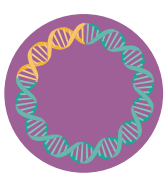


pDNA AMPLIFICATION. Plasmid DNA (pDNA) is amplified in host bacteria, typically *E. coli*, which grows in a single-use fermenter.

Plasmid purification

PURIFICATION

To achieve a high level of supercoiled plasmid.

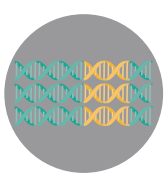


LINEARIZATION

With restriction enzymes that cleave DNA at specific sequences.

PURIFICATION

Recovery of the linearized plasmid.



ANALYTICS

Key technologies used to identify critical quality attributes and impurities are listed below.

Draft guidance is in process and therefore this is subject to change. Other technologies can be used.

Agarose gel electrophoresis: establish plasmid quality level & confirm linearity after restriction enzyme digest

Sequencing: plasmid identification

UV absorbance: pDNA quantification

Process-related impurity quantitation

qPCR and RT-qPCR: quantify residual host cell genomic DNA and RNA

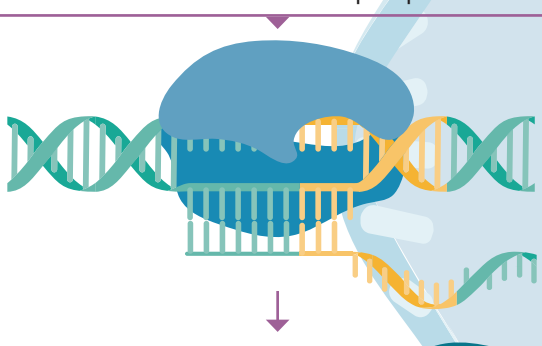
RP-HPLC: residual host protein and RNA detection

mRNA SYNTHESIS

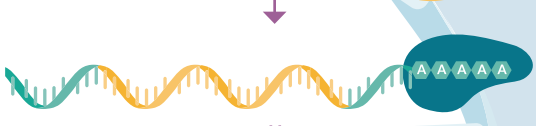
mRNA *in vitro* transcription and capping

mRNA is synthesized through the process of *in vitro* transcription (IVT) using linearized pDNA template

In vitro transcription, catalyzed by RNA polymerase

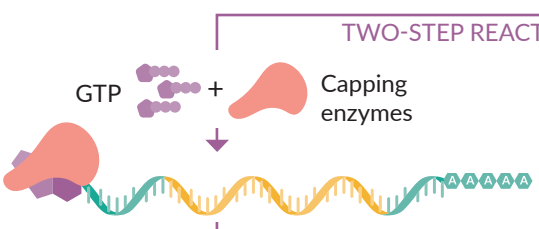


PolyA polymerase catalyzes the addition of the PolyA tail



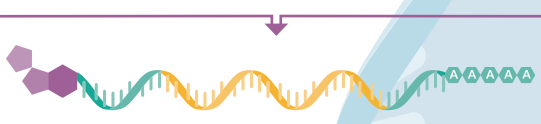
TWO-STEP REACTION

GTP + Capping enzymes



ONE-STEP REACTION

Cap analog



DNase I digestion of DNA template

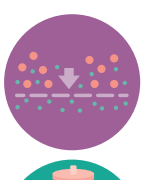
2–3 h

DOWNSTREAM

mRNA purification

mRNA is produced in a cell-free system using non-animal-derived raw materials. This simplifies downstream purification.

However, the reaction mixture contains impurities including enzymes, residual NTPs and DNA template, and aberrant mRNAs (dsRNA and truncated RNA) formed during the IVT.



ULTRAFILTRATION & BUFFER EXCHANGE

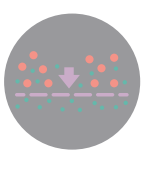
Reduce volume and remove small impurities

AFFINITY CHROMATOGRAPHY

Process related components such as truncated mRNA, DNA template, buffer components and NTPs

POLISH

Reduce dsRNA and uncapped RNA products from the final product



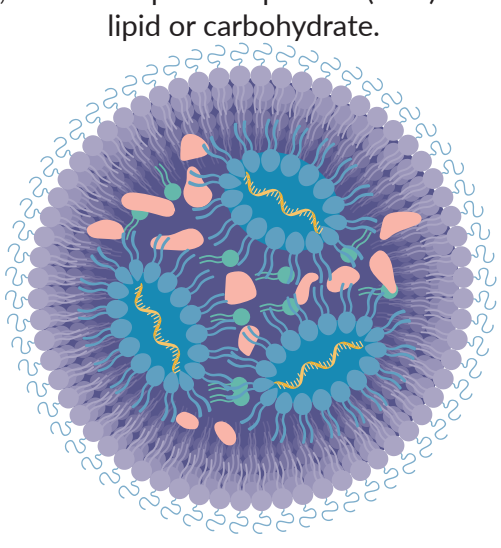
ULTRAFILTRATION & BUFFER EXCHANGE

Reduce volume and final 0.2 µm filtration

PURIFIED mRNA

Formulation, fill and finish

The purified mRNA is encapsulated in a drug delivery vehicle, such as a lipid nanoparticle (LNP) or another lipid or carbohydrate.



FINAL BUFFER EXCHANGE

FINAL FORMULATION & FILTRATION

Concentration adjustment and 0.2 µm sterile filtration

FILLING

Closed methods for aseptic filling of mRNA-based therapeutics reduce risk of contamination.

Packaging

The filled packages undergo final stage quality control and are stored in ultra-low temperature (below -80°C) freezers, ready for delivery to patients.

Impurities from IVT can reduce translation efficiency and cause unwanted immune responses.

Characterization & critical quality attribute testing: purified mRNA drug substance

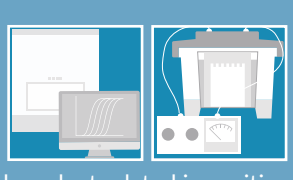
IDENTITY

Sequence confirmation: sequencing, RT-PCR

RNA CONTENT

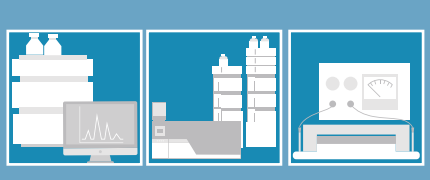
RT-qPCR, RT-dPCR, UV absorbance, fluorescence-based RNA-specific assays

PURITY



Process and product related impurities
Residual DNA template: qPCR
Protein & dsRNA: immunoblot

INTEGRITY



% intact & fragment mRNA: capillary gel electrophoresis
% 5′ capped: UPLC, RP-HPLC and LC/MS
% 3′ polyA: RP-HPLC
mRNA integrity: Gel electrophoresis

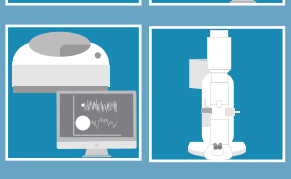
SAFETY & OTHER

Endotoxin, bioburden sterility, appearance

Characterization & critical quality attribute testing: mRNA–LNP drug product

Lipid content: LC/MS, HPLC

Particle size: Dynamic light scattering, electron microscopy



% RNA encapsulation: RiboGreen RNA assay, fluoresence-based mRNA assay

Lipid identity & impurities: LC-MS
Fatty acid analysis: HPLC

WEBINAR DIGEST

Simplifying lentiviral downstream processing with a novel affinity resin & robust analytical tools

Chantelle Gaskin & Suzy Brown

Due to its broad tropism and long-term, stable gene expression in non-dividing cells, recombinant lentivirus (LV) has become a vector of choice for many gene-modified cell therapies. The safety and efficacy of LV-based therapies depend greatly on optimized and controlled LV production. Downstream purification of LV particles presents unique challenges, and robust analytics are critical to verify both the recovery and infectivity of the purified product. This poster gives a condensed overview of a new affinity chromatography resin to purify VSV-G pseudotyped LV, as well as qPCR-based genomic and proviral infectious titer assays for analytical use.

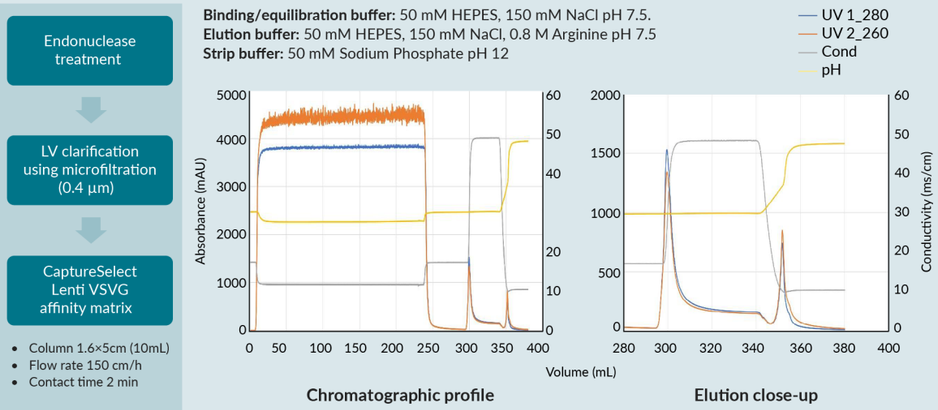
LENTIVIRAL VECTOR PURIFICATION

The purification of LV vectors is considered as a challenging process. It requires processing within narrow ranges of pH, temperature, conductivity, and shear and existing purification tools often end in low recovery or can affect infectious titers. Affinity chromatography has been the most requested method from the field to overcome these challenges. The newly launched CaptureSelect™ Lenti VSV-G Affinity resin was designed for specificity to VSV-G pseudotyped LV vectors. The resin provides high-level purification in a single step with gentle elution conditions at neutral pH to maximize infectious particle recovery. **Figure 1** shows the recommended chromatography conditions and the elution profile using the affinity resin. The elution was efficient and showed good compatibility with the enveloped virus particles, resulting in high concentrations of infectious particles in the elution fraction. As the process progresses, the total to infectious particle ratio decreases. This results in a >5-fold enrichment of infectious particles with a 50–60% recovery in the column eluate **(Table 1)**.

Table 1. Comparison of total particle to infectious particle ratios.

Sample	TP/mL	IP/mL	TP/IP ratio	Recovery	HCP removal	Total DNA removal
1. Feed	1.10×10	7.98×7	138			
1. Flow through	3.25×8	8.30×5	392			
1. Elution	4.44×10	4.42×8	100	50%	99%	80%
2. Feed	1.11×10	9.00×7	165			
2. Flow through	1.28×9	5.45×6	245			
2. Elution	2.6×10	4.66×8	71	58%	97%	97%

Figure 1. Representative chromatogram using recommended process conditions.



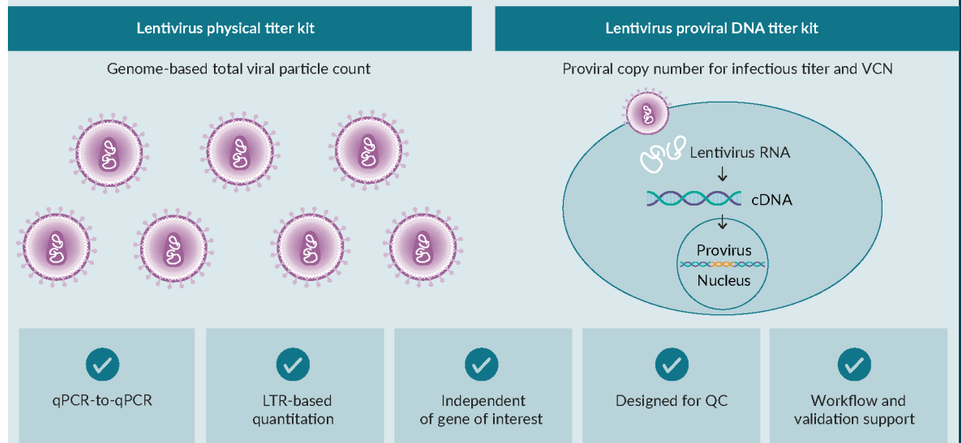
ANALYTICAL TOOLS FOR PURIFICATION PROCESS DEVELOPMENT

As LV vectors are used to transduce cells, they are an active ingredient in drug substances. Drug substances must be tested for critical quality attributes (CQAs) in-process and at lot release according to US FDA CMC guidance. For LV characterization and integration analysis, Thermo Fisher Scientific has recently introduced two new qPCR assays **(Figure 2)**. The ViralSEQ™ Lentiviral Physical Titer Kit is a one-step real-time RT-qPCR assay for genome-based LV titers, measuring physical titer in viral particles per mL. Additionally, the ViralSEQ™ Lentivirus Proviral DNA Titer Kit is a qPCR assay to measure integrated LV or proviral copies in transduced cells. It can be used to calculate infectious viral titers and vector copy number (VCN). Combined, these two assays provide a convenient method to compare qPCR to qPCR data, for total and infectious titers, as well as for measuring VCN for analytics across the LV workflow. Both assays are designed to provide robust performance and facilitate LV analytics, in-process development, and manufacturing in QC environments.

Watch the webinar here

Read the full transcript here

Figure 2. Overview of the ViralSEQ Lentivirus titer kits.



CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy 2023; 9(2), 149 • DOI: 10.18609/cgti.2023.021

In partnership with: ThermoFisher SCIENTIFIC

INTERVIEW

Establishing an end-to-end vector manufacturing solution for the gene therapy industry

Interview with **Dr Nicole Faust,** General Manager, Cell Line Development at Cevec, now part of Cytiva

NICOLE FAUST has more than 20 years of management experience in the biotechnology sector. Before joining CEVEC, she held leadership positions with Lonza and Taconic Biosciences. She has a scientific background in stem cell biology and holds a PhD in cell and molecular biology from University of Freiburg and an MBA from Educatis University, Switzerland. She is a member of the American Society for Gene and Cell Therapy and currently Chair of the Gene Therapy Advisory Committee of the Alliance for Regenerative Medicine (ARM).

Cell & Gene Therapy Insights 2023; 9(2), 235–240

DOI: 10.18609/cgti.2023.035

With demand for viral vectors from the cell and gene therapy space continuing to grow, is it time for a paradigm shift in vector manufacturing technology? In this interview, Nicole Faust (General Manager, Cell Line Development at Cevec, now part of Cytiva) discusses current challenges, potential solutions, and considers the future of the AAV manufacturing landscape.

Q The Alliance for Regenerative Medicine CEO Timothy D Hunt recently emphasized that

the cell and gene therapy field is clearly gaining pace, with up to 14 new approvals by regulatory authorities anticipated in 2023. What challenges is the industry currently facing, and how can the manufacturers of gene therapies adapt to keep up with this pace?

NF: Actually, the situation is even more challenging as we are not only seeing an increase in therapies, but we also have noticed a switch from ultra-rare and rare to prevalent and common diseases. This is evidenced by the fact that 58% of ongoing clinical trials with gene therapies are in these large indications. This all leads to an enormous demand for viral vectors that current vector manufacturing technologies are struggling to meet. The whole industry needs to prepare for a paradigm shift regarding the technologies used. We have seen a similar development in the past with monoclonal antibodies, which nowadays are successfully manufactured using standardized technologies based on stable producer cell lines.

Q Looking at current viral vector manufacturing, we see the industry working on a variety of different technologies and processes. Even for viral vector products that are filed for or entering clinical trials, the manufacturing methods are not yet fixed. This is a bit like building the plane while flying, isn't it?

NF: You're right – this is due to the lack of reproducible and, ultimately, standardizable large-scale manufacturing systems. The cell and gene therapy industry as a whole is still in its infancy. It started with treatments for rare and ultra-rare diseases where small quantities of vector material are sufficient not only for completion of trials but also for commercialization. The need for advanced production technologies wasn't that pressing. However, this is changing as the current standard manufacturing technology, transient transfection, clearly reaches its limits in the large scale.

Further, the pressure is not only increasing due to the high demand; I also believe the cell and gene therapy field will face much stricter requirements from regulatory authorities. While various manufacturing technologies are allowed today, we anticipate from other therapy fields that standardization will be required from the regulatory side. The aim here is to have a reliable technology that ensures the highest product quality possible, in a robust, reproducible, and optimizable format. The question now is how to best meet these requirements. The answer, in my opinion, can only be to establish a new industry standard via a production platform based on stable cell lines.

Q If I take your assumption that the industry will move towards a small number of standard manufacturing technologies in the future, with

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stable cell lines being one of them, can you give an overview on the current status of stable cell line systems for the most commonly used AAV vectors? What will the ideal stable cell line system need to look like to meet the future requirements of the industry?

NF: This is a good point – 'stable cell lines' is a term that is widely used in the industry and claimed by a number of companies. But a closer look at the underlying technologies reveals that the term refers to different approaches, such as production systems that still require the addition of helper viruses. With the need for consistency and further optimization options, a true stable manufacturing technology should be based on a producer cell where really every component needed for adeno associated virus (AAV) manufacturing is stably integrated into the genome of the cell. This means that for the manufacturing step – even at very large scale – no additional component is needed. As expression of AAV genes is toxic to the cells, an inducible system based on stable producer cells, such as ELEVECTA™, is very desirable. This way, viral vector production can be tightly controlled, and the production process can be scaled up to large stirred-tank bioreactors.

Q What is your view on the future of transient manufacturing systems?

NF: I believe transient transfection will stay relevant to the market. Especially in early research phases, where the therapeutic is not yet fully defined, vectors will continue to be produced using a transient approach. In addition, for therapies where smaller amounts of vectors are needed, the transient setting is a suitable option. It is flexible with respect to the specific vector produced, and the route to good manufacturing practice (GMP) material is initially slightly faster. We are therefore also optimizing our suspension HEK293 cell line, which forms the basis for ELEVECTA™, for transient transfection. This allows us to offer a platform for initial product development on a production cell with the same genetic background.

Q You mentioned that transient transfection is faster – and speed is of course highly relevant for therapeutic companies looking to enter clinical trials. Your view is that manufacturing technologies based on stable cell lines will become the standard technology for viral vectors. But as cell line development takes time, how do you address your clients' need for speed?

NF: Developing a stable producer cell line for a therapeutic takes time, but that does not necessarily mean that you have to wait until the producer cell line

"I believe the answer is to provide an integrated solution to the market by offering an end-to-end process for manufacturing clients. This starts with biology, meaning customized cell line development services, and continues via fully integrated process development..."

is finalized to get material. AAV material can already be produced after 6–8 weeks from a so-called stable polyclonal pool while developing the fully stable monoclonal producer cell line. Using this approach, you can kill two birds with one stone: you get material early and this material is based on the same parental cell line.

Q We have considered the future AAV manufacturing technology landscape – but what else is needed to transform the manufacturing industry into an affordable cure provider for patients in need?

NF: I believe the answer is to provide an integrated solution to the market by offering an end-to-end process for manufacturing clients. This starts with biology, meaning customized cell line development services, and continues via fully integrated process development (upstream and downstream) to improve manufacturing of a commercial-ready product. Optimization can be done on so many levels that, consequently, the best solution you can get is a system in which all components are optimally coordinated. With the acquisition of CEVEC, Cytiva has made an important step towards completing its end-to-end solution.

AFFILIATION

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Cevec, now part of Cytiva

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given her approval for this version to be published.

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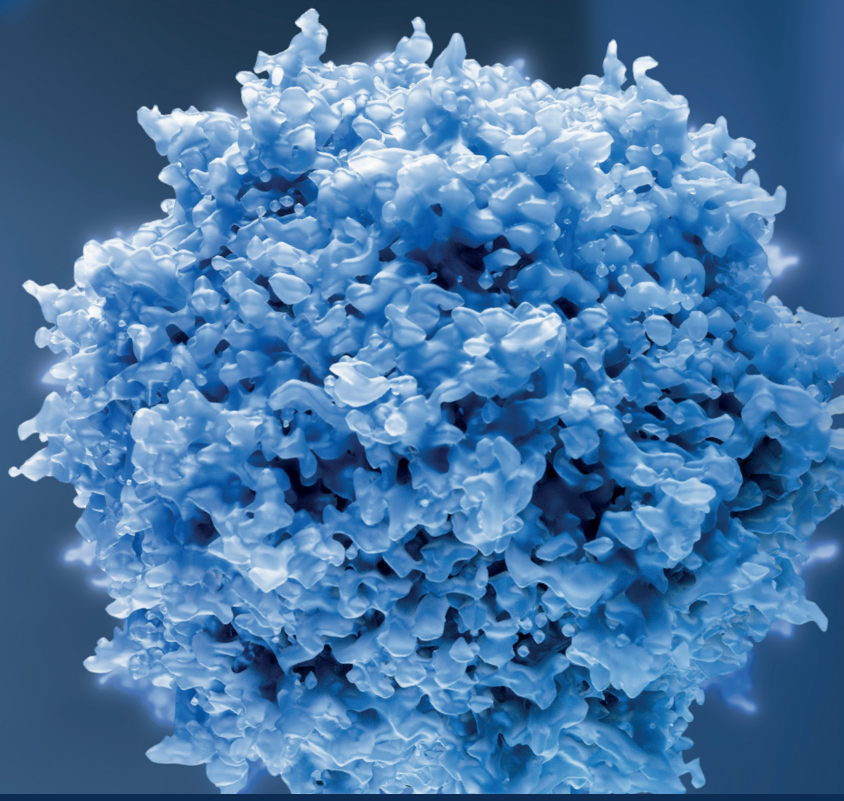


THE INDUCIBLE AAV PRODUCER CELL TECHNOLOGY

NO PLASMIDS · NO TRANSFECTIONS · NO HELPER VIRUS

cevec

now part of Cytiva

Ultra-rapid microbial detection in cell & gene therapy products: the closest you can be to real-time release

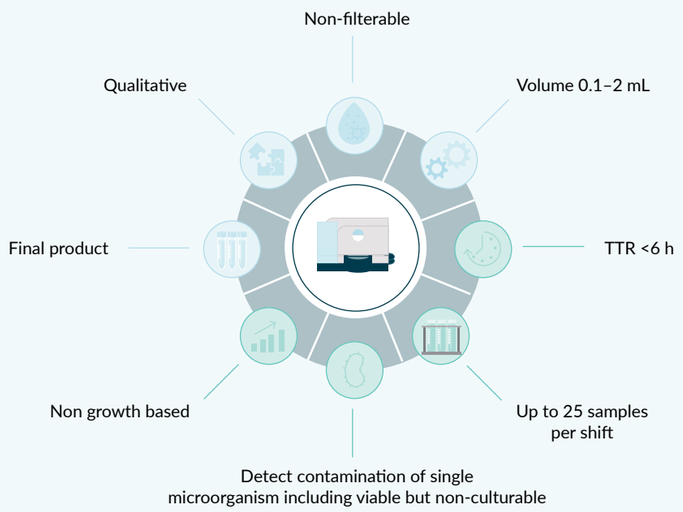
Félix A Montero-Julian PhD, Scientific Director, Pharma Quality Control Business, bioMérieux

When innovators are focused on shortening manufacturing process to meet patient demand for life saving therapies, there is a need for new quality control analytical method solutions that are fast enough to keep pace with faster manufacturing approaches. This poster explores a new ultra-fast sterility solution for cell and gene therapeutic products.

METHOD

The SCANRDI® is an ultra-rapid alternative technology for detecting microbial contaminants in drug products **(Figure 1)**. Designed to meet compendial testing standards, studies on limit of detection (LoD) and equivalency have been performed with a focus on species listed by the Pharmacopeias microorganisms. The most probable number (MPN) was used to demonstrate that the LoD of the SCANRDI® CELL-BURST is not significantly different from the LoD of a traditional plate counting method on ten compendial strains. To show the equivalency, we compared the proportion of positive results between both methods for all strains and all dilutions with a non-inferiority test of Farrington-Manning, with a 20% margin.

Figure 1. SCANRDI® CELL-BURST solution technological capabilities: a filtration based solid-phase cytometry.



CELL TYPES USED

Jurkat cells (cellular model used in R&D) – at a concentration of 10^7, 2×10^7, and 3×10^7 cells/mL

T cell lymphocytes – T cells at a concentration of 2×10^7 cells/mL

Final samples – CAR-T cells at a concentration of 5×10^6 cells/m

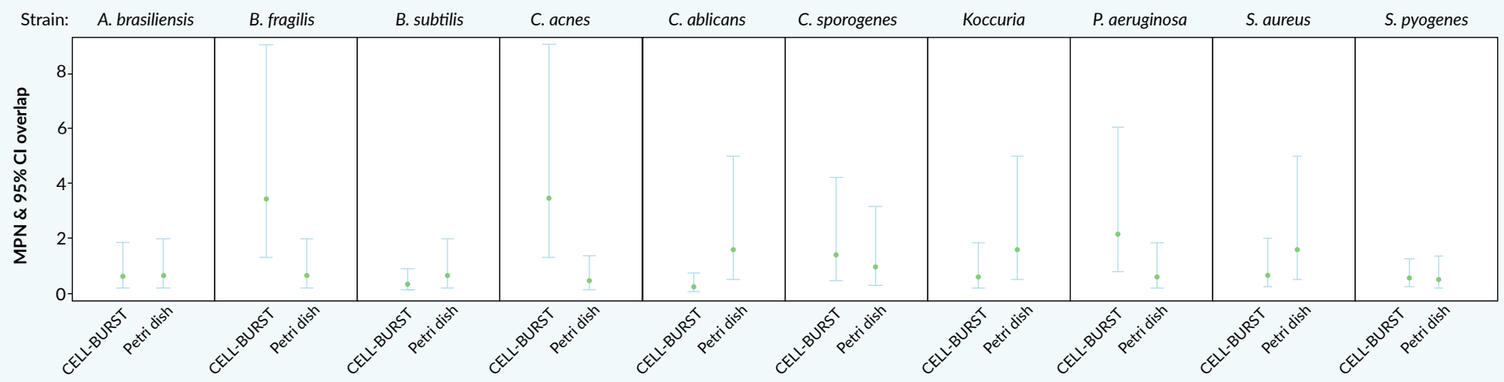
RESULTS

The MPN results **(Figure 2)** show that the confidence interval for both methods overlapped for each strain. Results demonstrated that there is no significant difference in LoD between the two methods. With a p-value inferior to 0.05 for the non-inferiority test of proportions, CELL-BURST is not significantly less sensitive than the petri dish method. The lower limit of the 95% confidence interval of the difference between detection proportions was -0.0689 (=-6.89%), which is greater than -20%.

CONCLUSION

The SCANRDI® CELL-BURST solution has a LoD that is not significantly different from a traditional counting method. Furthermore, the CELL-BURST was shown to be not significantly less sensitive than the petri dish method, considering a margin of 20%. These initial results allow us to proceed to full method validation and determine the equivalence with a traditional sterility method for cell-based products. This solution will facilitate sterility screening at various stages of the CGT manufacturing process in T cell-based products, with less than 6 h for time to result in a low-volume sample.

Figure 2. MPN & non-inferiority testing between the SCANRDI® CELL-BURST & petri dish methods for ten different microorganism strains.



CELL & GENE THERAPY INSIGHTS

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In partnership with:

WEBINAR DIGEST

Lentiviral titer determination: rapid & robust molecular methods suitable for validation

Unnati Dev, Product Manager, Pharma Analytics Team, Thermo Fisher Scientific

High-quality recombinant lentiviral vectors (LVV) are key for transgene delivery in many cell and gene therapies, including several of the FDA-approved CAR T-cell products. Critical to the success of these biologics are reliable methods to characterize and quantitate LVV. This poster highlights the challenges of current LV vector quantitation methods and describes two qPCR assays that enable quantitation and correlation of total and infectious lentivirus particles designed to facilitate LVV analytics in process development and manufacturing QC.

LVV IN CELL & GENE THERAPY

As of the first half of 2021, an estimated 288 lentiviral vector (LVV) cell and gene therapies were in the pipeline. To meet the growing demand, advances are being made in large-scale production to improve yields and turnaround and develop robust analytics to ensure vector quality and safety. **Figure 1** shows some of the key process elements and characteristics that LVV has in common with other viral vectors, as well as some that are unique to LVV.

QUANTITATION OF LV VECTORS

As LVVs are used to transduce cells, they are an active ingredient in drug substances, meaning they must be tested for identity, purity, strength, safety, and quality according to the FDA's CMC guidelines. There are several critical quality attributes for LVV, including titer, which is important for strength, quality, and safety testing.

Current methods of LVV quantitation include p24 ELISA, reverse transcriptase (RT)-qPCR or digital PCR, and particle counting. Regardless of the method used, manufacturers have identified quantitation challenges including poor reproducibility, high variation, difficulties optimizing the assays to complex matrix conditions, and inefficient recoveries. These challenges, coupled with the lack of an LVV reference standard, make it difficult to accurately quantify yields.

Furthermore, before patient cells are transduced, the infectious titers are tested in cell lines or healthy donor cells and the resulting integration frequency is measured as vector copy number (VCN). VCN is also measured in the final cell therapy product since high integration frequencies may pose a safety risk.

Figure 1. LVV manufacturing and analytics considerations.

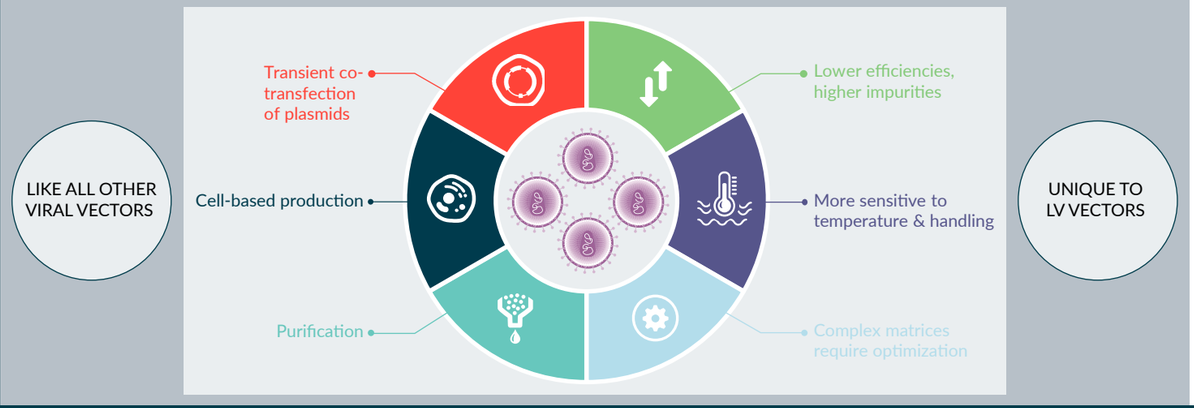


Figure 2. ViralSEQ lentivirus titer kits.

Methods for integration analysis include flow cytometry or fluorescent-activated cell sorting (FACS) for transgene expression, qPCR, or digital PCR to measure provirus integration, and cell-based assays to calculate infectivity.

In addition to the regulatory expectations for drug substance and drug product testing, there are also considerations around the validation of methods used for analytical testing. Performance characteristics evaluated for content/potency assays (e.g., titer) include specificity, working range, accuracy, repeatability, and intermediate precision.

APPLIED BIOSYSTEMS™ VIRALSEQ™ LENTIVIRUS TITER KITS

Thermo Fisher Scientific has developed robust integrated assay solutions that can be validated to give rapid, actionable results in-house. For LVV quantitation (total genomes) and integration analysis (proviral copy numbers), two qPCR assays have recently been introduced: the ViralSEQ™ Lentivirus Physical Titer Kit and the ViralSEQ™ Lentivirus Proviral DNA Titer Kit respectively **(Figure 2)**. The Lentivirus Titer Kits are designed to provide robust performance and facilitate LVV analytics in process development and manufacturing. Combined, the two qPCR assays provide a convenient method to compare and correlate data for total and infectious titers, and measure VCN, for analytics across the LVV workflow.

Watch the webinar here

Read the full transcript here

Cell & Gene Therapy Insights 2023; 9(2), 151 • DOI: 10.18609/cgti.2023.022

In partnership with: ThermoFisher SCIENTIFIC

FASTFACTS

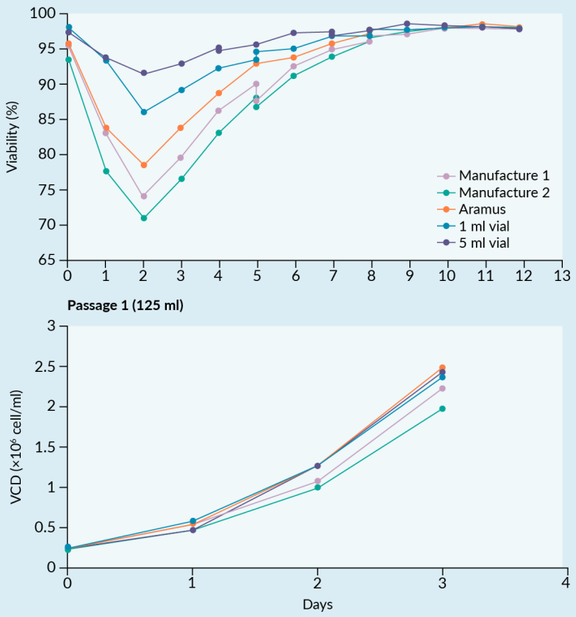
Benefits of upstream seed train intensification & high-density cell banking

Patrick Shu Miao

High-density cell banking (HDCB) involves freezing high concentrations of cells in large single-use bags. It increases productivity and reduces manufacturing costs by eliminating intermediate steps. HDCB also reduces the risk of contamination and the number of consumables needed, while enabling rapid cell recovery and high viability.

Aramus™ single-use fluoropolymer bags excel at HDCB, allowing cells to be frozen and stored at substantial volumes with a high cell concentration. After thawing, these large-volume, high-density cell cultures allow seeding direct into a bioreactor, thereby eliminating intermediate steps of the traditional seed train. The seed train process can be redesigned from vials and flasks to Aramus single-use fluoropolymer bags. The Aramus bag assembly is a closed system, which reduces the risk of contamination in the cell culture process, has lower surface energy, allowing recovery of every drop of valuable drug product, and has lower particulates per volume versus other bags. Additionally, Aramus ultra-pure low E&L profile has only one contributory element, resulting in reduced CAPA(s) due to unknown contaminants.

Figure 1. Cell viability over a 13-day CHO cell culture period (top) and cell density during the first passage of the same culture (bottom).



ARAMUS SINGLE-LAYER FLUOROPOLYMER ASSEMBLY FEATURES:

- Universal material chemical compatibility—resistant to solvents like DMSO, aggressive and highly concentrated chemicals
- Widest temperature operating range—fluoropolymer material maintains flexibility at -196°C
- Gamma-stable fluoropolymer film
- Built to be tough and 100% pressure tested, eliminating leaking welds and port fitments that can cause significant loss

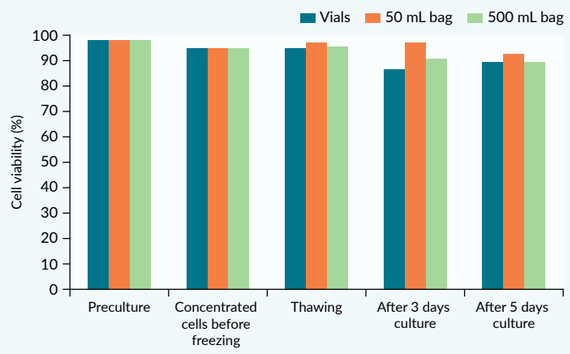
RECOVERY OF A HIGH-DENSITY CHINESE HAMSTER OVARIAN (CHO) CELL CULTURE

The recovery of a high-density CHO cell culture, post cryopreservation, was compared to alternative cryobags and 1 and 5 mL cryovial controls. The cells were frozen in liquid nitrogen for 2 weeks, then thawed and cultured for two passages up to 13 days. The results are shown in **Figure 1**. The Aramus bags demonstrated the quickest recovery among the different cryobags used and returned to a cell viability of >95%. In addition, the cells recovered from the Aramus bags showed a comparable viable cell density (VCD) to the vials and faster growth compared to alternative cryobags.

CELL VIABILITY AFTER THE FREEZE & THAW PROCESS

Viability of cells was measured before freezing and after thawing of the cells. Cultures were performed using glass vials or 50mL and 500mL cryobags. The results are described in **Figure 2**. Compared to glass vials, the fluoropolymer bags demonstrate comparable cell viability in the preculture as well as after thawing and culturing for 5 days.

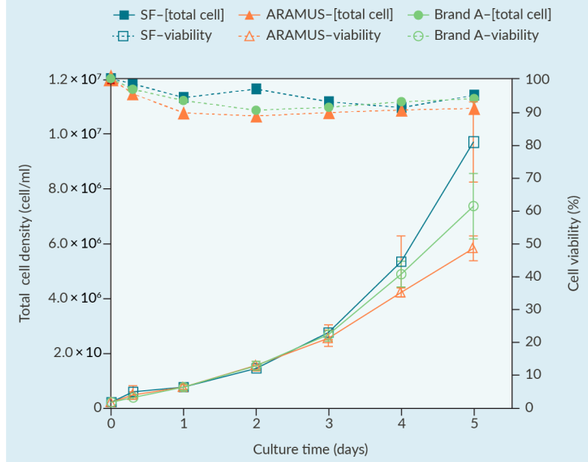
Figure 2. Cell viability during the various stages of the cell culture process (pre- and post-thawing).



CULTIVATION OF HEK293 CELLS

HEK293 cells were cultured for 5 days, and recovery was determined by measuring the total cell density and viability. Cell cultures were performed and compared between shake flasks (SF), Aramus- and brand A bags.

Figure 3. Cell density and cell viability in shake flask culture or cell culture performed using single-use bags.



The results are shown in **Figure 3** and demonstrate that the Aramus bags are suitable for cultivation of HEK293 cells.

CONCLUSION

Intensified HDCB using Aramus single-use cryobags can shorten cell culture times by weeks and reduce contamination risks by eliminating multiple manual cell transfer steps. It provides a wider window to start post-thaw seeding into the bioreactor, providing flexibility for scale-up production. Overall, the HCDB process leads to improved productivity, lower overall cost of goods, and better sustainability using fewer consumables.

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In partnership with: Entegris