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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

Induced pluripotent stem cells (iPSCs)

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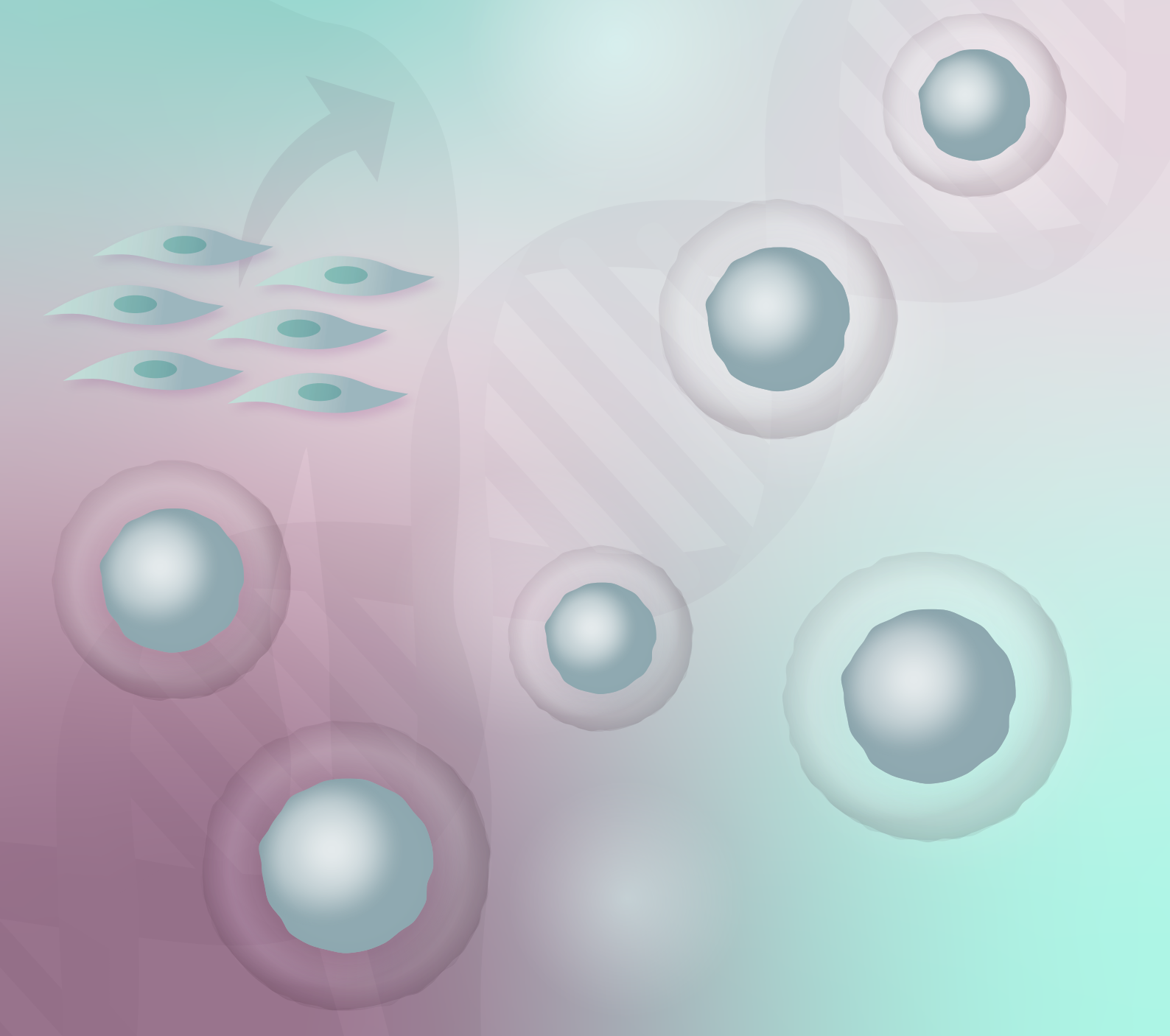

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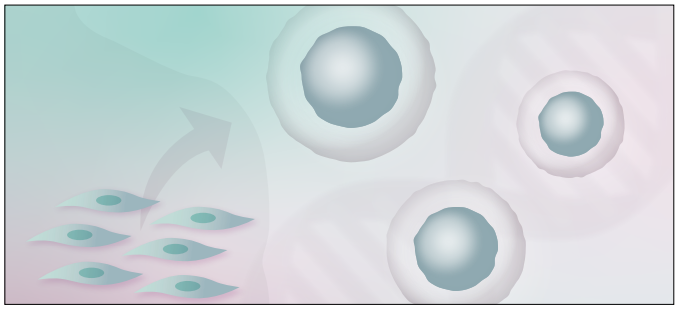

LATEST ARTICLES

CELL & GENE THERAPY INSIGHTS

INDUCED PLURIPOTENT STEM CELLS (iPSCS)

SPOTLIGHT

REVIEW

2D and 3D iPSC-CM models for studying cardiac toxicity and disease

Caleb W Heathershaw, Kiarash Shakeriastani, Olivia L Latham, Jacolby T Roddey, and Joshua T Maxwell

Advances in human induced pluripotent stem cell (iPSC) technology have enabled the generation of robust, cardiac-specific, *in vitro* systems for cardiac disease modeling, drug screening, and cardiotoxicity studies. Human iPSCs can be differentiated into iPSC-derived cardiac myocytes (iPSC-CMs), a model cell population able to recapitulate the phenotypes of complex cardiac diseases—mirroring both the observations made with *in vivo* animal models and the clinical manifestations seen in human patients. To improve the physiological relevance of iPSC-CM model systems, researchers are shifting from 2D to 3D models and incorporating a variety of cardiac specific cell types including cardiac fibroblasts, and vascular endothelial cells. In this paper, we will review the development of 2D and 3D iPSC-CM models, survey the various disease contexts in which these models have been utilized, and discuss the benefits and limitations of each strategy.

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THE DEVELOPMENT OF iPSC-CM MODEL SYSTEMS

Human iPSC technology has been established as one of the most significant advancements in recent medical science. At the same time, the ability to differentiate these cells into relevant cell types—like cardiac myocytes—has opened new avenues of cardiac research. Among all of the cell types differentiated from iPSCs, iPSC-derived cardiac myocytes (iPSC-CMs) are one of the most developed and well-characterized model systems. In 2D iPSC-CM culture, iPSCs are differentiated into a monolayer of cardiomyocytes CMs on a matrix-coated flat surface, allowing for easy observation and manipulation. In contrast to embryoid body formation protocols, efficient iPSC-CM differentiation can be driven by sequential treatment with growth factors that target Wnt signaling like activin A and BMP4 [1]. Cells can be easily removed from plasticware for purification of myocyte fractions or other downstream applications. Critically, the extensive progress made in iPSC technology not only circumvents the ethical concerns involved with

using human embryonic stem cells, but also enables the generation of patient-specific iPSCs [2]. The spontaneous, stable beating of iPSC-CMs makes them particularly valuable for *in vitro* cardiac disease models as well as long-term cardiotoxicity studies [3,4]. Moreover, patient-specific iPSC-CMs are crucial for developing reproducible cardiotoxicity assays, including high-content drug screening and population-level toxicology studies [5–7]. Ultimately, these cardiotoxicity assays provide platforms for personalized drug-screening and toxicity predictions and targeted drug development to reverse and/or mitigate drug-related injuries, as well as enable the correlation of human of human genetic information with cellular phenotypes [8].

ESTABLISHMENT OF 2D iPSC-CMS AS A CARDIOTOXICITY STANDARD

In addition, being used for basic research that mechanistically explores cardiac disease, iPSC-CMs have been established as the benchmark for preclinical *in vitro* cardiotoxicity testing through the initiation of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) program, a standardized assay for evaluating the proarrhythmic potential of drugs [7]. The CiPA assay quantifies the effects of drugs on crucial ventricular ion channels and overall myocyte function [7]. Recent use cases of CiPA include evaluations of antimalarial drugs repurposed for COVID-19 treatment and evaluations of novel anti-seizure medications. To examine if artesunate and pyronaridine (antimalarial drugs) could cause cardiac arrhythmias, researchers measured action potential disruption and checked for the production of torsades de pointes, a fatal heart arrhythmia. The researchers found that the repurposed drugs have low levels of arrhythmogenic potential [9]. Similarly, levetiracetam, an anti-seizure medication, underwent the same process to check for proarrhythmic behavior and QT prolongation. Levetiracetam was also found to not promote proarrhythmic heart function [10].

3D iPSC-CM MODELS IMPROVE PHYSIOLOGICAL RELEVANCE

3D culture models have improved drug discovery and disease modeling by mimicking native microenvironments more accurately than conventional 2D models, potentially improving the preclinical predictive power of iPSC-CM systems [11]. When suspended in 3D, cells form an extracellular matrix (ECM) and self-organize into structures that increase cell–cell and cell–ECM interactions, while also allowing for the formation of macrostructures including chambers and capillary-like vasculature [12,13]. IPSC-CMs have been integrated into multiple types of 3D structures. In broad terms, these structures fall into two categories: self-organizing culture and scaffolded culture. Self-organizing structures include spheroids and organoids, while scaffolded constructs include microtissues and engineered heart tissues (EHTs). Both self-organizing cultures and scaffolded constructs have been employed in another type of culture system: microfluidic devices known as hearts-on-chips. The following paragraphs will survey these systems, their fabrication, and their impacts on physiological relevance.

Spheroids

Spheroids are scaffold-free 3D models that incorporate iPSC-CMs in a self-adherent, suspended, culture [14]. Their capacity to replicate physiological function of tissues *in vivo* makes them a particularly valuable tool in disease modeling as they integrate metabolic, genotypic, and pharmacological input into functional output in the form of spheroid contraction [15]. Spheroids can be created using multiple types of scaffold-free methods including hanging drop

culture and static suspension culture [16]. These methods differ in terms of their benefits and limitations, including process difficulty, size control, uniformity, scalability for mass production, and labor requirements [17]. The hanging drop method is an affordable approach for generating spheroids with consistent size and shape. In this technique, a small droplet of cell suspension is placed on the underside of a culture dish lid where gravity facilitates cell aggregation [18]. Growing iPSC-CMs in this method enables the cells to form spheroids without interacting with a solid substrate [19]. Gravity-induced cell settling, cell–cell or cell–ECM interactions, and subsequent growth contribute to structural integrity of spheroids [20,21]. In static suspension culture, cellular adhesion is inhibited by coating U-bottomed wells with non-adherent, hydrophilic, often proprietary substrates that force the cells into suspension [16,22]. This method requires less hands-on technical management of cells, but does require more expensive plasticware.

Organoids

Human cardiac organoids (COs) are 3D miniature organ-like *in vitro* models used to study the heart's biochemistry, physiology, and pharmacology. Although spheroids and organoids are quite similar, constructs are considered organoids if they mimic the structural features or intracellular interactions of the myocardium [23]. COs are often created by co-culturing iPSC-CMs with other cells found in the myocardium like endothelial cells (ECs), fibroblasts (FBs), or smooth muscle cells (SMCs) [6]. Cardiac organoids not only can replicate the human heart's physiological conditions, but they can also be constructed to mimic the layered structure, vasculature, or even the chambers of the heart, making them a potential model for evaluating diseases that impact the structural features of the heart on the micro scale [24–27].

The signaling pathways that drive ultrastructural development can only be modeled in 3D. Development of multi-chambered cardiac organoids require 3D environments and Wnt modulation for self-directed assembly (although note that these cardiac myocytes were derived from embryonic stem cells, not iPSCs) [28]. Modulation of Wnt signaling in a cardiac organoid resulted in development of chambers that were bounded by endothelial cells [29]. Other developmental pathways, like NRP2 cardiogenesis signaling, have been modeled in 3D cardiac epicardial organoids [30].

Microtissues

Although spheroids, organoids, and microtissues are terms used often interchangeably, microtissues can be said to incorporate multiple cell types within a scaffold. Scaffolded approaches can utilize a myriad of components, such as hydrogels, biofilms, or even magnetic particles [16]. Hydrogels, extensively used as cell scaffolds in matrix-based 3D cultures, improve cell-matrix interactions by embedding cells within a structure, crucial for processes including cell adhesion, proliferation, differentiation, gene expression [31]. Scaffolds can also be composed of ECM components. For example, Matrigel® is an ECM-based scaffold composed of laminin (60%), collagen IV (30%), entactin/nidogen, perlecan, heparin sulfate and growth factors [32,33]. This dissolved basement medium allows the organoids to generate complex structures that mimic human tissues [18,34].

Microtissues cultures promote maturation through cell–cell interaction. An extensive study by Giacomelli *et al.* found multiple aspects of increased maturity in spheroid microtissue models that incorporated ECs and FBs. Transmission electron microscopy revealed enlarged mitochondria (indicative of mature energy consumption), organized sarcomeres with Z-lines,

I-bands, H-zones, and caveolae lipid transport sacs. Additionally, transcriptomic comparison between 2D iPSC-CMs, iPSC-CM spheroids, and human adult and fetal heart samples suggested that the model maturation reflected native tissue genes regulating ion channels, sarcomeric proteins, and other genes. Additionally, patch-clamp electrophysiology showed faster transient repolarization and increased action potential amplitude, demonstrating that these transcriptomic changes resulted in mature functionality [35].

Engineered heart tissues

Engineered heart tissues (EHTs) are 3D cultures that integrate cells into a mechanically ordered environment that modulates mechanical stress, fluid pressure, or electrical stimulation. For example, in one engineered heart tissue model, iPSC-CMs were seeded into interlayered 3D printed polycaprolactone channels that reflected the alignment patterns of native cardiac muscle. These cells were found to have phenotypically mature calcium handling, ion channel related gene expression, and sarcomeric structure [36]. In a more dynamic engineered heart tissue model, researchers were able to mimic the mechanical forces of preload (filling stretch) and afterload (ejection pressure), leading to more aligned myocytes and improved contractility. This model was critical for evaluating diseases whose phenotypes progress due to mechanical stimuli, as in the case of desmosome-linked diseases. They found that phenotype of a patient iPSC-CM desmoplakin-driven arrhythmia was only recapitulated in the mechanically stimulated engineered heart tissue, not in 2D or static 3D systems [37].

Hearts-on-chips

Hearts-on-chips models incorporate either unscaffolded or scaffolded 3D cultures into microfluidic systems that allow for continuous mechanical stimulation, continuous noninvasive observation, and instantaneous electrophysiological feedback. For example, a scaffolded heart-on-chips model incorporated iPSC-CMs and fibroblasts into a fibrin hydrogel suspended between pulsatile walls, mechanically trained by 1 Hz uniaxial strain. This system included electrophysiology probes for measuring electrical activity directly, allowing researchers to use the model for accurately predicting QT-alterations and evaluating pro-arrhythmic drugs [38].

SUPPORTIVE CELLS IMPROVE PHYSIOLOGICAL RELEVANCE

The integration of iPSC-CM models with non-myocyte support cells provides an opportunity for critical cell-cell interactions and maturation of the myocytes, improving the overall physiological relevance of iPSC-CM models. The specific ratio of cells used in culture varies but typically reflects the proportion of cells found in the heart [15]. The next paragraphs will walk through these support cell types and how they have improved cardiovascular modeling.

Fibroblasts

Fibroblasts are known to regulate the fibrosis state of the heart, including through the p38-MAPK pathway and TGF-β1 activation [39]. Investigation of these pathways in iPSC-CM 3D coculture aids in modeling collagen deposition and fibrosis *in vitro*, as stimulated by TGF-β1 and treated with anti-fibrotic drugs [40]. Additional cell–cell interactions between iPSC-CMs and cardiac fibroblasts were found to improve maturation through increased connexin 43 gap junctions and altered cyclic AMP cascades [35]. Interestingly, deriving fibroblasts from arrhythmogenic cardiomyopathy patients

was sufficient to recapitulate arrhythmogenic features, even when the iPSC-CMs were derived from healthy donors [35]. Fibroblasts can be transdifferentiated into myofibroblasts by addition of TGF-β1 in models of cardiac fibrosis. Fibroblasts in 3D models can be differentiated from iPSCs, ESCs, transdifferentiated from MSCs and epicardial cells, or expanded from primary cell sources [41–43].

Epicardial cells

Epicardial cells have been included in a cardiac spheroid model as a supportive cell, in addition to their use as a cardiac fibroblast precursor. Their inclusion promoted maturation in the model, but, as this model also included fibroblasts, the independent effect of epicardial cells remains unclear [42]. Epicardial development from iPSCs has also been explored as a method for better modeling the pathogenesis of congenital hypertrophy and fibrotic remodeling [30].

Endothelial cells

Endothelial cells are known to regulate the inflammation, vascularization, and redox states of 3D IPSC-CM models. Endothelial cells have been found to line microvascular networks within cardiac spheroids, reflective of human heart tissue [44]. In the same study, endothelial nitric oxide synthase knock out within endothelial cells was found to reduce doxorubicin induced cell death, demonstrating the efficacy of endothelial cell modification in treating iPSC-CM injury models. In an ischemia-reperfusion injury (IRI) heart-on-chip model, extracellular vesicles isolated from endothelial cells were found to alleviate cell death, increase CM respiratory capacity, and reduce losses in contraction capacity [45]. Also, endothelial cells can form a permeable barrier which mediates cardiotoxicity as seen in multiple heart-on-chip microfluidic studies [46–48].

Smooth muscle cells

Smooth muscles cells, in conjunction with endothelial cells and fibroblasts, were found to increase spheroid size, prevent apoptosis, improve sarcomere maturation, and affect cardiac myocyte bioenergetics in a cardiac spheroid model [49]. However, the effects of smooth muscle cells specifically on cardiomyocytes have not yet been shown in a 3D model. Transplantation of iPSC-derived smooth muscle cells monoculture spheroids into ischemic limb injured mice have been shown to improve perfusion and arterial density [50].

Macrophages

Macrophages are known to function in immune and developmental roles within the heart. Currently, there are few studies on macrophage interaction with 3D iPSC-CM models. One study using human embryonic stem cell-derived cardiac microtissues found that $LYVE1^{+}$ hESC-derived macrophages induced sarcomeric protein maturation, reduced microtissue apoptotic stress, and enriched efferocytic genes. This model indicates that macrophages provide benefits in early cardiac maturation and trigger macrophage specification, key steps in the development of the human heart [51]. The effects of mature polarized macrophages in 3D models have not yet been explored.

Mesenchymal stem cells

Mesenchymal stem cells have been included in 3D spheroid models of cardiac fibrosis. MSCs improved CM maturation and generated fibroblasts that became profibrotic myofibroblasts in response to TGF-β1 [41]. MSCs have also been proposed as a therapeutic agent for various cardiovascular diseases, including ischemia-reperfusion injury. MSC-derived exosomes were found to decrease cell death and improve function

in ESC-derived chambered cardiac organoids that were exposed to ischemic conditions [52].

DISEASES STUDIED IN iPSC-CM MODELS

Modeling of genetic cardiac disease

Over the last decade, the development and use of genetic engineering technologies in conjunction with 2D human iPSC-based disease modeling has led to more in-depth investigations of a wide variety of complex genetic cardiac diseases. In particular, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system [53] can be utilized to remove disease-specific mutations from iPSC-derived cardiac myocytes (iPSC-CMs), thereby creating isogenic variants that enable the in-depth analysis of genotype-phenotype associations with cardiac ion channelopathies [54,55]. Furthermore, comparative studies utilizing these genetically engineered iPSC-CMs provides critical insight into the molecular mechanisms underlying the pathogenesis of disease-specific mutants in a controlled environment [56]. iPSC-CMs are uniquely capable of recapitulating patient-specific complex cardiac genetic diseases, mirroring observations in both *in vivo* animal models and human patients, and ultimately facilitating the development of novel therapies [57]. In the next sections, we will highlight several studies regarding iPSC-based disease modeling for genetic arrhythmias and cardiomyopathies.

Long QT syndrome

Long QT syndrome (LQTS), a genetic cardiac arrhythmia affecting 1 in 1000 births, arises from mutations in genes encoding proteins crucial for voltage-gated ion channel function in the heart [58]. Mutations in these genes cause channelopathies that disrupt ion conductance and action potential (AP) propagation in cardiac cells, ultimately prolonging ventricular repolarization and producing the characteristic 'long' QT interval seen on an electrocardiogram [58]. Although this disorder initially presents with sinus bradycardia, it can worsen and cause life-threatening ventricular fibrillation (torsades de pointes), cardiac arrest, or sudden cardiac death (SCD) if left undiagnosed and untreated [58]. Over 500 mutations have been linked to LQTS, but a majority of cases are caused by mutations occurring in the *KCNQ1*, *KCNH2*, and *SCN5A* genes, which encode the pore-forming α-subunits of cardiac voltage-gated potassium (K^+) and sodium (Na^+) ion channels, and are associated with the LQTS1, LQTS2, and LQTS3 variants, respectively [59]. *KCNQ1* and *KCNH2* mutations disrupt the slow and rapid delayed rectifier K^+ currents (I_{Ks} and I_{Kr}), which are the key outward K^+ currents responsible for the repolarization and prolonged depolarization phases of ventricular APs [59]. On the other hand, *SCN5A* mutations diminish the rapid, inward Na^+ current (I_{Na}) that defines the upstroke velocity of the ventricular AP [59].

iPSC-CM models have emerged as valuable tools for studying LQTS, particularly focusing on the LQTS1, LQTS2, and LQTS3 variants [54,55,60]. iPSC-CM models with *KCNQ1* mutations have demonstrated a decrease in the number of functional KCNQ1 channels, resulting in diminished I_{Ks} current [61–63] and abnormal electrophysiological properties, such as prolonged AP duration (APD) [64] in LQTS1 iPSC-CMs. Similarly, LQTS2 iPSC-CMs with *KCNH2* mutations [65–68] and LQTS3 iPSC-CMs with gain-of-function *SCN5A* mutations [69–72] also displayed increased APD due to impaired K^+ channel function and reduced I_{Kr} current. Notably, both LQTS1 and LQTS2 models showed disrupted outward K^+ currents and abnormal calcium (Ca^{2+}) handling, which can be reversed by Ca^{2+} channel antagonists [71,73]. However,

the overlap of these genetic mutations with other conditions, such as Jervell and Lange-Nielson and Brugada syndromes, presents challenges in using patient-specific iPSC-CMs for precise disease modeling of LQTS.

Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is genetic cardiac disease characterized by stress-induced ventricular arrhythmias, particularly ventricular tachycardia or fibrillation [74]. CPVT affects approximately 1 in 10,000 people and it is estimated to cause about 15% of all SCDs in affected young people [75]. There are two main types of CPVT, both of which are caused by pathogenic variants in genes encoding essential Ca^{2+} handling proteins in cardiac myocytes. CPVT1 is caused by mutations in the ryanodine receptor type 2 gene (*RYR2*), which encodes a Ca^{2+} channel that controls Ca^{2+} release from the sarcoplasmic reticulum (SR) during ventricular systole [75]. CPVT2 results from mutations in the calsequestrin-2 gene (*CASQ2*), which encodes a sarcoplasmic Ca^{2+}-binding protein involved in Ca^{2+} handling during myocardial excitation–contraction coupling [73]. Identification of *RYR2* and *CASQ2* mutations in CPVT patients highlights the consequential role that intracellular Ca^{2+} dysregulation plays in cardiac myocytes.

iPSC-CM models have been utilized to investigate several CPVT-associated genes, including *RYR2* and *CASQ2* [76–79]. These CPVT iPSC-CM models effectively recapitulate the stress-induced arrhythmic phenotype observed in patients, allowing arrhythmias to be induced by stressors such as adrenergic agonists and pacing [80–83]. In both CPVT1 and CPVT2 iPSC-CM models, pacing has been shown to induce delayed afterdepolarizations (DADs), resulting in a negative inotropic response in the heart [78,80–82]. It has been hypothesized that the aberrant effects of adrenergic stimulation in CPVT are caused by altered CaMKII signaling [84]. Several studies have investigated the functional abnormalities in CPVT1 and CPVT2 iPSC-CMs, however more research is needed to fully elucidate the mechanistic basis of these abnormalities, especially irregular Ca^{2+} signaling [67,79,83]. Other studies have also indicated that iPSC-CM models can be effectively utilized for patient-specific drug screening [85–87]. Although the use of iPSC-CMs for *in vitro* CPVT models has been controversial due to the immature phenotype often displayed by CPVT iPSC-CMs compared to controls, several studies have validated this approach by demonstrating improved outcomes following treatment with dantrolene (a non-specific RyR antagonist), accurately reflecting clinical responses observed in CPVT patients [88,89].

Dilated cardiomyopathy

Dilated cardiomyopathies (DCMs) are a heterogeneous group of non-ischemic myocardial diseases that produce functional and structural abnormalities in the heart [54]. Clinically, DCM presents with left ventricular dilation and impaired systolic function, which can eventually lead to heart failure [90]. DCM can be idiopathic or caused by many different factors such as genetic mutations, metabolic and autoimmune disorders, infectious diseases, environmental toxins [90]. Despite these diverse etiologies, DCM is the most common type of cardiomyopathy, affecting approximately 1 in 250 individuals [54,55]. Familial DCM (FDC) is most commonly associated with autosomal dominant inheritance of pathogenic variants in over 20 genes encoding proteins that are crucial for proper Ca^{2+} handling, sarcomere structure and function, and cytoskeletal organization [91]. Approximately 15–20% of DCM cases are caused by truncating mutations in the titin gene (*TTN*), which encodes a sarcomeric protein that provides structural stability,

helps sense mechanical stress, and facilitates force transmission in cardiac myocytes [92,93]. Although not as common, mutations in lamin A/C gene (*LMNA*) gene (which encodes the nuclear lamina structural proteins, lamin A and lamin C) are associated with one of the most malignant types of DCM distinguished by early-onset conduction delay that contributes to sinoatrial and atrioventricular node dysfunction and atrial fibrillation, which can lead to ventricular arrhythmias and SCD [91,94]. Other implicated FDC-related genes encode key Ca^{2+} handling and regulatory contractile proteins such as myosin heavy chain-β (*MYH7*), cardiac troponin T (*TNNT2*), cardiac myosin-binding protein C (*MYBPC3*), and phospholamban (*PLN*) [90].

Utilizing patient-specific iPSC-CMs to generate *in vitro* models of DCM has enabled researchers to accurately recapitulate the contractile dysfunction typically seen in DCM patients [95,96]. DCM iPSC-CMs serve as an investigative tool to develop novel therapeutic strategies, as well as provide insight into the molecular pathogenesis of specific mutations in *LMNA* [94,97–99], *TNNT2* [100–102], *PLN* [103], *MYH7* [104], and *TTN* [105,106]. Many of these studies have reported sarcomere disorganization, decreased contractile force, and impaired Ca^{2+} handling, all of which correspond with the clinical presentation of DCM-induced heart failure [107]. Furthermore, iPSC-CM models have demonstrated that β-adrenergic blockers and Ca^{2+} antagonists can attenuate the DCM phenotype seen *in vivo*, including increased apoptosis and sarcomere disorganization [101,108]. Notably, targeted gene correction in pathogenic iPSC-CMs has successfully reversed the *in vitro* disease phenotype [103,109].

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM), a myocardial disease affecting approximately 1 in 500 people [110], can be characterized by left ventricular and/or septal hypertrophy without a causative hemodynamic burden [54]. The clinical manifestations of HCM are highly variable, but often involve an increased risk of atrial fibrillation and left ventricular systolic dysfunction that can lead to reduced cardiac output and SCD, particularly in young adults [111]. While over 1400 mutations are linked to HCM, most are found in genes encoding cardiac sarcomere components, with pathogenic variants in *MYH7* and *MYBPC3* accounting for approximately 80% of clinical HCM cases [111,112].

Patient-specific iPSC-CM models have been shown to effectively recapitulate several pathognomonic phenotypes observed in patients with familial HCM, including abnormal Ca^{2+} handling, sarcomere disarray, cardiac myocyte hypertrophy, hypercontractility, and arrhythmias [113–116]. HCM iPSC-CM models have also elucidated changes in signaling pathways resulting from these genetic mutations, including the endothelin-1 [117], canonical Wnt [114], and calcineurin/NFATc4 pathways [114,115,117]. Specifically, nuclear localization of NFATc4 and irregular Ca^{2+} handling were identified by the aforementioned studies as determinants of cardiac myocyte hypertrophy. Using CRISPR/Cas9 technology, researchers have been able to generate isogenic lines of iPSC-CMs with pathogenic variants in *MYH7* [114,115] and *MYBPC3* [116–118] to investigate the pathogenic mechanisms of HCM. Moreover, these studies reported that mutant iPSC-CMs were in a hypercontractile state due to increased tension and delayed relaxation kinetics. Other studies have utilized HCM iPSC-CM models to gain insight into mutation-specific arrhythmogenic events associated with specific electrophysiological abnormalities. For example, some mutations are primarily known to elicit DADs [115], while others produced early afterdepolarizations [116], or no DADs at all [114].

Of note, conflicting results were found across studies in familial HCM, which may

be cause for concern. For example, some studies reported decreased expression of RyR2 and sarcoplasmic reticulum calcium-ATPase 2a (SERCA2a) (the primary Ca^{2+} channels responsible for release of SR Ca^{2+} stores and subsequent active reuptake of cytosolic Ca^{2+} back into the SR, respectively) [114]; while others report increased levels of these proteins [116]. While the underlying causes of these heterogeneities remain unclear, they may be attributable to the relative immaturity of iPSC-CMs compared to native cardiac myocytes [119], as well as discrepancies in study logistics, such as measurement time points.

Modeling of cardiotoxicity and environmentally induced cardiac disease

In this section, we will turn our focus to cardiac toxicity studies and environmentally induced cardiac disease models. Incubation of iPSC-CMs with various concentrations of drugs and compounds or incubation in the presence of environmental stress (such as hypoxia) has proven to be a key experimental technique for assessment of cardiac injury and toxicity. Similar to genetic disease modeling, non-genetic disease and toxicity modeling have become useful tools for identification of disease mechanisms, toxicity profiles, and therapeutic targets.

Cardiotoxicity

Chemotherapy drugs, like carfilzomib and melphan, are known to have adverse health effects after prolonged periods of treatment, including cardiotoxicity [120]. Using iPSC-CMs, researchers have attempted to decipher the mechanisms of this cardiotoxicity. Carfilzomib caused structural, morphological, and functional changes in the heart, significantly affecting Ca^{2+} handling at the single myocyte level, leading to an overall dysregulation in overall contractile properties of the heart. Contractile dysfunction is accompanied by significant down regulation in genes that account for contractile mechanisms, integrin pathways, and extracellular matrices, followed by mitochondrial disturbance and oxidative stress [121]. Melphan produced similar responses on the disruption of calcium handling, oxidative stress measurements, along with the interruption of P53 enzymes [122].

Doxorubicin is an anthracycline chemotherapeutic drug used to treat a wide range of cancer types due to its tumor suppressant abilities [123]. However, many anthracyclines used have been linked to severe cardiotoxicity [124]. Specifically, Doxorubicin cardiac toxicity is characterized by a sharp increase in oxidative and nitrosative stress. The release of reactive oxidative and nitrogen species has acute and chronic implications that often lead to heart failure [125]. This is also paired with a calcium flux that causes dysregulation in cardiac myocyte contractile properties and a lowered ATP output [126]. iPSC-CMs have been used as *in vivo* models to confirm mechanisms of doxorubicin and to understand patient differences in toxicity [127]. Researchers generated patient-specific iPSC-CMs which expressed variable responses to doxorubicin. In engineered heart tissues, not only did patient-derived iPSC-CMs reflect clinical function, but also the pharmacokinetic profile of doxorubicin [128]. The variations in cardiotoxicity aligned with familial clinical results. These findings supported patient specific iPSC-CMs as a reliable method for evaluating possible patient to patient differences in cardiotoxic response [127].

Hypoxia (myocardial infarction and ischemia reperfusion injury)

Ischemic heart disease is the leading cause of death in the USA [129]. One of the deadliest expressions of ischemic heart disease is a myocardial infarction (MI). In MI, a coronary or microvascular blockage causes

permanent damage to the heart muscle due to inadequate oxygen supply, resulting in redox imbalance, myocardial cell death, and scarring. Tissue reperfusion is employed clinically to prevent further ischemic injury; however, the reperfusion of oxygen causes a secondary injury known as IRI, characterized by oxidative stress, mitochondrial dysregulation, apoptosis, and ultimately, cardiovascular complications. iPSC-CMs are well suited as a model system for mimicking ischemia reperfusion injuries. When exposed to prolonged hypoxia and reoxygenation, iPSC-CMs undergo a reduction in beat frequency and an unordinary alignment of sarcomeres, as well as increase expression of clinical biomarkers of IRI like troponin T, lactate, HIF1α, and MyBPC3 [130]. Additionally, apoptosis and an influx of reactive oxygen species-driven injuries developed [131]. However, these studies were conducted in 2D with iPSCs supported by mouse embryonic feeder cells. In more recent 3D models, similar changes have been observed including changes in contractile function, mRNA expression, apoptosis, and ROS [132]. Unlike in 2D studies however, the creation of a 3D hypoxic gradient is possible, allowing for probing of different zones analogous to infarct zones [133]. These results validate iPSC-CMs as clinically relevant models for modeling MI and as a tool for evaluating potential therapeutics in myocardial repair.

As shown in Table 1, many diseases have been evaluated in 3D iPSC-CM models [29,30,35–41,43–45,47,48,106,132–166]. These studies represent all current 3D iPSC-CM disease model systems, including the systems discussed in previous paragraphs. However, the list of compounds evaluated for cardiotoxicity is non exhaustive. Many of these studies have also been carried out in 2D, however, with the advent of 3D cell culture models the complexity and thus the relevance of the outcomes have been re-examined in 3D cultures.

THE LIMITATIONS AND BENEFITS OF 2D AND 3D iPSC-CMS

Patient-derived iPSC-CMs are attractive candidates for use in 2D systems because they are readily available, easily obtained with minimally invasive techniques, and can be cultured *in vitro* for extended periods of time (weeks to months) [167]. These characteristics sharply contrast with the rapid dedifferentiation and decreased viability of isolated adult ventricular myocytes in culture [54]. iPSC-CMs respond to drugs in a similar manner as native cardiac myocytes, making them extremely relevant for personalized drug screening [4]. iPSC-CMs are also able to recapitulate complex, patient-specific phenotypes of various cardiac diseases, mirroring observations seen in both *in vivo* models and in patients within clinical settings [56]. Lastly, iPSC-CMs can be used lieu of adult ventricular cardiac myocytes (isolated from human or non-human animal sources) to form architecturally-complex cardiac structures that mimic the *in vivo* cardiac tissue microenvironment, potentially reducing the need for pre-clinical animal studies [168].

Although there are many benefits associated with using iPSC-CMs for cardiac disease modeling, drug efficacy testing, and cardiotoxicity panels, there are also several limitations. In particular, iPSC-CMs display an immature, fetal-like phenotype in comparison to adult ventricular myocytes which limits their use [119]. This 'immaturity' is characterized by a fetal gene expression profile, disorganized sarcomere structure and function, a partially depolarized resting membrane potential, and spontaneous, asynchronous beating—a feature absent in adult atrial and ventricular cardiac myocytes [169]. In addition, iPSC-CMs persist in a fetal metabolic state that favors glucose over fatty acid utilization for energy production. Therefore, metabolic changes in iPSC-CMs are often undetectable under steady-state conditions, limiting their use

TABLE 1

Diseases studied in 3D iPSC-CM models.

Disease or injury	Model systems used	Key findings	References
Cardiotoxicity (doxorubicin, verapamil, quinidine, terfenadine, fexofenadine, isoproterenol, isoprenaline, propranolol, atorvastatin, valproic acid, acetaminophen, norepeniphrine, bisphenols, microplastics, etc.)	Spheroid, organoid microfluidic system, engineered heart tissue	EHTs reflected human doxorubicin-induced pharmacokinetics, structural phenotypes, and miRNA expression; microplastics induced oxidative stress, apoptosis, and collagen accumulation in organoids	[29,38,44,47,134–147]
Myocardial infarction and ischemia reperfusion injury	Spheroid, organoid, microfluidic system	Spheroids recapitulated *in vivo* MI-induced changes in contractile function and mRNA expression	[36,45,132,133,148–151]
Cardiac fibrosis	Spheroid, engineered heart tissue, microfluidic system	Heart-on-chip exposed to TGFβ reproduce collagen, BNP, and tensile phenotypes; mechanical compaction of EHT reflected fibrotic myocardium	[39–41,152]
Cardiac hypertrophy	Organoid, engineered heart tissue	Epicardiods mimic signaling changes of congenital and stress-induced hypertrophy; electrically and mechanically conditioned EHT reflects patient-specific LV hypertrophy and drug response	[30,153,154]
Duchenne muscular dystrophy (DMD)	Engineered heart tissue, organoid	Patient-specific COs recapitulate DMD phenotype and RNA expression	[155–158]
Dilated cardiomyopathy	Microtissue	Microtissues linked titin mutations to dilated cardiomyopathy	[106]
Hypertrophic cardiomyopathy	Engineered heart tissue	Patient-specific EHTs recapitulated mutant hypertrophic phenotype	[159–161]
Arrhythmogenic cardiomyopathy	Microtissue, engineered heart tissue	Microtissues with healthy iPSC-CMs, but mutant patient fibrobroblasts recapitulated arrhythmogenesis	[35,37]
Pregestational diabetes-induced congenital heart defect	Organoid	Organoids in diabetes-like conditions reflected clinical scRNA, ROS, and lipidomics profiles	[29,162]
Hypertensive disorders of pregnancy	Spheroid	Spheroids exposed to patient plasma induced changes indicative of preeclampsia	[163]
SARS-CoV-2 myocarditis	Organoid	Organoids infected with SARS-CoV-2 reflect myocarditis injury	[164]
Cardiovascular complications of rheumatoid arthritis (RA)	Microtissue	Patient-specific microtissues developed inflammation and capillary structures seen in RA	[43]
Inflammation (TNFα)	Microfluidic system	Heart-on-chip stimulated with TNFα reflected inflammatory electrophysiological changes	[48]
Cryoinjury	Organoid	Immature organoids regenerated after cryoinjury without fibrosis or hypertrophy	[165]
Spaceflight and microgravity	Spheroid	Spaceflight altered spheroid gene expression and protein levels linked with metabolism and cell survival	[166]

to study metabolically-driven cardiac diseases [53]. Morphologically, these immature iPSC-CMs resemble smaller, round, uninucleate fetal cardiac myocytes [4]. They also do not fully engraft or integrate electromechanically into host myocardium, potentially altering native myocardial architecture and leading to functional disruptions like post-engraftment electrical instability and arrhythmogenicity [169]. While useful for characterizing cellular functions, traditional 2D *in vitro* cell culture models lack the native physiological microenvironment (including cell–cell and cell–matrix interactions and intra- and inter-cellular signaling), thus, failing to reflect the complexity of *in vivo* tissue organization [170]. Therefore, 2D iPSC-CM models may be less physiologically relevant than *in vivo* or 3D models like cardiac spheroids and organoids.

While classic cell culture forces cells to spread longitudinally across flat surfaces like flasks and dishes, in 3D cultures cells interact in all directions as *in vivo*, bridging the gap between 2D culture and native tissue and fostering a deeper understanding of cardiac biology [171]. Critically, both scaffolded and unscaffolded 3D iPSC-CM models promote myocyte maturation though interactions with other myocytes, supportive cells, ECM components, mechanical forces, or even electrical stimulation [35,144,172,173]. Multiple key hallmarks of mature cardiac myocytes have been seen across 3D systems, including improvements in calcium handling, sarcomere organization, transcriptomic changes, and changes in cardiac action potential [36,174,175]. In addition to the more mature phenotype expressed in 3D iPSC-CM models, 3D models allow for the study of disease that impact structural features of the heart like vascularization [176,177] and fibrosis [40,158].

While 3D iPSC-CM models address some limitations of 2D models, 3D models face a few unique challenges. Critically, oxygen can only sufficiently diffuse 200 μm deep into tissues, meaning that larger organoids can develop hypoxic necrotic regions [176]. This can lead to uneven contractility or fibrotic remodeling of cardiac constructs. However, this disadvantage can be utilized in the study of hypoxia-driven disease. For example, cardiac organoids inherently develop an oxygen diffusion gradient that reflects the gradient found in the native heart after myocardial infarction. In organoids, this gradient can recapitulate key aspects of myocardial infarction including fibrotic remodeling and impaired calcium handling [133]. Because of their size (50–500 μm in diameter), 3D iPSC-CM models also can be used to generate gradients of cytokines, signaling molecules [178]. Additionally, 3D cultures that incorporate multiple cell types complicate the study of cell-specific analysis, although analysis of integrated function is improved [179]. The final limitation of 3D iPSC-CM models is universal to all *in vitro* research: cells do not recapitulate all structural aspects of native physiology, particularly ultrastructural features and variation in cell type distribution across tissues. However, by integrating support cell types into 3D iPSC-CM models the field is able to model aspects of human heart disease in human cells, thereby boosting physiological relevance, bridging preclinical gaps, and bringing patients closer to safe, effective treatments for heart disease.

TRANSLATIONAL INSIGHT

Induced pluripotent stem cell-derived cardiac myocytes (iPSC-CMs) hold tremendous promise for revolutionizing cardiovascular research and therapy. These cells possess the remarkable ability to differentiate into various cardiac cell types, including cardiac myocytes. This unique characteristic makes them invaluable tools for studying heart development, disease modeling, drug discovery, and regenerative medicine. Advancements in the utilization

of iPSC-CMs and other cardiac cell types have yielded 2D and 3D iPSC-CM models for *in vitro* use. Although both offer unique advantages for cardiovascular research, ultimately the choice between 2D and 3D models depends on the specific research question and the desired level of physiological relevance needed for the study. 2D models are valuable for high-throughput screening and studying basic cellular mechanisms, while 3D models provide a more physiologically relevant environment for disease modeling and drug discovery. As iPSC-CM technology continues to advance, these models will play an increasingly important role in advancing our understanding of heart disease and developing new therapies. Furthermore, the recent elimination of the FDA mandate for preclinical animal testing suggests an inevitable shift toward decreasing reliance on animal studies. The 3D models such as spheroids and organoids described here are moving into the mainstream with their ability to recapitulate the complex structure and multicellular organization of human organs alone or in combination with other organ systems.

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CELL & GENE THERAPY INSIGHTS

INDUCED PLURIPOTENT STEM CELLS (iPSCS)

SPOTLIGHT

COMMENTARY

Choosing the best path to patients: how to decide between in-house and outsourced iPSC therapy development and manufacturing

Laura Koivusalo, Anni Mörö, and Ross Macdonald

Induced pluripotent stem cells (iPSCs) offer the potential of creating life-changing regenerative medicine products for many current unmet needs. To ensure iPSC-based therapies have the possibility to reach their breakthroughs potential, we as developers should ensure that they actually can be accessible to patients—and that means considering also the market demands of the over-burdened healthcare systems across the world. In this article, we offer some viewpoints and considerations around the accessibility of therapies from the cost perspective, debating the alternative models of development and manufacturing of iPSC-based therapies.

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Induced pluripotent stem cells (iPSCs) are, by definition, capable of producing any cell type in the human body—and as such they are regarded as the ideal starting material for life-changing regenerative medicine products. However, if iPSC therapy developers want to ensure that patients can one day benefit from their innovations, simply navigating the emerging regulatory pathway alone to market approval is not enough. To ensure patient access, developers should keep the end in mind—ensuring that these revolutionary treatments can also be manufactured at reasonable cost and relevant scale.

The potential for one-time curative treatments challenges the current healthcare paradigm, which is built to support pill-per-day medications. Most cell and gene therapies are clearly justified by their enormous economic and social benefits over existing treatments. Think for example, a curative treatment for type 1 diabetes over life-long supply of insulin. Still, the high price of cell and gene therapies continues to scare the European healthcare system, even leading to products being completely removed from the European market due to lack of reimbursement (e.g., Zynteglo™) [1,2].

But why do developers need to fetch million-dollar price tags to make their products profitable? Complex and/or bespoke manufacturing processes leading to extremely high cost-of-goods are often offered as the likely culprit. Also, the entire venture-backed biotech business model is built around high return on investment—which thrives on creating blockbuster medicines. With the slow market uptake of novel cell and gene therapies, investors lack the incentives to invest in this promising field. By designing products that can ultimately survive the market conditions, developers can show a route to profitability to potential partners and investors.

As a case example, we will talk about the iPSC-based cell therapy for limbal stem cell deficiency currently being developed at StemSight. In this article, we will discuss the decision-making process behind the development and manufacturing of StemSight's product and how those decisions are seeking to ensure that the product meets the demands of commercial markets.

CONSCIOUS ABOUT COSTS

To understand the ways in which developers can seek to lower the costs of their product, it is important to first identify the different sources of costs. A recent review by Madrid et al. depicting challenges and considerations for iPSC-derived cell therapies, the authors categorized different indirect and direct costs [3]. While the direct costs are directly transferred to the price of the therapy, the indirect costs need to be covered by the profit margins. We have illustrated these cost types in Figure 1 below.

In iPSC-derived cell therapies, the first major cost to consider is the starting material iPSC line. From a pricing access perspective, at StemSight, we have chosen to work with a single iPSC line as the basis of our products, which are allogeneic and 'off the shelf' to reduce both CMC complexity and cost-of-goods. We secured the iPSC line from a third party, already manufactured under GMP control, thereby further reducing costs and development time. In selecting the iPSC line, careful consideration was placed especially on available documentation regarding, e.g., donor screening and starting materials, as well as the commercial terms of use of the cell line. While the quality control burden is significantly higher for an allogeneic donor and the resulting iPSC master cell bank (MCB) than for an autologous iPSC used only for a specific patient, we believe that ultimately, the approach will improve the wide-spread adoption of our therapy and lower the costs in manufacturing. When the starting material procurement costs become a fixed cost, they do not play such a significant role in the final product pricing. Additionally, the allogeneic iPSC therapy approach means that the cell differentiation process (i.e., manufacture of the finished product) can be done in large batches, taking advantage of the economies of scale.

Process complexity for iPSC expansion and differentiation plays also a significant role in optimizing for scalability and costs. At StemSight, we have undertaken extensive optimization work to simplify our manufacturing processes and to optimize reagent consumption for maximum cell yields. Simpler processes translate to reduced costs of reagents and consumables, fewer failed batches and reduced burden for quality control documentation.

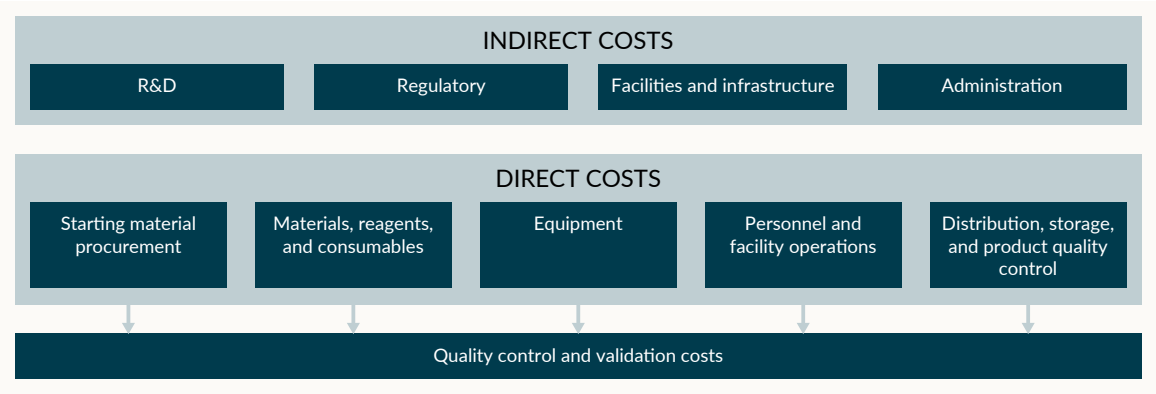
BUILD IT OR BUY IT?

Research and development costs are arguably the cost type for which there is largest variation between different developers and assets. For developers looking to streamline their R&D and using their investors' cash wisely, they want to maximize their chances of getting to key data points needed for preclinical and clinical development.

External service providers including CROs and CDMOs give promise of

FIGURE 1

Indirect and direct costs contributing to finished product cost.



Adapted from Madrid *et al.* [3].

accelerating and simplifying the development process of iPSC products to the clinic with their cGMP facilities and expertise of their team. For investors, this outsourcing route is introduced as a way of derisking the development of iPSC therapy products, potentially leading to reduced costs and lower capital requirements. However, in view of the typically very high costs in engaging a CRO and/or CDMO, does this always hold true?

Many iPSC-derived therapy developers have chosen the strategy to aim first for rare indications to accelerate their market access and to shorten their R&D timeline. When looking for a CRO with prior experience in the specific indication as well a new modality like an iPSC-derived cell therapy, this can greatly limit the options available. More often than not, developers need to work very closely with the CRO to ensure that the intended preclinical disease model, handling of the product and sample processing and analysis methods are all aligned for gaining the wanted results. There is also a risk that the process may inadvertently educate the CRO with consequent erosion of competitive advantage and IP.

At StemSight, we first followed the obvious outsourcing trend with a major preclinical development milestone. At the time, our company did not have access to suitable local infrastructure for preclinical studies, so we searched for a reputable and experienced preclinical CROs in our therapeutic area. However, significant challenges in the disease model development, communication across time zones and, ultimately, sample processing and analysis delays, caused us to pursue an in-house strategy instead. For us, the ability to perform the preclinical proof-of-concept work resulted in improved control over our R&D milestones, rapid sample analysis, as well as accumulation of valuable hands-on know-how, which is crucial to truly understands the bottlenecks and risks of your product in the hands of a CRO, or eventually the doctor using it in a clinical setting.

For manufacturing of the iPSC MCB, and potentially the final product, outsourcing is also an important consideration. Building a standalone manufacturing facility is certainly a huge investment. It is also expensive to maintain, with yearly running costs estimated from $1.5 million to $2.5 million [4,5]. As of now, it seems highly unlikely that any cell and gene therapy product would be able to fill an entire manufacturing facility at least in the first years of operations. However, during the biotech boom initiated by the Covid-19 pandemic,

several GMP grade clean rooms were established around the world. With capital pulling back after the boom, biotech developers were forced to look critically at their capital expenditure. Instead of companies owning their own GMP manufacturing facilities, using a CDMO becomes more appealing as a flexible option for using resources and staff only when needed. However, working with a CDMO has the same caveats as employing a CRO, i.e., losing control over your timelines and process-related IP. Additionally, the technology transfer costs of even a simple 2-week manufacturing process can be as high as $1.5–2.5 million [3].

As a third alternative, a rental-based use of clean room suites could be an attractive option, at least in the first clinical trial phases. Some developers working closely with academic institutes might have access to nearby GMP production facilities within universities and university hospitals. Additionally, there have been other initiatives to provide clean room facilities, where developers can bring their own processes and manufacturing staff. One such initiative is the Canadian Centre for Regenerative Medicine (CCRM), which has hubs at different locations around the world. The newest hub is CCRM Nordic located near Gothenburg in Sweden, where they are building large GMP manufacturing clean rooms for external companies to use. Another similar model is offered by the Cell and Gene Therapy Catapult in the UK, where developers can gain access to state-of-the-art facilities, equipment and process development expertise, while maintaining the ownership of their own process knowhow and IP. Specifically, these initiatives like the CCRM and CGT Catapult are designed to bridging the translation gap between academic research and commercial-ready manufacturing.

There are of course several aspects that should be considered when weighing the options around manufacturing plans. The scale of manufacturing needed depends on the target indication, the number of cells needed per patient dose, number of patient doses needed, and whether manufacturing is done in batches or per patient. In StemSight's case, the current batch size in one differentiation run corresponds to approximately 100 patient doses, and the process is built with a further scale-up option. For StemSight's product, the number of cells required for a dose is only 10^6 cells, and patient numbers are relatively low. With batch manufacturing and subsequent cryostorage of the therapeutic cells, running manufacturing campaigns can be easy to handle and predict. However, for developers who require higher orders of magnitude, they should start to work with systems for manufacturing scale-up in the early development to avoid significant delays in technology transfer to a new culture system.

Additionally, the duration and complexity of the process affects costs of both of rental time as well as technology transfer costs in out-sourcing. In case manufacturing needs are continuous, or difficult to predict, then owning a manufacturing site starts to become a realistic option. Having full control over the manufacturing of a product can also be an asset for potential future partnering discussions, and a way to protect important process-related IP and know-how.

NURTURING IN-HOUSE TALENT PAYS OFF

What drives companies to look at out-sourcing parts of their development and manufacturing, or using consultants, can also be a result of an overall skills shortage in the industry. In their 2023 report, the CGT Catapult estimated that the cell and gene therapy industry will need an overall increase of 63% in workforce by 2028 [6]. 73% of the experts interviewed reported major skills shortages especially in manufacturing and regulatory affairs [6].

Using external experts when needed provides companies with some budget flexibility, compared to having staff on the payroll. However, thinking about personnel as just one row in a cost calculation grossly overlooks the fact that talented people are worth their weight in gold. Especially in a newly developing industry like iPSC therapies, attracting and retaining key talent can become an important asset for any company. If the company is successful in recruiting experts in the iPSC therapy field that reduces the need for outsourcing as well, ensuring that all the knowhow gained during development remains with the company.

Small biotech companies can rarely compete with salary alone to attract talent, but there are other points that might tip the scales for potential employees. Building revolutionary therapies can offer a clear sense of purpose that resonates even with seasoned experts, attracting them to the biotech's cause. While the taxation laws on offering stock options or shares vary between countries and jurisdictions, implementing employee ownership in the company can incentivize, balancing the salary compromise early on. To ensure that the company has access to top talent, flexible hybrid work practises can also grow the pool of talent and enable search for key talent globally.

Building an iPSC therapy company in one of the global biotech hubs, like Boston or Basel, might make fundraising for early-stage companies easier, but the war for talent can also be fierce. Going against the stream and setting up in an area where there is a source of skilled talents but very little competition, can also pay off in the long run. In StemSight's case, the company is located right next to Tampere University in Finland, an academic institute with a 20-year history of working with pluripotent stem cells and a focus on hands-on cell culture skills training. With close ties to the university, StemSight has a steady influx of inbound workforce.

With the availability of highly-skilled talent and access to manufacturing infrastructure in the Nordic region, it is likely set as a potential new hub for global iPSC therapy innovations.

TRANSLATION INSIGHT

To ensure widespread adoption of new iPSC-based therapies when they reach the market, developers need to be aware of the full value chain from development strategies, through the regulatory pathway and all the way to patients and payors. One critical component in the adoption and access to iPSC-based therapies comes from the healthcare systems and payors' perception of value for money. With the increased rate of new approvals of cell and gene therapies, there is also an increased pressure to limit their pricing. The costs associated with the end product are defined by parameters that are chosen relatively early in the development process. Being mindful of these aspects early on, developers can ensure that their products have higher probabilities of success also after market approval.

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CELL & GENE THERAPY INSIGHTS

INDUCED PLURIPOTENT STEM CELLS

SPOTLIGHT

Investing in early-stage stem cell therapy developers

Stijn Heessen and Kristian Tryggvason

VIEWPOINT

"Providing a future, healthy, and competitive commercial cell therapy landscape depends on young companies having early access to sufficient capital to advance new transformational and complementary cell therapies."

Cell & Gene Therapy Insights 2025; 11(2), 183–186 · DOI: 10.18609/cgti.2025.023

What are venture capital investors expecting to see from early-stage stem cell therapy developers, and are they focusing on the right attributes? At Alder Therapeutics, we spend considerable efforts in formulating answers to these questions and we find that fulfilling expectations of potential investors is equally important as educating them about the specifics of developing stem cell therapies.

At a first glance, the prerequisites for investing in any early-stage company seem similar and modality agnostic. Whether you are developing a conventional small molecule, an antibody or peptide drug, a cell or gene therapy, or a nucleic acid-based

product, you will need to convince any potential investor that there is a future commercial model that will create value, that you have a world-class management team and scientific advisory board, and that you thoughtfully plan capital allocation to reach meaningful value inflection points with a clear pathway to Return on Investment.

Whether you deliver your 6-minute pitch live during a paid time slot at an investor conference or if you manage to get a 15-minute Teams call, investors quickly focus on the unmet medical need you are solving, on the uniqueness and competitiveness of your program, and whether you have the scientific data to back up your claims. While these are some of the key features any investor will use to judge the validity of your drug development proposition, it is our observation that investors looking to invest in stem cell therapy start-ups often underestimate the time and effort developers should spend initially on establishing a future-proof production process.

Specifically, what differentiates a stem cell therapy start-up from developers of other tried-and-tested modalities, is the strong conviction that in cell therapy the 'process' is considered the 'product'. This paradigm means that early-stage companies should spend significant efforts on mitigating all CMC-related risks. These efforts should include choosing a suitable and affordable cellular starting material generated in accordance with current GMP, gaining access to and continuous supply of xeno-free raw materials and establishing a robust and reliable differentiation method of the cellular starting material into a specific target cell type. Also, early developers should build a manufacturing framework devoid of highly manual traditional labor-intensive processes and early on they should explore dose and batch size requirements and a suitable comparability strategy.

It is our conviction that taking a proactive approach to CMC risk-mitigation is at the heart of building a sustainable and 'investable' cell therapy company. However, it has also been our experience that an early focus on mitigating manufacturing process risks does not necessarily convert into relevant development-driven inflection points that investors will normally be looking for. This may not only create misalignment between what an investor and the developer deem relevant capital allocation, but it can also result in an undervaluation of reaching significant CMC milestones. Often this seems to originate from the flawed idea that for young cell therapy companies 'having CMC under control' is considered a given, and that early capital should rather be allocated to reaching more conventional, non-CMC-related developmental milestones like preclinical mode-of-action or safety studies.

At Alder, we use a derisking development approach that directly couples early CMC risk-mitigation to an early understanding of the commercial potential. At the cost-end of this equation, we start with defining a quality target profile and associated critical quality attributes, which provide early insights into the process and help identify key parameters for scaling the manufacturing, which is directly linked to intended therapeutic dose. We also undertake a detailed manufacturing process evaluation early-on to define priority actions that can help maximize process success and we prioritize early advice from various regulatory authorities to understand whether our development concepts are globally acceptable. At the opposite return-end of the same equation, we explore the market opportunity by means of an early health economics and market access analysis collecting information on the epidemiology, competitor landscape, market size, and reimbursement potential. Only if the equation of commercial value versus the cost of risk-mitigation yields

a net positive result, we consider that a program is worth pursuing and that it can attract investor capital.

The recent publications on clinical proof-of-concept breakthroughs of allogeneic induced pluripotent stem cell (iPSC)-based cell therapies in Parkinson's disease, epilepsy, heart failure, and diabetes provide validation of the original thesis that stem cell therapies may help change the treatment paradigm from symptomatic to curative. A phrase often heard in this context is: 'The cell-therapy field is where the biologics field was 10–15 years ago'. Given how biologics are today considered first-line treatment in various indications, at Alder we are convinced that stem-cell therapies will equally constitute standard treatment regimens in the not-too-distant future.

Providing a future, healthy, and competitive commercial cell therapy landscape depends on young companies having early access to sufficient capital to advance new transformational and complementary cell therapies. It will thus fall on the starting companies to further educate the investment community on the particular attributes of cell therapy development and how it differs from developing other, well-established therapeutic modalities.

BIOGRAPHIES

Stijn Heessen is the Chief Operations Officer (COO) and co-founder of Alder Therapeutics, Hoorn, The Netherlands. As COO, he is responsible for business development, contracting, regulatory and clinical strategy, and health economics and market access activities for Alder's cell therapy pipeline. Prior to Alder, he spent more than 12 years in senior global business development and licensing positions in the pharmaceutical sector, working for companies such as Perrigo Consumer Selfcare International, Sanofi Consumer Healthcare, and Boehringer Ingelheim. His experience has seen him lead international cross-functional teams and close numerous multi-million euro licensing deals. He has also held Regulatory Affairs Manager roles at both GlaxoSmithKline and Boehringer Ingelheim, where he worked closely together with marketing teams to support the launch of new products. Stijn has an MSc in biomedical sciences from Utrecht University, Utrecht, The Netherlands a PhD in Cell and Tumor Biology from the Karolinska Institute, Solna, Sweden, and held post-doctoral positions at the Ludwig Institute for Cancer Research and the Netherlands Cancer Institute.

Stijn Heessen PhD, Chief Operations Officer, Alder Therapeutics, Hoorn, The Netherlands

Kristian Tryggvason has more than a decade of experience in the cell therapy field has seen him found a successful cell therapy reagents company, as well as license a promising cell therapy product to a large pharma company. He is founder and Chief Executive Officer (CEO) of Alder Therapeutics, Stockholm, Sweden. As CEO, he is responsible for driving preclinical development of the company's promising pipeline of pluripotent stem cell therapies, in addition to managing all PR, marketing, and fundraising activities. Prior to Alder, he founded Swedish cell culture solutions company BioLamina, growing it to a profitable €100 million enterprise with 70+ employees and more than €17 million in annual sales across 35 countries. He has also held business development and project management positions in the pharmaceutical sector, where he created patent strategies, out-licensed cell therapy products, and gained broad exposure to the clinical, regulatory, and commercial aspects of drug development. Just like the other member of the Alder Team, Kristian blends his deep business knowledge and experience with rigorous scientific training. He holds an MSc in Molecular

Biology from the University of Oulu, Oulu, Finland, a PhD in Cellular and Molecular Biology from the Karolinska Institute, Solna, Sweden, and an MBA from the Copenhagen Business School, Copenhagen, Denmark.

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INDUCED PLURIPOTENT STEM CELLS

SPOTLIGHT

Developing autologous iPSC-based therapies for Parkinson's disease treatment

INTERVIEW

"...the industry still faces significant challenges in iPSC manufacturing, particularly regarding the scale up and automation of differentiation processes."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Kim Raineri**, Chief Technology Officer, Aspen Neuroscience, about the development of novel induced pluripotent stem cell (iPSC)-based therapies for Parkinson's disease. They also discuss the regulatory, safety, and technical challenges in advancing iPSC-based therapies, as well as the company's goals for future clinical trials.

Cell & Gene Therapy Insights 2025; 11(2), 169–174 · DOI: 10.18609/cgti.2025.021

Q What are you currently working on at Aspen Neuroscience and what are the company's main aims?

KR Aspen Neuroscience is a clinical-phase company focusing on autologous induced pluripotent stem cell (iPSC) therapies. Our lead program is in Parkinson's disease (PD), and we are currently conducting a Phase 1/2a clinical trial from

our cleared IND in 2023. For this particular treatment, we produce autologous dopaminergic precursor cells, which are transplanted directly into the putamen of the patient's brain. The goal is for these cells to engraft and relieve the motor symptoms of PD. In our first cohort, we have already presented safety and tolerability data, which is very encouraging. Our current focus is to treat the remaining patients in this clinical trial to further validate both the surgical and long-term safety, as well as identify signs of biological efficacy.

Q How is Aspen Neuroscience working to scale out manufacturing to maintain its autologous approach?

KR At Aspen Neuroscience, we have a three-stage manufacturing process. For autologous cell therapies, we begin with a small biopsy of the patient's own skin cells. The biopsy is then processed to create a fibroblast cell bank. Subsequently, the fibroblasts are then reprogrammed into several iPSC lines for each patient. Through our proprietary genomic platform, we then carefully select the best iPSCs to advance to the final differentiation process, where we produce dopaminergic precursor cells tailored for the patient. At every stage of the manufacturing process, we cryopreserve the cells, which allows for rigorous quality control and ensures we are only advancing the best cells to the next stage, ultimately aiming to provide the highest therapeutic potential for patients.

To scale out manufacturing, we are working to automate these processes, particularly the iPSC production stage. We recently announced a collaboration with Cell X Technologies to develop an automated GMP system for iPSC manufacturing. The generation of iPSCs is a highly technical process, typically executed by experienced scientists in research laboratories, and our goal is to transition to an automated platform. The system being developed combines robotics, liquid handling, and optics within an environmental chamber equipped with incubation and reagent management. It is entirely computer-driven and utilizes machine learning (ML) algorithms to select cells, including positive selection (picking) and negative selection (weeding).

In conjunction with that, we are building a manufacturing facility in San Diego, CA, USA to accommodate systems such as the Cell X Technologies platform as we prepare for larger-scale trials. Our ongoing development efforts aim to transition our current manual processes to automated systems, with plans to incorporate devices such as the Cell X Technologies platform in future clinical trials.

Torrey Pines Manufacturing Facility.

"...the industry still faces significant challenges in iPSC manufacturing, particularly regarding the scale up and automation..."

Q What is the effectiveness of the existing tools and enabling technologies for iPSC-derived therapy manufacturing, and what gaps remain?

KR There are several enabling technologies and proprietary tools for reprogramming iPSCs under GMP conditions. However, I think the industry still faces significant challenges in iPSC manufacturing, particularly regarding the scale up and automation of differentiation processes.

Currently, most cell culture automation platforms are designed for the CAR-T cell market, particularly autologous cell therapies. These platforms typically focus on small-scale (such as 1 L) suspension cultures, which are not well-suited for iPSC generation, as these cells are an adherent cell culture modality.

This gap is precisely why Aspen Neuroscience has made a dedicated effort to identify and develop automated solutions tailored to small-scale cell culture for autologous adherent cells.

Q To what degree can platform opportunities for the end-to-end manufacture of iPSCs, including closed, automated processing, be applied?

KR Although our lead programs focus on PD, the platform we are developing with Cell X Technologies will allow us to differentiate iPSCs into multiple cell types. In particular, we plan to utilize this platform in our preclinical studies for discovery programs. Instead of starting with a manual cell culture process, we are taking a forward-thinking approach by initiating some of these discovery programs in the automated platform.

Q What are the critical CMC challenges faced in the development of an iPSC-derived autologous cell therapy? How can these hurdles be overcome?

KR One of the key challenges is ensuring comparability between the manual and automated manufacturing processes. Comparability is crucial when making process changes, and it can be particularly challenging depending on the nature and magnitude of the change in automation. Major changes to the process could lead to unintended consequences on the critical process parameters or quality attributes (CQAs).

To address this hurdle, we are working to automate the activities we have already perfected in the manual manufacturing process, instead of introducing entirely novel

"Our goal is to keep the process as consistent as possible when transitioning from manual to automated techniques."

approaches. Our goal is to keep the process as consistent as possible when transitioning from manual to automated techniques.

For example, we are still performing adherent cell culture at similar scales in comparable cell culture dishes and continuing with positive and negative selection (picking and weeding) in the same way. This approach lowers the hurdle, as we are simply adding automation around a process that is already established instead of doing something completely novel. We have already shown that automated processes can produce comparable cells that meet our CQAs.

Another CMC challenge in the cell and gene therapy space is product characterization, which is also essential for comparability. There are still gaps in the technology available today to fully characterize cell therapy products. Traditionally, developers have used analytical techniques such as flow cytometry and PCR for cell characterization, but these methods have limitations.

For us specifically, one unique challenge that we have solved is that our drug product is a precursor cell instead of a mature neuron, which can make characterization very complex. Once our precursor cells are transplanted, we hope they mature, engraft, and provide motor symptom relief through dopamine production. Therefore, to better characterize our cells from each batch, we have explored techniques such as whole genome sequencing and RNA sequencing, along with ML algorithms. These tools help us assess the identity, maturity, and potential for engraftment as well as dopamine release capabilities of our cells. Aspen has invested significant effort in this area, building a bioinformatics platform to support these efforts and crafting assays that could potentially serve as potency assays.

Q What, in your view, are the key regulatory challenges in the iPSC field? And to what extent have the existing safety concerns been addressed?

KR Beyond characterization of the cells and their potency, another key challenge in the iPSC-derived cell therapy space is the materials used in the differentiation process. Since the field is still relatively young, many of the components and reagents used in iPSC differentiation lack proper sourcing or testing or are not produced under GMP standards. It is incumbent for developers and sponsors to thoroughly review the materials they are using in their manufacturing processes and ensure that these materials meet regulatory standards, ideally before filing an IND.

Regarding safety, there has been significant progress in the field, especially in addressing tumorigenicity concerns. Typically, it is required in IND-enabling studies to demonstrate a lack of tumor formation in certain *in vivo* models. Additionally, it is essential to test the final drug product for residual iPSCs. There have been attempts to improve the sensitivity of these assays to ensure no residual iPSCs are present in the final product.

At Aspen Neuroscience, we have completed both of these safety tests and are confident in the strong safety profile of our iPSC-derived cell therapy product.

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Q What are your key goals and priorities, both for yourself and for Aspen Neuroscience as a whole, over the next 1–2 years?

KR As we look ahead, it is an exciting time for Aspen Neuroscience. We look forward to completing the dosing of our patients in the Phase 1/2a clinical trial and testing out a new commercial formulation. We are also focused on expanding our capabilities for future clinical trials and preparing for a much larger pivotal trial.

It has been an exciting journey over the past 2.5 years since we filed our IND and began the clinical trial. Looking ahead, we are committed to making a meaningful impact on PD treatment.

BIOGRAPHY

Kim Raineri has deep global experience in the cell and gene therapy industry, biologics and medical device spaces, with a distinguished track record of innovation and implementation of Good Manufacturing Practices (GMP). Prior to joining Aspen Neuroscience, San Diego, CA, USA, Kim served as Chief Manufacturing and Technology Officer and a member of the executive team at AVROBIO, a Boston-based leader in the gene therapy space. Before AVROBIO, Kim was vice president of operations for Nikon CeLL innovation Co., Ltd, a Japanese contract development and manufacturing organization. During his tenure, he established the company as the preferred provider of custom process development and manufacturing services for cell and gene therapy products in the Japanese market. Previously, Kim held management positions at Lonza, serving as the business director for cell therapy contract manufacturing operations in Singapore for 5 years, and prior to that as Director of Operations at Lonza's Maryland facilities. Kim was also previously the Senior Manager of the Tissue Processing Lab at CryoLife Inc. He holds a BS from the University of Miami, Coral Gables, FL, USA and an MBA from Kennesaw State University, Kennesaw, GA, USA.

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CELL & GENE THERAPY INSIGHTS

CONTENTS VOLUME 11 · ISSUE 2

RAW/STARTING MATERIALS

ANALYTICS

CELL & GENE THERAPY INSIGHTS

RAW/STARTING MATERIALS

REVIEW

Raw and starting materials for cellular and gene therapy products

Gary C du Moulin

One of the fastest growing classes of therapeutics is represented by the cellular and gene therapy (CGT) sector. Many non-genetically modified cell therapies, *in vivo* gene transfer therapies and therapies with gene modified cells are moving from investigational phases to marketed products at an increasing rate. The sourcing and quality of raw and starting materials becomes an essential element if support of this robust pipeline can continue to meet the needs of our patients. This review is intended to assist the developers of these therapies in better understanding the definitions and classification of raw and starting materials as interpreted by US or European regulators. Phase appropriate qualification of raw and starting materials critical to CGT manufacturing processes are described with examples of starting and ancillary materials, especially allogeneic and autologous cell sources subject to recent US FDA and European regulatory interpretation. Best practices are described to better address and mitigate sourcing, variability and supply continuity risks and those Chemistry, Manufacturing and Control (CMC) requirements to support IND clinical investigations. The reader is introduced to regulatory guidances recently issued by FDA, ISO and USP pertinent to raw and starting materials. The review concludes with points to consider in addressing the realities of the supply chain ecosystem.

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As of October 2024, 37 cellular and gene therapy products have been approved by the US FDA, including therapies for sickle cell anemia and hemophilia. There continues to be great hope and expectation in the prospect that advanced cancers and genetic diseases can be addressed through advancements in cellular and gene therapy [1]. In order to encourage innovation and momentum within the biopharma industry, the FDA has established expedited development designation programs for advanced therapies including a Breakthrough Therapy designation (BTD), Regenerative Medicine Advanced Therapy (RMAT) designation, and Fast Track (FT) designation [2]. The European Union has also promoted a program for accelerated assessment for Advanced Therapy Medicinal Products (ATMPs) [3]. Despite

constrained pipelines and financial pressures one crucial aspect and essential common prerequisite of successful development of cellular and gene therapies in each of these regulatory designations continues to be the sourcing and quality of raw and starting materials [4–7]. Regulatory authorities continue to identify concerns regarding safety, efficacy, and consistency of raw and starting materials [4].

While batch to batch consistency is essential for successful and long-term commercialization of cellular and gene therapies, many biological raw materials are inherently variable. It is crucial to establish a robust process for selecting raw and starting materials, including manufacturers of plasmid DNA and viral vectors, in which all aspects of production from qualified suppliers are well designed and controlled.

Regardless of the phase of development, sponsors must address the source and testing of raw and starting materials. Regulators urge that quality built into these materials in early phases of development helps insure better preclinical and clinical data outcomes and can accelerate a positive regulatory assessment [8–12]. Early risk assessment and selection of high-quality materials without the risks of chemical toxicity or presence of adventitious agents can build confidence and minimize chances of mid- to late-phase changes that could necessitate an arduous change control process that might require additional validation studies, comparability assessments, or additional clinical studies [8,13–16].

DEFINITIONS IN CLASSIFYING RAW MATERIALS

Depending upon the governing regulatory agency, materials used in cellular and gene therapies are typically categorized into starting materials, ancillary materials, or raw materials (Table 1).

ICH Q3A defines starting material as a *"material used in the synthesis of a new drug substance that is incorporated as an element into the structure of an intermediate and/or of the new drug substance. Starting materials are normally commercially available and*

TABLE 1

US and EU regulatory definitions relating to materials used in the manufacture of cellular and gene therapies.

Raw materials	Ancillary materials (not globally recognized, referred to as raw material in EU)	Starting materials
USP <1046> and USP <1047>: ALL materials used in the manufacturing of CGT that MAY or MAY NOT remain in the final product (including Ancillary Materials); EMA Part IV of the Annex to Directive 2001/83/EC: materials used during the manufacture of the active substance and NOT intended to form part of the active substance shall be considered as raw materials; EP 5.2.12 General chapter: raw materials of biological origin for the production of cell-based and gene therapy medicinal products	*USP* <1043>, specific for CGT; ICO/TS 20399 Biotechnology Ancillary materials; NOT intended to be in the final product, used as processing aids that exert their effect on the therapeutic substance; FDA other terms: ancillary products, ancillary reagents, processing aids, processing reagents	*USP* and/or *EP*; Material used in the synthesis of new drug substance that is incorporated as an element into the structure of an intermediate and/or new drug substance (ICH Q3A); EMA Part 1 of the Annex to Directive 2001/83/EC: materials from which the active substance is manufactured or extracted; FDA and EMA

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of defined chemical and physical properties and structure" [17]. Examples of starting materials for cellular and gene therapies could include donor cells and tissues, cell or virus banks, plasmids needed to generate viral vectors, or linear DNA used as a template for *ex vivo* transcription into mRNA.

FDA regulatory guidance, EU directives, and ISO Standards are consistent in the definition of raw materials (also called ancillary materials in the USA) [7,15,18–24]. 'Ancillary materials' are defined as those materials used in processing but are not intended to be present in the final product [4,24]. A partial list of raw materials is shown in Box 1.

Where a material is composed of multiple materials such as culture media, all components are considered ancillary materials. These materials must demonstrate lot to lot consistency with respect to identity, purity, storage, stability, traceability, biosafety, and performance [11,14,20,22–24].

Excipients, by definition, are raw materials intended to be part of the final product and as such are not considered ancillary materials. The term, 'ancillary materials' is not globally recognized in the EU although the ISO International Standard 20399 defines ancillary materials as *"materials that come in contact with the cellular therapeutic product during cell processing but are not intended to be part of the final product formulation"* [24]. Raw materials as defined by the *US Pharmacopeia*'s informational chapters, <1046> and <1047> are considered to be materials used in manufacturing including those defined as ancillary materials [25,26]. The *European Pharmacopeia* Chapter 5.2.12 defines raw materials of biological origin whereas the *US Pharmacopeia* in Informational Chapter <1043> *"Ancillary materials for cell, gene, and tissue-engineered products"* introduced the term 'ancillary materials' as a specific subset of raw materials [21,23,27].

PHASE APPROPRIATE QUALIFICATION OF RAW AND STARTING MATERIALS CRITICAL TO CELL AND GENE THERAPY MANUFACTURING PROCESSES [4–9,11,12,24,27]

The production of a cellular or gene therapy product can comprise hundreds of raw and starting materials, and a multitude of manufacturing steps including open and closed manual or automated operations performed by many personnel in a variety of manufacturing establishments. Quality control analytics supporting these processes also require a host of media, reagents, and consumables. The sponsor's quality assurance systems are tasked with responsibility and control of the immense challenge of selecting and qualifying these raw materials, not only in the sponsor's own facilities, but in all facilities in which raw materials are being produced for a given product [28,29]. Donor eligibility for patient cells and tissues as starting materials of biological origin only add to the level of scrutiny and qualification required before they can be confidently used in manufacturing [30,31]. Phase appropriate GMP clinical investigations mandate that all raw and starting materials are sufficiently qualified to ensure safety and potency prior to the onset of human clinical trials. Mitigating risk, determination of material specifications, and maximizing the consistency of these materials at an early product development cycle is a huge responsibility. To illustrate these challenges, examples of starting and ancillary material are presented below.

Starting material: autologous cells—US guidance

For the manufacture of autologous products, donor eligibility determination under 21 CFR 1271.50 and donor screening or testing under 21 CFR 1271.75, 1271.80, and 1271.85 is recommended but not mandatory

BOX 1

Partial list of commonly used ancillary (raw) materials used in the manufacturing of cellular or gene therapy products IAW ISO 20399.

- Salts
- Culture media
- Enzymes
- Transfection reagent
- Cytokines
- Activation agents
- Immunomagnetic beads
- Novel polymers
- Hydrogels
- Fetal Bovine serum
- Nanoparticles
- Sterile buffered solutions
- Growth factors
- Antibodies
- Anticoagulants
- Cryoprotectants
- Non-mammalian cells
- Human serum albumin
- Plasmids
- Viral vector
- Antibiotics
- Recombinant insulin

In accordance with ISO 20399 ancillary materials do not include: cells that are starting materials, intermediates, or the final form of a cellular therapeutic product, feeder cells, additives used post processing, scaffolds, or non-biological consumables, e.g., beads, dishes, tissue culture flasks, bags, tubing, pipettes, needles, or other plastic ware that come into contact with the cell or tissue, apparatus, or instruments. IAW ISO 20399 viral vectors and plasmids are considered ancillary materials.

for starting materials. However, the manufacturer must include the applicable required labeling of this starting material with the statement 'FOR AUTOLOGOUS USE ONLY'. If donor screening and testing is performed under 21 CFR 1271.75, 1271.80, and 1271.85 the manufacturer must prominently label the product with the statement, 'NOT EVALUATED FOR INFECTIOUS SUBSTANCES'. FDA also recommends that the manufacturer include a minimum of two unique identifiers (e.g., donor identification number [DIN], product tracking number, etc.) to minimize the potential for mix-ups [30–32].

Starting material: allogeneic cells—US guidance

T cells from allogeneic donors should meet the requirements of relevant national laws and regulations. Donor eligibility for starting materials used for allogeneic cell and gene therapy products produced in the US is based upon donor screening and testing results in accordance with 21 CFR 1271.75, 1271.80, and 1271.85. Screening will entail the review of relevant medical records to determine risk factors or clinical evidence of relevant communicable disease agents and diseases, or communicable disease risks associated with non-human cells or tissues (xenotransplantation). Review of medical records in accordance with 21 CFR 1271.3(s) will include a current interview of the donor's medical history interview, physical assessment of a cadaveric donor, or the physical examination of a living donor. Testing will be performed on the donor's blood by FDA-licensed, approved, or cleared test kits by a certified Clinical Laboratory Improvement Amendments (CLIA) certified laboratory no later than 7 days before or after the recovery of the cells or tissues. Specimens for testing of donors of peripheral blood stem/progenitor cells or bone marrow may be collected up to 30 days before recovery [30-33].

Procedures for acquiring, transporting, sorting, testing, and preserving donor cells should be established and validated. Specifications and requirements for characteristics, culture conditions, generation, growth characteristics, preservation status and conditions, and quality testing of donor cells must be documented. Cell banks should be established that will preserve cell characteristics and production consistency for the clinical application. Testing

standards for cell banks should be established to meet basic safety, quality control, and efficacy requirements.

SUBSTANCES OF HUMAN ORIGIN (SOHO) REGULATION: EUROPEAN GUIDANCE

The European Union regulates the standards of quality and safety of starting materials used in the manufacture of medicinal products under Directive 2002/98/EC for the collection, testing, processing, storage and distribution of human blood and blood components. This legislation is known as the 'Blood Directive'. Cells and tissues are similarly regulated as starting materials under Directive 2004/23/EC known as the 'Tissue Directive'. Together these two directives are known as the 'BTC' (Blood, Tissues and Cells) legislation. In the USA, starting material requirements across the country are in full alignment with 21 CFR 1271, *Human Cells, Tissues and Cellular and Tissue Based Products*, whereas in Europe elements of donor screening, donor testing and standards for source material collection vary from country to country. However, in July 2022, the European Commission adopted a proposal for a new BTC regulation which would repeal and replace the current blood and tissue directives with the goal of increasing harmonization with other global statutes. After consultation, agreement, and adoption, the finalized regulation came into force with a transition period of two to three years, depending on particular provisions. The new BTC legislation repeals Directives 2002/98/EC and 2004/23/EC and is entitled *Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for substances of human origin intended for human application*, published in July 2024 in the official Journal. The act is both binding in its entirety and directly applicable in all Member States. It is to be fully implemented by August 7, 2027 [34].

STARTING MATERIALS: VIRAL VECTORS

Viral vectors that are used to transduce cells can be considered ancillary products IAW ISO or *USP* standards because these materials are not expected to remain in the final product [24,27]. However, others may consider viral vectors, transposons, genome editing components or plasmid DNA used in the production of viral vectors as starting materials from which the active substance is manufactured [11]. Whether considered starting or ancillary materials the production and quality control of vectors for genetic modification should meet the current (and still evolving) FDA or EMA requirements for current GMP or principles of GMP [4,8,18,19,35–42]. Two challenges jeopardize compliance with cGMP regulations. These include sourcing of the materials and management of the supply chain. The starting critical raw materials commonly used for vector production typically comprise cell lines, transfection reagents, media components, modifying enzymes, growth or adhesion factors, serum, and plasmid DNA [19]. Each component of viral vector production must undergo a rigorous program of testing for identity, purity, bacterial endotoxin, sterility, biological activity or other testing to reduce the risk of introducing adventitious agents into the manufacturing process [19,40,41]. Each component must be procured from a qualified vendor and should be accompanied by a product certificate of analysis, certificate of origin and a certificate establishing corporate compliance to the requirements of ISO 9001. A limited number of suppliers can produce cGMP grade plasmid DNA and even fewer can produce transfection reagent of cGMP grade. For example, depending upon the type of transfection application plasmid DNA manufacturers maintain their own manufacturing processes and have different criteria for assessing quality and testing [19]. This also poses regulatory challenges

as European directives consider plasmids for vector manufacturing as a starting material for ATMPs whereas in the USA, plasmids are referred to as intermediates [8,15].

As part of IND documentation submission describing production and purification procedures of vectors, regulators will review the reagents and components relating to the manufacture of gene therapy vectors. A description of all production and purification procedures including the cell culture and expansion steps, transfection or infection procedures, harvest steps, hold times, vector purification, and concentration or buffer exchange steps used during these processes will be examined. Any in-process testing to ensure vector quality will also be reviewed [8].

Sponsors should be cognizant of changes to recombinant starting materials occurring in the manufacturing process. Risk evaluations should be performed on any proposed change. Changes at the level of the cell starting material may warrant assessment of comparability between the two methods to measure the impact of pre and post change on different batches and on process controls on the quality of the released final product [16]. These studies should be carefully monitored by the sponsor to insure a consistent and reproducible characterization process.

EMA guidelines on quality of genetically modified cells recommends that quality of materials and reagents should follow guidance of Ph. Eur. General Chapter 5.2.12 as well as measures taken to ensure viral safety to minimize risk of transmitting agents within any reagent or material of animal origin of Transmissible Spongiform Encephalopathies (TSE) [21]. FDA provides similar guidance in the CMC information for human gene therapy IND applications [8].

A framework for enhancing the quality and consistency of the ancillary materials has been provided in the documentary standard ISO 20399:2022, *Biotechnology—Ancillary materials present during the production of cellular therapeutic products and gene therapy products* [24]. Under development is ISO 16921-2: *Biotechnology—Gene Delivery Systems—Part 2: Guide for Methods for the Qualification of Viral Vectors*, which provides guidance for determining the physical and functional titer of viral vectors that should be central in ensuring consistent characterization of viral vectors [41].

Ancillary materials: cell culture media

Classical media available to research cell biologists was adequate to support the well understood fibroblastic cell lines. However, clinical application of cell and gene therapies now require high quality sophisticated media formulations. These applications require functionally complex media components including serum-free formulations and defined media. Media suppliers must upgrade their manufacturing processes with robust GMP controls to ensure lot to lot consistency and stability. Prior to entering into a quality agreement, sponsors of cell and gene therapy developmental programs should qualify these manufacturers as critical suppliers through procedures that include external onsite audits to assess the environmental control, cleanliness policies, process control procedures, documentation and data integrity systems capable of providing complex media formulations that comply with applicable GMP regulations in accordance with regulatory guidance [43].

BEST PRACTICES TO UNDERSTAND AND MITIGATE SOURCING AND SUPPLY CONTINUITY RISK: APPLYING A QUALITY BY DESIGN RISK ASSESSMENT PROCESS FOR RAW AND STARTING MATERIALS

The FDA encourages the adoption of quality by design (QbD) principles in the

development and manufacturing of drug products, especially for the assessment of raw and starting materials [44]. FDA emphasizes quality must be built into the manufactured product. As a science and risk based systematic approach, the application of QbD emphasizes predefined objectives with increasing product and process understanding. FDA's guidance in assessing risk defines four basic elements:

- The product is designed to meet patient needs and performance requirements.
- The impact of starting raw materials and process parameters on the product quality is understood.
- Critical sources of process variability are identified and controlled with appropriate control strategies.
- The process is continually monitored and updated to allow for consistent quality over time [44–48].

Elements of QbD include the determination of critical quality attributes (CQAs) defined as those attributes that must be controlled within predefined limits to ensure that the product can consistently meet the goals of safety which includes sterility, efficacy, stability, and performance. Risk assessments conducted in accordance with ICH Q9 link attributes of the raw materials and process parameters with the CQA. Those process parameters impacting CQA are termed Critical Process Parameters (CPPs). CPPs must be controlled throughout the manufacturing process and may include such parameters as processing time, temperature, pH, etc. [44–48].

A Critical Material Attribute (CMA) is a physical, chemical, microbiological, or biological characteristic or property of an input material that should be within an acceptable range, limit, or distribution to ensure the anticipated quality of that in-process material, excipient, or drug substance. Identification of CMAs and CPPs are linked to CQAs. CMAs can significantly impact pharmaceutical unit operations, process consistency, and product quality attributes. In other words, the properties of the raw material can influence the quality of the cell therapy product. Hence, material properties need to be tested and CMAs need to be defined and controlled [48].

A risk-based approach should be used as early as possible to define the most important characteristics for each raw material. Determine the CQAs of the cell therapy product to evaluate the potential effect of the raw materials on the quality of the therapeutic product. As such, the supplier should be asked for supportive analytical or validation data to analyze variabilities and impact upon the cell therapy product. These data should be trended from lot to lot to ensure that the raw material quality remains within a prescribed and robust control limit.

The qualification and testing of ancillary (or raw) materials should be based upon a quality risk management strategy. In creating a robust raw material qualification program requires attention be focused in five areas:

- Ancillary material identification and sourcing,
- Selection and suitability for use in manufacturing
- Characterization
- Vendor qualification
- Quality assurance and control [27]

USP < 1043> *Ancillary materials for cell, gene and tissue-engineered products, for information, qualification and risk*

classification defines four risk categories that facilitate the identification, analysis, and evaluation of potential risks. Through this process, a Risk Evaluation Matrix can be constructed which can quantify the risk and facilitate decision making. This informational Chapter defines the four tiers as follows.

Tier 1 represents low risk highly qualified material. Examples of such materials could include, licensed biologic, drug, or medical devices, e.g., recombinant insulin or human serum albumin for injection. Tier 1 also addresses use of licensed components or materials but utilized 'Off Label' in the manufacturing process. The use of recombinant insulin as a component to serum-free cell culture media formulations is but one example. Qualification and risk reduction activities are noted in the description of this risk tier.

Tier 2 represents well characterized material produced under an established quality system, be well suited for CGT product manufacturing. Examples of this risk tier might include recombinant growth factors, cytokines, or immunomagnetic beads.

Tier 3 represents materials intended for research use only (RUO), not necessarily intended for CGT products and require more qualification than materials identified as tier 1 or tier 2. A product manufactured in an academic or pilot stage manufacturing setting would likely need to have its quality profile upgraded for later stage clinical trials and eventual commercialization. Such materials may include tissue culture media process buffers, novel polymers, scaffolds, or hydrogels.

Tier 4 represents high risk minimally qualified materials. In such cases, the manufacturer would need to conduct extensive qualification, perform adventitious agent testing, or work with the manufacturer to upgrade the manufacturing process to comply with cGMP standards. Examples of such materials might include animal or human cells used as feeder layers or animal derived polymers, scaffolds or hydrogels.

Details regarding the implementation of a qualification strategy for raw materials may also be found in *European Pharmacopoeia* Chapter 5.2.12 entitled, *Raw materials of biological origin for the production of cell-based and gene therapy medicinal products.* This general chapter has recently been revised by the *European Pharmacopoeia* Commission to align with the new approach to gene therapy providing a standardized way of controlling medicinal products produced through gene transfer. General chapter 5.14 *Gene transfer medicinal products for human use* has been replaced by general monograph 3186 and an accompanying General Chapter 5.34 entitled, *Additional information on gene therapy medicinal products for human use* [21,49,50].

NATIONAL INSTITUTES OF HEALTH: REGULATORY KNOWLEDGE GUIDE FOR CELL AND GENE THERAPIES—NIH SEED INNOVATOR SUPPORT TEAM

The National Institutes of Health established for its award grantees a Small Business Education and Entrepreneurship Development Program known as SEED. The SEED Innovator Support team helps NIH awardees build a business and explore their life science innovation potential. While designed for awardees of NIH grants, the internal experts in regulatory affairs provide useful guidance to all innovators to lower regulatory hurdles. The SEED Innovation Support team published a guide which emphasizes regulatory and compliance knowledge entitled, *Regulatory Knowledge Guide for Cell and Gene Therapies* [51]. This document should provide smaller organizations with useful and practical information to construct a robust quality system.

Bioprocess development and manufacturing procedures to support IND clinical investigations: Chemistry, Manufacturing, and Controls considerations by FDA [2,8,18,28–31,33,35,51–54]

The IND package should include the following assessments:

- Process/manufacturing consistency, batch-to-batch variability, and complexity of manufacturing
- Effect of CMAs and CQAs on CPPs (in process testing and variability)
- Understanding of mechanisms of action and their links to measurable product attributes
- Risk–benefit analysis of safety and efficacy

The CMC is among the most critical components of CGT product development and IND/BLA applications. An incomplete CMC package can be a significant impediment in expediting the development of CGT products. Regulatory assessment of CGT product testing occurs on a case-by-case basis, depending on current scientific knowledge, regulatory precedents and experience with similar products and indications, the phase of product development (e.g., preclinical, Phase 1, end-of-Phase 2), and the benefit–risk profile in the target patient population. The FDA has shown considerable flexibility in CMC regulatory requirements for CGT products; however, one should be aware that these requirements usually increase and become progressively more stringent as the product development program advances toward marketing.

Manufacturing of CGT products to support IND studies and BLA submission involves many materials, procedures, and challenges [2,8,18,28,30,31,33–35,52–54]. Therefore, FDA requires thorough descriptions of processes and procedures, controls, and testing. Retaining a CMC consultant available to facilitate the development of the overall CMC strategy, including upstream and downstream unit operations can be valuable in advancing to an IND/BLA enabling phase-appropriate clinical trial. Well designed, early-phase clinical trials are essential in establishing the regimen and design of the late-phase clinical trials. This can also shorten the development period [9].

During Phase 1 studies and early investigational studies, sponsors may follow the Phase 1 GMP guidance, which outlines CBER's expectations for Phase 1 studies [9]. This guidance provides information on how the manufacturer's quality unit controls and documents operations in the facility, as well as how product quality is maintained. In this case, a risk assessment may be used to qualify materials. FDA also recommends that sponsors verify critical materials such as plasmids or cell banks, which could jeopardize the manufacturing process and delay clinical development [8,9,17,28–31].

At the start of Phase 2 clinical trials, there is an expectation that manufacturers will operate under GMP requirements outlined in accordance with 21 CFR 211. Compliance with GMP requires that all starting and ancillary materials used in the manufacture of the drug product are tested to verify identity when they come into the GMP facility. 21 CFR 211.84, codifies compliance to specifications of identity, purity, strength, and quality. As an alternative, a sponsor may rely on qualified vendor information of a Certificate of Analysis for confirmation of purity, strength, and quality. If the vendor performs a specific identity test, the sponsor still retains the responsibility to verify identity. Verification of identity can help build confidence in the supplier through a history of quality assurance and testing verification. As part of vendor qualification sponsors should be performing

a risk assessment for suppliers of critical ancillary and raw materials.

RECENTLY ISSUED REGULATORY GUIDANCE PERTINENT TO RAW AND STARTING MATERIALS

ISO 20399:2022 Biotechnology—ancillary materials present during the production of cellular therapeutic products and gene therapy products [24]

Ancillary materials (AMs) refer to materials that come into contact with the cellular therapeutic product during cell processing but are not intended to be part of the final product formulation. ISO 20399-2022 was prepared by ISO technical Committee ISO/TC 276, Biotechnology to replace ISO/TS 20399-1:2018, ISO/TS 20399-2:2018, and ISO/TS 203-3:2018. The main changes for this new ISO standard included the merging of the three parts of the ISO 20399 series, changes to the definition of key terms including 'ancillary product' and 'cellular therapeutic product' and addition of key concepts, animal derived components, mutual responsibilities and qualification. Most importantly,

BOX 2

Recommendations of responsibilities and responsible parties.

Responsibilities of the supplier:

- Provide documented evidence that the AM is safe with respect to source-relevant animal disease (e.g., BSE/TSE)
- Prepare and submit a master file for AM
- Assess the stability of the AM
- Inform the AM user of any changes that will very likely or with certainty impact the AM (under a quality agreement)
- Conduct an assessment of the AM container closure system
- Provide a Certificate of Analysis (CoA), Certificate of Origin (CoO), and Safety Data Sheet (SDS) for the AM

Responsibilities of both the AM supplier and the AM user:

- Conduct characterization testing of the AM and prepare a specifications document (e.g., identity, purity, functionality, viral contamination, and animal origin)
- Execute a quality and supply agreement

Responsibilities of the AM user:

- Provide user requirement specifications to the AM supplier
- Conduct a risk-based AM supplier qualification process, generally including initial screening onsite audit formalized approval, continuous monitoring/oversight
- Determine if biocompatibility, biodistribution, cytotoxicity, or adventitious agent testing is needed (or if testing results are available from the AM supplier, if applicable)
- Conduct a risk assessment for the use of an AM, based on information provided by the AM supplier, or in collaboration with the AM supplier
- Establish similar assurances and plans for alternative suppliers
- Qualify the performance of the AM in the intended application
- Confirm the CoA test result (s) critical to the cell product (e.g., functional assay)
- Assess the effect of lot-to-lot variation of the AM on the final cell product
- Establish and implement a qualification plan for the use of an AM

Extracted from ISO 20399:2022 *Biotechnology—ancillary materials present during the production of cellular therapeutic products and gene therapy products* [24].

there was a revision and rearrangement of requirements and recommendations with emphasis on clarifying responsibility of involved parties and emphasis of a risk-based approach. This standard is a good starting point in planning and developing raw material acquisition program during early phase product development. Box 2 derived from the standard describes the responsibilities of the supplier and user in developing a robust program for managing raw material supply.

US FDA voluntary consensus standards recognition program for regenerative medicine therapies (October 2023) [53]

The FDA issued a Guidance for Industry in October 2023 the *Voluntary consensus standards recognition program for regenerative medicine therapies*. This program is designed to identify and recognize standards that can facilitate the development and assessment of regenerative medicine therapy products regulated by CBER when such standards are appropriate. Modeled after a similar program enacted by CDRH's Standards and Conformity Assessment Program, it is CBER's position that the use of these Voluntary Consensus Standards can help sponsors more efficiently meet regulatory requirements. The benefits of this program will:

- Provide a mechanism to confirm early in a product's development whether FDA will recognize a standard.
- Allows sponsors to streamline their product development processes upfront using recognized standards, saving time and reducing the chance that the processes will need to be revised later.
- Reduces guesswork in selecting appropriate standards for use by highlighting standards vetted by FDA, increasing regulatory predictability.
- Improves efficiency of regulatory review, enabling products to get to market faster.

As of June 14, 2024, CBER has recognized 19 standards emanating from the following standards setting organizations: ASTM, ISO, ANSI, and PDA. Recently International Standard ISO 20399, entitled, *Biotechnology—Ancillary materials present during the production of cellular therapeutic products and gene therapy products* was added to the list of recognized standards [24].

US FDA Draft Guidance for Industry: Considerations for the use of human and animal derived materials in the manufacture of cellular and gene therapy and tissue-engineered medical products (April 2024) [54]

In order to minimize the risk of human and animal derived materials during product manufacturing the US FDA has issued this Draft Guidance for Industry. The guidance covers reagents, feeder cells and excipients and those materials used to manufacture these ingredients that come into direct contact with the CGT starting material. The use of these materials at any point in the manufacturing process can affect the safety, potency, purity, and stability of the final product. This guidance emphasizes that these materials should be thoroughly characterized and described in regulatory submissions.

The guidance also provides instruction as to the information on these materials needed for regulatory submissions, specifically in the IND or Common Technical Document (CTD) organizational structure. FDA requests that a list of all materials used in manufacturing and a description of the quality and grade of these materials

should be provided in tabular format. CGMP regulation require identity testing of materials and specific tests should be used. FDA clearly states in this guidance that *"although the production of an investigational drug for use in a Phase 1 study is exempt from compliance with 21 CFR part 211, manufacturers must follow statutory cGMP required under section 501 (a)(2)(B) of the FD &C Act and you should consider implementing identity testing, even during Phase 1 clinical investigations, in order to minimize any unintended compromise to product safety or quality."* Each material should identify the manufacturer, catalogue number, source (e.g., human, animal, bacterial, insect), grade and stage at which the material is used in the manufacturing process.

US PHARMACOPEIA GENERAL TEST AND GENERAL INFORMATION CHAPTERS

For over 200 years the *US Pharmacopeia* has provided standards to ensure the quality and safety of pharmaceutical products. The *USP* works with regulators and developers of cellular and gene therapies to solve problems that can be addressed through standardization. These standards take the form of documentary best practices as General Information chapters or standardized methods as General Test chapters. *USP* also produces physical reference standards for sponsors to aid in calibration of assay methods and for system suitability testing to monitor assay performance. FDA and *USP* work together to identify areas for monograph or general chapter development where there is a need for quality issues to be addressed. These interactions lead to a more efficient standards development process and become an integral part of the patient safety framework. The Federal Food, Drug and Cosmetic Act (FDCA) expressly recognizes *USP* quality standards for medicines.

To support cell and gene therapy development *USP* has produced a number of General Test Chapters and General Information Chapters specific for cell and gene therapy product development. FDA has also issued a number of guidances including many that deal with starting, raw and ancillary materials, e.g., allogeneic cells, human and animal derived materials. *USP* citations appear in many FDA guidance documents including *USP* <63> (Mycoplasma test), *USP* <71> (Sterility Test), and *USP* <1223> (Validation of alternative microbiological methods). The International ISO standard 20399, *Biotechnology—Ancillary materials present during the production of cellular therapeutic products and gene therapy products* refers to *USP* <1043> (Ancillary materials for cell, gene, and tissue-based products) and *USP* <1240> (Virus testing of human plasma for further manufacture). A listing of relevant 'Best Practices' General Test and Information Chapters is shown in Box 3.

ADDRESSING THE REALITY OF THE SUPPLY CHAIN ECOSYSTEM

The continuing development of standards emanating from the *US Pharmacopeia* and with the issuance of ISO 20399:2022 supporting the needs of these critical therapeutic modalities for our patients, suppliers of raw and ancillary materials should establish strict quality management systems to meet international standards of quality in accordance with ISO 9001:2015. In qualifying raw material suppliers, experienced auditors retained by the developer can assess the culture of quality of the supplier and commitment of senior management in ensuring the quality of its products and services. To assess this commitment questions should be asked to determine the following:

- Has the supplier made a strategic decision ensuring that quality is

BOX 3

General test and information chapters in the *US Pharmacopeia* providing 'Best practices' guidance for the development of CGT products.

General Test Chapters:

- *USP* <1>Injections
- *USP* <61> Microbial Enumeration Tests
- *USP* <63> Mycoplasma Tests
- *USP* <71> Sterility Test
- *USP* <72> Respiration-Based microbiological Methods for the Detection of Contamination in Short-Life Products—USP NF 2025 Issue 2 (Feb 2025)
- *USP* <73> ATP Bioluminescence-Based Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products—USP NF 2025 Issue 2 (Feb 2025)
- *USP* <74> Solid Phase Cytometry Based Rapid Microbial Methods for the Detection of Contamination in Clear Aqueous Solutions—USP PF 49(5) (In Development)
- *USP* <77> Nucleic Acid Amplification Tests—USP PF 48(5) (In Development)
- *USP* <85> Bacterial Endotoxins
- *USP* <86> Bacterial Endotoxins Test Using Recombinant Reagents
- *USP* <89> Enzymes Used as Ancillary Materials in Pharmaceutical Manufacturing
- *USP* <90> Fetal Bovine Serum—Quality Attributes and Functionality Tests
- *USP* <92> Growth Factors and Cytokines Used in Cell Therapy Manufacturing
- *USP* <127> Flow Cytometric Enumeration of CD 34+ Cells
- *USP* <785> Osmolality and Osmolarity
- *USP* <788> Particulate Matter in Injections
- *USP* <790> Visible Particulates in Injections
- *USP* <791> pH

General Information Chapters:

- *USP* <1023> Evaluation Strategy for Trace metals in Cell Culture Media Used in the Manufacture of Recombinant Therapeutic Proteins
- *USP* <1024> Bovine Serum
- *USP* <1043> Ancillary Materials for Cell, Gene, and Tissue Engineered Products
- *USP* <1046> Cell Based Advanced Therapy and Tissue Based Products
- *USP* <1047> Gene Therapy Products
- *USP* <1050> Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
- *USP* <1071> Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products—A Risk Based Approach USP NF 2025 Issue 2 (Feb 2025)
- *USP* <1114> Microbial Control Strategies for Cell Therapy Products (In Development)
- *USP* <1223> Validation of Alternative Microbiological Methods
- *USP* <1240> Virus Testing of Human Plasma for Further Manufacturing

guaranteed through a third-party audited, efficient, and standardized system for its production processes?

- Has a clear organizational structure with department functions been established, specifying the relevant responsibilities and authorities of each position and necessary interactions to ensure that a quality policy and objectives have been implemented?
- Has the organization adopted a problem-solving strategy such as

the PDCA (Plan, Do, Check, Act) cycle with risk-based thinking that can proactively respond to market competition and continuously strive to improve its overall efficiency through an understanding of the needs and expectations of its customers?

- Does the quality policy of the supplier focus on the customer's needs and understand the applicable statutory and regulatory requirements to be an important and valued partner in the field of cellular and gene therapies?
- Is the company willing to cooperate with customers to create a collaborative environment for the continued development and improvement of the therapy?
- Can the organization produce GMP-grade products in a pharmaceutical-grade manufacturing plant, designed and controlled in compliance with relevant domestic and international regulations?
- Are the systems that underpin GMP including a material system, manufacturing system, facility/equipment system, packaging/labeling system, and laboratory quality control system established and documented by robust standard operating procedures?
- In response to the challenges that impede the growth of the cell and gene therapy industry can the company provide customers with enhanced professional support to address anticipated or unanticipated concerns such as fluctuations in the quality of GMP materials, vulnerabilities in the supply chain, or regulatory uncertainties?

CONCLUSIONS AND SUMMARY

In order to understand and meet regulators' expectations, the manufacturer of a cellular and gene therapy product should establish a Quality Management System that includes a raw materials qualification program. This entails the creation of a robust raw materials risk assessment process, including supply chain risks. Identification of CQAs, CMAs, and CPPs by defining how each raw material is used, especially those most critical for the process. Ensure quality upfront by auditing the supplier and by reviewing the supplier's certificates of compliance and supportive data. Identify those reliable raw material suppliers that can provide consistent quality with a reliable and sustainable supply chain. Have a plan to review your quality agreements and standard operating procedures that are in place for material and vendor qualification. Forecasted needs should be shared with your suppliers to ensure they can cope with increasing demand.

Begin early-stage process development with preclinical or GMP grade raw materials allowing for a seamless transition from preclinical to clinical development and beyond. If possible, avoid 'For Research Use Only' materials.

Develop and validate assays for source materials especially those identity tests for critical ancillary materials as early as possible in the pre-clinical product development process. Understand the assay's parameters and the point at which variability can occur. This makes it possible to create an assay protocol that promotes comparable inter-laboratory results. Using known reference and patient samples establish acceptance criteria before clinical trials. Evaluate lot-to-lot consistency especially when transferring technology and whenever the manufacturing process changes. This will lead to better decision-making at each step along the translation process and more confidence that an observed effect is reproducible in later clinical investigational phases.

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CELL & GENE THERAPY INSIGHTS

RAW/STARTING MATERIALS

Streamlining the supply chain: insights on raw material sourcing and quality by design

INTERVIEW

"...maintaining quality by design is especially difficult in CGT because we have variable starting material."

Abi Pinchbeck, Editor, BioInsights, speaks to **Lara Silverman**, Founder and Principal Consultant, LIS BioConsulting, discussing the critical role of supply chain management in early-stage cell and gene therapy companies, including raw and starting material sourcing strategies. They cover the importance of QbD in advanced therapies, regulatory expectations surrounding the supply chain, and how evolving technologies can help drive down manufacturing costs.

Cell & Gene Therapy Insights 2025; 11(2), 205–210 · DOI: 10.18609/cgti.2025.025

Q What are you working on right now?

LS Currently, I am working with multiple clients that are struggling with tech transfer into their CDMOs. It is a difficult phase in the development pathway of a company. Oftentimes, investors push to speed up timelines, and sometimes this leads to attempting tech transfer to a CDMO too soon, before the researchers have a solid understanding of the key drivers of their process. That being said, they do have to move

"A decade ago, it was challenging to find high-quality reagents, especially of the kinds used in cell therapy. Now, media suppliers are working with earlier-stage companies and getting these reagents into the processes earlier."

forward, and it is that push and pull with investors that can keep us moving at a good pace. Otherwise, researchers can spiral in research forever. That push and pull is a normal, natural, and welcomed part of the process.

Q Your consulting work often revolves around helping early-stage cell and gene therapy (CGT) companies. When it comes to raw and starting materials, what are the common challenges you see these companies facing during their development phase?

LS Most groups I work with are early-stage spinouts from universities or groups that have not necessarily taken something into the clinic yet. Many people take reagent sourcing for granted. There is a lack of education around raw material attributes and what quality actually means. It is not for a lack of wanting; these groups want to source good reagents, but there is a lack of knowledge around what they should source.

Q Can you share your perspective on the current state of the supply chain for raw materials in the CGT space? Are there any specific bottlenecks or challenges that companies should be particularly mindful of?

LS Compared to 10–15 years ago, there is a great deal more availability of high-quality reagents, and much more understanding from media suppliers of what cell therapy companies need. A decade ago, it was challenging to find high-quality reagents, especially of the kinds used in cell therapy. Now, media suppliers are working with earlier-stage companies and getting these reagents into the processes earlier. This is fantastic, as comparability challenges can be large in the event you need to change reagents later on.

Another thing I have noticed is that there are fewer low-quality reagents available. If you are looking to source reagents, many have different quality attributes, testing, and virus information available, which makes the transition process much easier. Overall, both the supply and the quality of the supply have improved. I saw a couple of bad incidences 5–10 years ago where single-source reagents were not available due to a manufacturing issue. Now, I do not hear of that as much. There is more redundancy and more awareness from suppliers about the need to have continuity.

There is also more awareness from researchers around the risks with human and animal components. People are stripping out fetal bovine serum more and more. A lot of folks are still using human albumin, which comes with its own issues, but getting the bovine and porcine reagents out earlier is simplifying things down the road.

"Developers need to understand and work with suppliers to know how many batches they make, and consider talking to vendors about custom manufacturing."

Q With the increasing demand for raw materials driven by the growth of CGT, how can companies maintain a sustainable and scalable supply chain without compromising on quality?

LS There is often R&D folklore with hard rules around how to supply and exchange media in a culture process. It is often taken for granted that you must change media fully every 2 days. If there is the time and the budget, maybe you can experiment to find out that you only need to do half a media exchange every 4 days, for example, or that there is another way to handle ancillary materials that significantly reduces the volume.

People often do not have the time and money to perform a design of experiments (DoE) study for their media. If this is not completed early, it is difficult to do down the road. If a process demands liters and liters of media, it is difficult to not only source that, but also to store it. Particularly for allogeneic products or anything that requires large amounts of media and processes you intend to scale up significantly, early optimization and considering your volume of media early is critical.

Another key requirement is to risk assess reagents and identify backup sources for the critical reagents, or ones you need in large quantities. Sometimes, reagents are made in small batches, and if there is only one batch available, a backup source from a different vendor will be required. Developers need to understand and work with suppliers to know how many batches they make, and consider talking to vendors about custom manufacturing.

Q How does quality by design (QbD) impact the selection, testing, and qualification of raw materials in the development of CGT?

LS The reality is that QbD is not employed nearly enough in CGT, especially in the early stages, due to a lack of awareness and education. Many people who were brought up in the CGT space might have not been exposed to QbD. There needs to be more implementation of robust QbD in our space, as it is expected by the regulators.

The truth is that maintaining QbD is especially difficult in CGT because we have variable starting material. QbD was originally created for completely controlled processes. We have to recognize that in CGT, we have unique challenges related to the starting material. However, QbD can actually bring down costs; if you spend the time and money to implement it, it can focus your team on the high-risk reagents, and reduce wasting time on other reagents that will not hugely impact a process.

In development, QbD principles should be applied to raw materials immediately, before Phase 1. These principles allow the identification of key raw material attributes that will be monitored, tested, and qualified. All of those things—monitoring, testing, and

"I have seen companies make seemingly innocent reagent changes, but the results are catastrophic..."

qualifying—take time and money. As an early-stage company, you have to be judicious in terms of where you place your focus and attention. QbD is a tool that lets you pick where to place your attention. Without a structured approach to prioritization, it can become overwhelming and very expensive. It is a necessary activity right out of the gate.

Q How important is it for early-stage companies to develop a robust raw material sourcing strategy early in their product development lifecycle, and what advice do you give to companies that may be unsure about when to start this process?

LS A vast majority of early-stage companies in CGT leverage CDMOs. My advice is to lean into the expertise of your CDMO and into their systems to manage raw material sourcing. A great line of questioning when interviewing a potential CDMO is around their sourcing team and strategy, because this is likely is outside of the expertise of your internal staff and something that you will need to leverage at the CDMO.

You cannot start too soon with this. I have seen companies make seemingly innocent reagent changes, but the results are catastrophic, for example cells completely not growing, due to a lack of characterization data to understand the impact of changing a reagent.

My advice would be to spend money on GMP reagents early on. Switching from a research-use only (RUO) to the GMP reagent can lead to differences in excipients, stability, and manufacturing processes. It is key to understand the difference between the RUO and GMP reagents and it is worth spending money on the GMP if there are any differences between those two reagents.

Q The transition from preclinical to clinical phases is a critical time for any CGT company. How would you advise developers align their raw material strategies with regulatory expectations from agencies, including the FDA, during this stage?

LS The *USP* <1043> is an excellent guide for selecting and managing reagents. The lower the risk score, the less headache a reagent will likely be. Ensuring reagents come from reputable companies that provide robust documentation is absolutely critical. The *USP* guide helps to inform and educate on what those things are.

An important note when selecting your excipients is that there is a higher regulatory burden on these than on ancillary materials. Picking either *USP* or compendial grade reagents or those that have a Drug Master File (DMF) will simplify your life down the road, because more regulatory information is needed on the manufacturer production and testing of those excipients.

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Q Finally, as the field continues to evolve, what emerging trends or technologies do you predict will influence the raw material landscape for CGT in the next 3–5 years? How can companies stay ahead of these trends to ensure a smooth path from development to clinic?

LS As more characterization tools come to market, especially process analytical tools (PATs), and as QbD becomes more widely used and executed properly, there will be a much better understanding of what drives process success. It will also help us manage our raw material procurement in a much more streamlined and sophisticated way.

We can then start to focus our energy on the variables that impact our process versus the ones that distract us. That means overall cost will come down. As manufacturing costs come down along with these new technologies, novel media components will become less expensive as they themselves will be manufactured with less expensive techniques and analytics.

As an industry, we recognize these medicines are too expensive right now. A lot of that cost is driven directly by the cost of goods and manufacturing. I am seeing progress in terms of creative thinking around how to bring costs down in our manufacturing efforts in regards to reagents. This is a trend we need to see in the industry if we are going to start treating larger patient populations and providing these products to a much broader market.

BIOGRAPHY

Lara Ionescu Silverman is Founder and Principal Consultant at LIS BioConsulting, Salt Lake City, UT, USA, which has provided strategic advising and consulting services to early stage cell and gene therapy companies since 2021. Additionally, Dr Silverman is a strong advocate for women in biotech, and runs a Women in Science community called 'I Wish I Had Known', with monthly roundtable webinars interviewing women in science. Dr Silverman spent 10 years as the Head Scientist at an allogeneic cell therapy company prior to running her own consulting business. She received her PhD from University of Pennsylvania, Philadelphia, PA, USA in Bioengineering and her BSE from Princeton University, Princeton, NJ, USA in Chemical Engineering.

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CELL & GENE THERAPY INSIGHTS

ANALYTICS

Navigating analytical and regulatory hurdles in early-phase clinical cell therapy development

INTERVIEW

"Patient safety and regulatory compliance remain the ultimate priorities in my work."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Vaibhav Patel**, Director of Quality Assurance and Regulatory Affairs at the University of Minnesota, about the analytical and regulatory challenges of cell and gene therapies, particularly in early phase development. This includes the adaptation of analytical methodologies from early phase into commercial manufacturing and the increasing emphasis on post-marketing surveillance data as well as other regulatory hurdles.

Cell & Gene Therapy Insights 2025; 11(2), 377–380 · DOI: 10.18609/cgti.2025.044

Q What are you working on right now? Could you describe your role at the University of Minnesota and your work in the quality assurance and regulatory affairs of cell and gene therapies (CGT)?

VP Currently, I work at the University of Minnesota, where I am Director of Quality Assurance and Regulatory Affairs. Mainly, this facility serves all the investigators at the university who wish to manufacture products suitable for clinical

trials. My role involves ensuring that the investigational products are manufactured in compliance with the US FDA's GMP and Good Tissue Practice standards for CGT. My work primarily focuses on early-phase clinical trials—Phase 1 and 2 INDs—where I ensure the quality and regulatory alignment for patient safety and successful trial progression.

Q What are some of the key analytical challenges that you face when transitioning cell therapies between clinical trial phases?

VP During transition between early clinical phases (Phases 1 and 2) in cell therapy development, the main challenges that we encounter revolve around product variability. Unlike traditional biologics, cell therapy has inherent donor-to-donor variability and complex biological behaviors. This makes analytical characterization a little more challenging than traditional biologics, especially when it moves into a later phase.

Another challenge is assay reproducibility. Early-phases are exploratory in nature. As we know more about the product, our methods need to evolve accordingly. Reproducibility, robustness, and precision are all essential when it comes to convincing regulatory agencies of a product's suitability. Sometimes it can be challenging with the evolving nature of the product, as well as the assay.

Another major challenge within the industry at the moment surrounds potency assays. Working in late phase trials, we need more buy-in from regulatory agencies because this is an evolving field, and we need to improve our practices regarding potency assays.

Q How can analytical methodologies be adapted to address changing requirements effectively as a product moves through the later stages of development and into commercial manufacturing?

VP When CGTs progress from early to later stages of development, analytical methods must evolve to address the regulatory expectations with regard to process scale-up and commercial manufacturing challenges. Because of this, product critical quality attributes (CQAs) have the potential to change, which means the methods need to evolve, ensuring that they remain compliant. It is important to continually qualify or validate these methods to match the pace of drug development.

In the long run, the adoption of automated processes during development would reduce the variability and ensure scalability for commercial production. However, not all companies can afford automation now.

Q What role do new technologies, such as process analytical technologies (PAT), play in ensuring the manufacturing consistency and safety of cell therapies?

VP PAT has the potential to revolutionize cell therapy manufacturing particularly by enabling real-time monitoring, control, and optimization of CQAs.

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"Early engagement with regulators may help streamline some of the decisions and approaches companies use during development."

Given the complexity and variability of these live cell products, this plays a role in real-time process monitoring, which allows for the continuous monitoring of CQAs during production. This drives better product outcome, and it is easy to prove that you have improved process control with PAT. It also leads to a reduction in batch failures, giving you the outcome that you want. It is data-driven; with continuous real-time process monitoring, you have the data to help make the decisions needed to achieve the desired outcomes over time. PAT will also assist in regulatory compliance.

Q With regulatory agencies increasingly emphasizing the importance of post-market surveillance, what analytical strategies do you think will be critical in monitoring the long-term safety and efficacy of cell therapies once they are commercially available?

VP Recently, regulatory agencies have been placing greater emphasis on long-term safety monitoring for CGTs. This is because these types of therapies may have delayed adverse effects, durability concerns, or immune responses. As such, some of the post-marketing surveillance can include long-term patient monitoring. Companies may need to implement a framework to track patient response and efficacy as well as safety profile over an extended period of time, which is required by regulatory agencies.

Another possibility is registry-based tracking, which means developing patient registries for gene modified therapies and using these for systemic safety tracking and signal detection. This is something that is evolving. In the near future, we may see companies coming up with innovative approaches for long-term safety, and will hopefully see improvement in the way companies approach long-term monitoring.

Q In your experience, what are the most common regulatory hurdles in analytical development for cell therapies, and how can developers prepare for them from early development through to approval and beyond?

VP One of the main analytical challenges is the potency assay. Many cell therapies lack well-defined potency markers, which can lead to regulatory delays. All developers should work on establishing some relevant potency assays early in development. You may have multiple assays, but in later stages, you will need proper validation of all those assays.

Another challenge is CMC; ensuring a robust, scalable, and reproducible manufacturing process. During early stages of development, you have limited understanding of the product compared to in later stages. Early engagement with regulators may help streamline some of the decisions and approaches companies use during development.

Q What are your key goals and priorities over the next 1–2 years?

VP My focus over the next 1 or 2 years is primary patient safety, as well as strengthening the regulatory strategies for emerging CGT. Patient safety and regulatory compliance remain the ultimate priorities in my work.

Another goal is to establish and support effective collaboration between academia, industry, and regulators to accelerate these advanced therapies for patients.

BIOGRAPHY

Vaibhav Patel has over 13 years in pharmaceutical quality assurance. He has established himself as a leader in drug development, particularly in cancer therapies, biologics, and gene therapies. Currently, as the Director of Quality Assurance and Regulatory Affairs at the University of Minnesota, Vaibhav ensures FDA compliance for Phase 1 and 2 INDs, specializing in advanced therapies. His work is instrumental in maintaining rigorous GMP standards and navigating complex regulatory requirements to drive innovation in life-saving treatments. Previously, Vaibhav led the quality assurance team at Elucida Oncology, where he pioneered the Quality Management System (QMS) and directed GMP facility start-up for ELU001, a first-in-class nanomedicine. His oversight throughout ELU001's development was critical in meeting FDA standards and advancing the product toward clinical trials, offering new therapeutic options for patients with limited treatments. Vaibhav's expertise also extends to Chemistry, Manufacturing, and Controls (CMC) and compliance strategies for mitigating nitrosamine impurities. His thought leadership on these topics has made him a sought-after speaker, including at the 9th CAR-TCR Summit. Known for his forward-thinking approach, Vaibhav's contributions continue to shape the future of pharmaceutical quality assurance, advancing patient safety and the efficacy of new therapies.

Vaibhav Patel, Director of Quality Assurance and Regulatory Affairs, University of Minnesota, Minneapolis, MN, USA

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CELL & GENE THERAPY INSIGHTS

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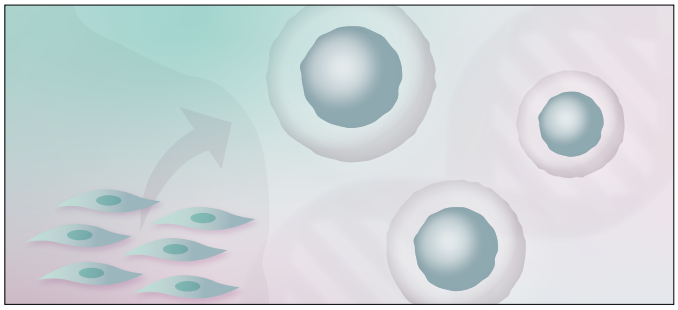

LATEST ARTICLES

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Achieving robust and scalable AAV empty/full capsid separation for gene therapy

Julio Huato Hernandez and Mark Schofield

Efficiently separating empty and full capsids to ensure product purity and vector safety remains a crucial challenge for the adeno-associated virus (AAV)-based gene therapy field. This article highlights the importance of efficient separation techniques, such as ion exchange chromatography, and the development of a two-step elution strategy. A case study is outlined that highlights batch-to-batch reproducibility and fast flow rates suitable for large-scale manufacturing.

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ION EXCHANGE CHROMATOGRAPHY FOR EMPTY/FULL CAPSID SEPARATION

A major challenge for AAV-based gene therapies is that the genetic payload is not always packaged within AAV capsids during upstream production, potentially compromising vector safety, as noted by the FDA [1]. To ensure product purity, it is crucial to adopt efficient full capsid separation techniques.

Alternative empty/full capsid separation techniques include ultracentrifugation, which offers high resolution. However, this method has limited scalability, requires manual handling, and is prone to operational variation. As an alternative, ion exchange chromatography can be utilized for the removal of empty capsids. Isoelectric point (pI) differences between full and empty capsids (ranging from 5.9–6.3) allow separation using anion exchange chromatography (AIEX). This technique offers high flow rates and scalability. Additionally, resin-based techniques can be prepacked and have flexible sizes with unique packing.

OPTIMIZATION OF A TWO-STEP ELUTION STRATEGY

Here, Mustang™ Q membranes and Capto™ Q resin were utilized for empty/full capsid separation. Process development for this separation began by implementing small discrete elution steps (Figure 1A). These steps, incremented by 0.5–1 mS/cm, were used to identify precise separation conditions.

Subsequently, a visual inspection of the 260/280 nm ratios for each peak was

performed. The goal was to detect when the absorbance at 260 nm became higher than at 280 nm, signaling a high concentration of full capsids due to the DNA payload's absorbance at 260 nm. In the chromatogram illustrated, an enrichement of full capsids is evident as absorbance at 260 nm exceeded 280 nm in the second and third peaks.

However, for large-scale manufacturing, implementing such small discrete steps proves challenging. To address this challenge, a two-step elution strategy was optimized. The first step involved eluting the empty capsids, while the second step isolated the full capsid population containing the product of interest (Figure 1B). This process development approach established an efficient path for transitioning from rapid process development to a large-scale-manufacturing-friendly two-step elution method.

ASSESSING SCALABILITY OF MUSTANG™ Q XT MEMBRANE FOR AAV8 CAPSID SEPARATION

A design of experiments was performed with Mustang Q membrane to understand the robustness of the separation [2]. The results from the models were then used to select an optimal operating point, followed by confirmation runs at a small scale. Afterward, the process was scaled up using a Mustang Q XT 140. It was observed that the second peak achieved high enrichment and there was excellent scalability from the 0.86 mL XT Acrodisc™ bench-scale capsule to the 140 mL process scale capsule. The vector genome yield was found to be enriched multiple fold.

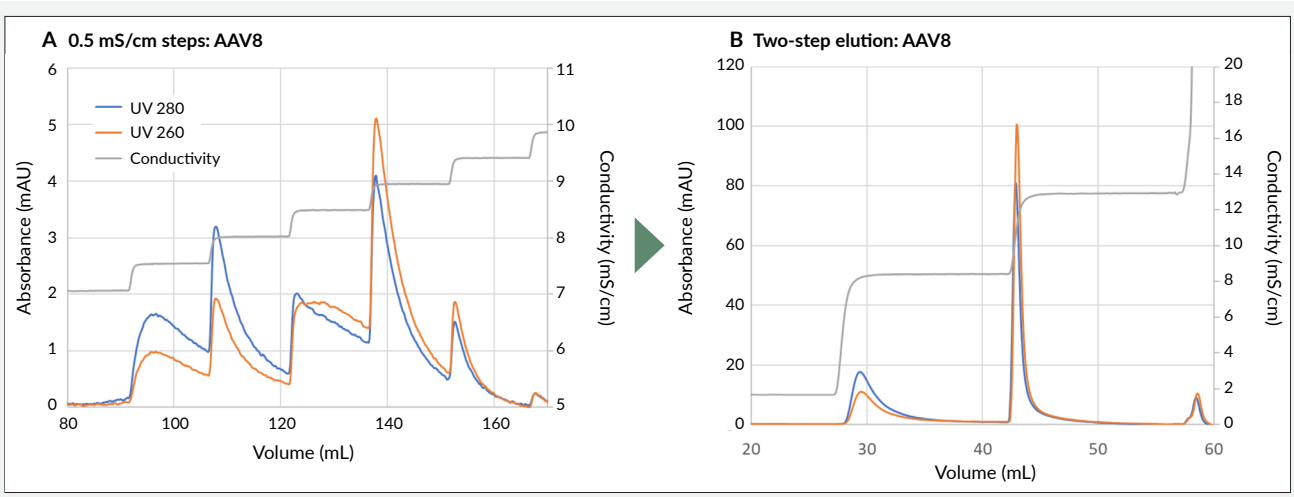
The percent of full capsids, measured by mass photometry, ELISA, and ddPCR, showed successful empty/full capsid separation at both scales. As shown in Figure 2, the chromatograms from both scales appear nearly identical, with very similar UV area ratios of 1.2, confirming the successful scale-up of Mustang Q XT 140 membranes with AAV8.

EVALUATING MUSTANG Q MEMBRANE APPLICABILITY ACROSS VARIOUS AAV SEROTYPES

In order to evaluate whether the enrichment of full AAV capsids with Mustang Q

FIGURE 1

Development of a two-step elution strategy for empty/full capsid separation using Mustang Q membranes and Capto Q resin, with small discrete elution steps identified in (A) and corresponding chromatogram (B) showing the separation of full capsids.



membranes is applicable across various serotypes, the same process development approach was used to investigate AAV5 and AAV9 serotypes. Firstly, the 0.5 mS elution steps were carried out, and two-step elution methods were utilized for both serotypes. As illustrated in Figure 3, successful separation and enrichment were observed for both AAV5 and AAV9. In the case of AAV5, for example, the second peak percent of full capsids, measured by mass photometry, was found to be 83%.

EVALUATING BATCH-TO-BATCH REPRODUCIBILITY

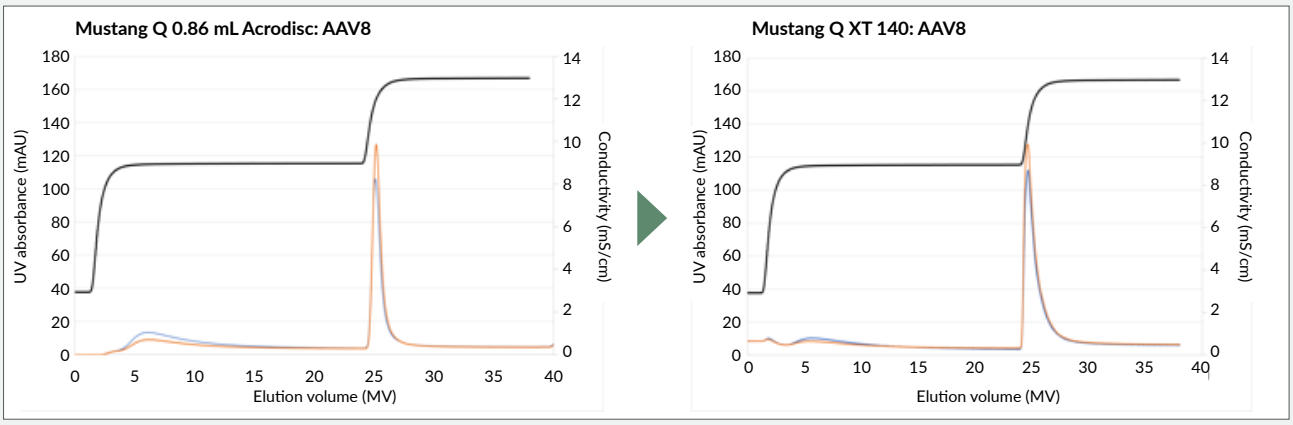
Having demonstrated efficient empty/full capsid separation with Mustang Q membranes across several serotypes and at scale, the next step was to investigate batch-to-batch reproducibility, as it is critical for the successful manufacturing of AAV-based products. To achieve this, a 1 mS step approach was performed on three different batches of AAV5, using three industry-relevant feedstreams. The feedstreams included human embryonic kidney (HEK) suspension made in a stirred tank bioreactor, HEK adherent culture, and SF9 suspension made in a stirred tank bioreactor. Three different Mustang Q membrane batches, labeled as batch 1, batch 2, and batch 3, were tested with these three feedstreams. A total of nine experiments were conducted.

The batch-to-batch variability was then assessed by analyzing the chromatograms for each feedstream. As shown in Figure 4, the Mustang Q membrane demonstrated robust reproducibility across all three batches and the three different feedstreams. Notably, despite the differences in behavior between the feedstreams, reproducibility was consistently maintained.

Furthermore, the batch-to-batch variability was quantified by analyzing the UV 260/280 ratios for all runs across the three

FIGURE 2

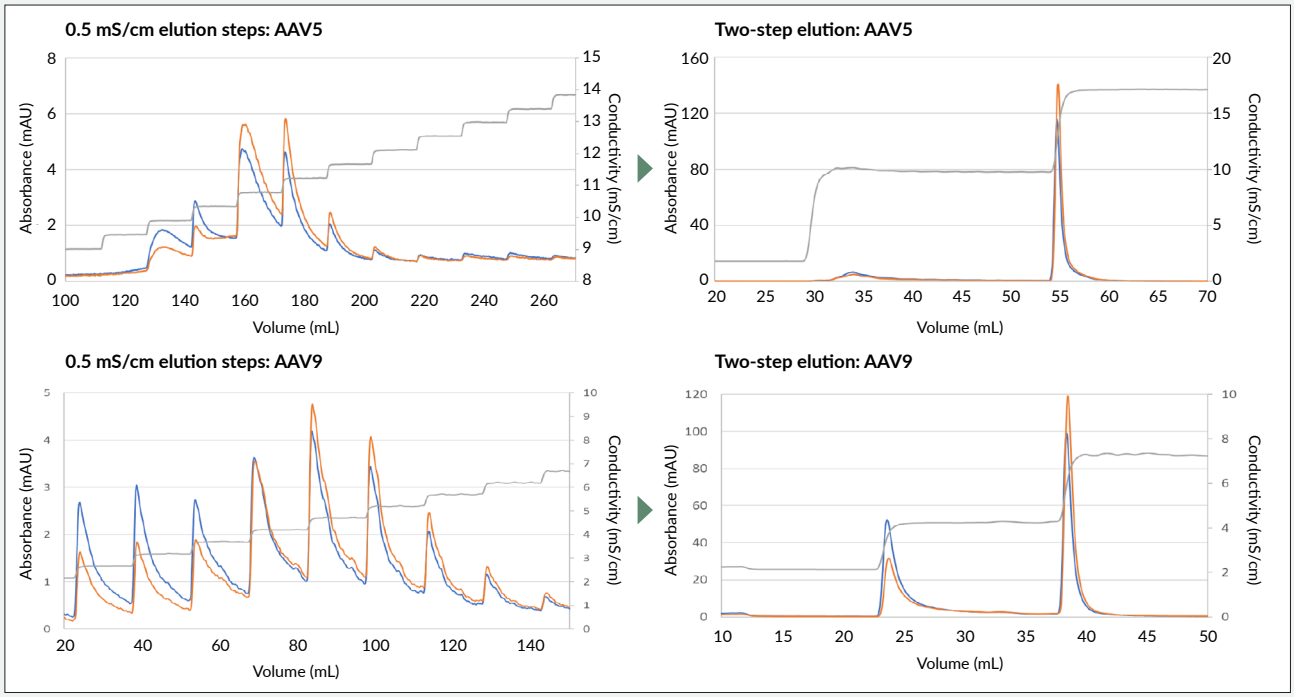
Chromatograms from small-scale and large-scale Mustang Q XT 140 membrane systems.



Metric on full peak	Mustang Q XT 0.86 mL capsule	Mustang Q XT 140 mL capsule
Vector genome yield (%)	71	93
Percent full (ELISA/ddPCR)	68	50
Percent full (Mass photometry)	74	56
Enrichment (fold)	3.4	2.5
UV area ratio	1.2	1.2

FIGURE 3

Chromatograms depicting successful enrichment and separation of full AAV5 and AAV9 capsids using the Mustang Q membrane system.



Mustang Q membrane batches. For each chromatogram, the ratios were calculated for both the main empty peak and the main full peak, as shown in Table 1. The 260/280 ratios across all batches demonstrated highly reproducible empty/full capsid separation performance. While performance was different between feedstreams, the reproducibility across batches remained virtually identical, highlighting the high reproducibility and robustness of Mustang Q membrane.

ANALYZING THE EFFICIENCY OF RAPID CYCLING

Beyond analyzing the batch-to-batch variability of Mustang Q membranes, another goal was to evaluate whether this system could achieve fast flow rates. A potential challenge that could arise is the need to process AAV feedstreams that exceed the capacity of a particular Mustang Q membrane capsule size, without having enough material to sufficiently challenge the larger capsule size. To address this challenge, a proposed solution was to utilize a specific capsule size and cycle it several times back-to-back at high processing flow rates of up to 10 MV/min. This approach was tested by performing nine cycles using three different buffer sets to demonstrate the feasibility and reproducibility across varying buffers. The results illustrated in Figure 5 show efficient AAV capsid separation on Mustang Q XT5 membranes across the nine runs. The 260 nm chromatograms are overlaid for all runs with a total runtime of 180 min, demonstrating the reproducibility of this rapid cycling approach.

Subsequently, the results from each rapid cycle run were grouped by the three independently made buffer sets. ELISA and ddPCR were used to quantify vector genomes and total capsids across all runs. It was observed that the percent of full capsids for each experiment across all buffer sets

FIGURE 4

Chromatograms for the evaluation of batch-to-batch reproducibility of Mustang Q membrane across three AAV5 feedstreams.

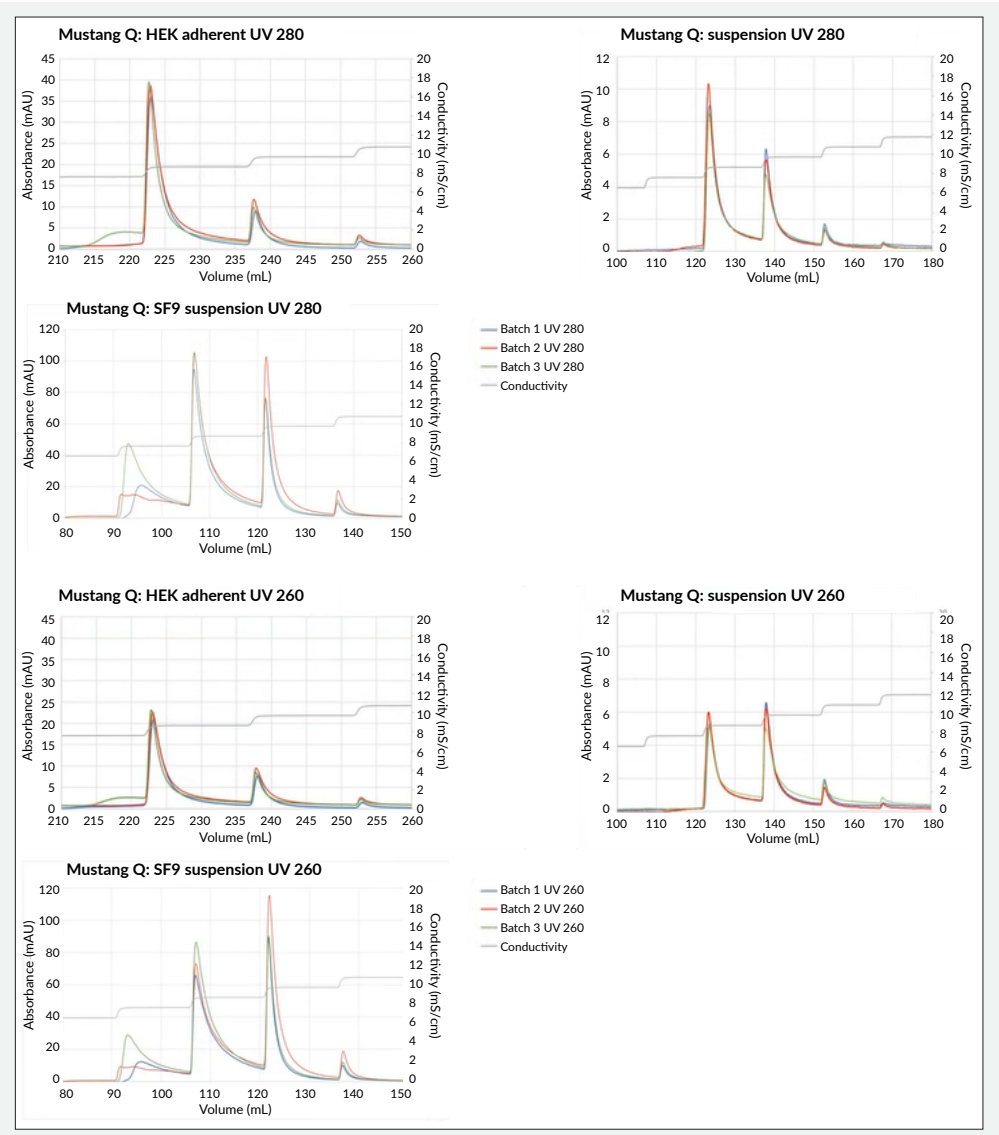


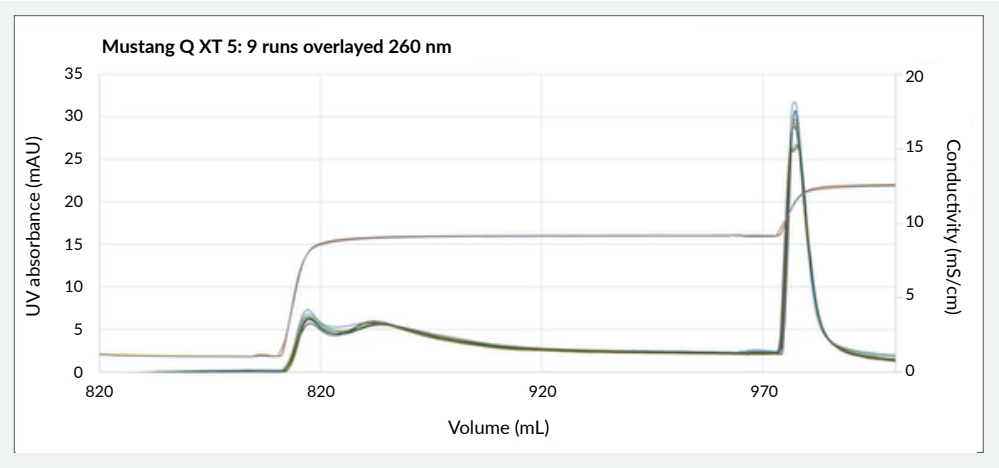
TABLE 1

UV 260/280 empty and full peak area ratios, averages, and standard deviation for batches 1–3, on all feedstreams.

Feedstream	Empty peak UV 260/280 ratios: batch 1, batch 2, batch 3 (Mustang Q average, STD, RSD)	Full peak UV 260/280 ratios: batch 1, batch 2, batch 3 (Mustang Q average, STD, RSD)
Suspension HEK	0.56 0.58 0.59 (0.57 ± 0.016, 3%)	1.08 1.09 1.11 (1.09 ± 0.014, 1%)
Adherent HEK	0.61 0.59 0.58 (0.59 ± 0.017, 3%)	0.83 0.79 0.83 (0.82 ± 0.023, 3%)
Suspension SF9	0.68 0.69 0.82 (0.73 ± 0.077, 11%)	1.18 1.15 1.18 (1.17 ± 0.026, 2%)

FIGURE 5

Evaluation of Mustang Q XT5 membrane's ability to achieve fast flow rates.



was very similar. The same trend was seen for capsids/mL and vector genomes/mL. In conclusion, reproducible results were achieved across all nine experiments when performing rapid cycling, demonstrating the robustness of the Mustang Q membrane.

CONCLUSION

Both Mustang Q membranes and Capto Q resin provide scalable, platformable, and efficient separation of empty and full AAV capsids. Although distinct operating spaces and conditions are required for each serotype, this approach is platformable and consistent across different serotypes. Based on the experiments outlined in this article, the performance of the Mustang Q membrane is consistent across batches and can be used to achieve fast flow rates, demonstrating its reliability and robustness for large-scale applications.

Q&A

Mark Schofield

Q Can rapid cycling at 10 MV/min be carried out with all the Mustang Q XT membrane capsule sizes?

MS We designed a scalable range of products where the devices operate consistently across different capsule sizes, from 1 mL to 5 L. All devices have the same ratio of hold-up volume to membrane area, ensuring scalability between different sizes. These devices can all be operated at 10 MV/min and are expected to behave in the same way.

Q Would reproducibility using the Mustang Q membrane in Acrodisc™ 0.86 mL syringe filters translate to larger devices in the XT range?

MS Instead of making separate membranes for each filter size, we produce one series of membrane batches that can be used in all the filters. As a result, the same membrane is used in both small and large filters. Testing the small filters is a great way to establish proof of principle for the larger filters. Additionally, larger filters have a significantly greater membrane area, which likely helps average out minor differences that may occur in smaller filters, resulting in reduced variability in the larger filters.

Q What is the processing capacity of Mustang Q membrane?

MS The capacity for Mustang Q membrane is approximately 1×10^{15} capsids/mL, which is consistent with what we expect from a convective media. Unlike resins with diffusive pores, Mustang Q membranes do not rely on diffusion for binding. Instead, all the binding occurs on the surface, and the membrane has a large surface area available for this process. As a result, Mustang Q membranes achieve very high capacities, potentially even higher than some resins, which may have diffusive pores.

Q What factors come into consideration when choosing between Capto™ Q resin or Mustang Q membranes?

MS Each product has its place in the purification toolbox for developers. Choosing between them can be difficult and perhaps the best way to decide is to test both. They each behave differently and offer unique features, meaning one might be more suitable than the other for a specific separation.

From what we have observed, Capto Q resin might perform slightly better when the separation is very challenging. For example, if one starts with a very low percentage of full capsids and a five- or tenfold enrichment is required, Capto Q resin may offer better results in these deep separation challenges.

On the other hand, Mustang Q membranes have several advantages. As mentioned, its higher capacity, approximately 1×10^{15} capsids/mL, is about an order of magnitude higher than that of some resins. Furthermore, rapid cycling with Mustang Q membranes at 10 MV/min allows for multiple cycles in a short amount of time. Lastly, Mustang Q membranes enable process intensification because you can cycle quickly and get a result in minutes.

Q Can the response surface DoE approach also be applied to Capto Q resin?

MS Yes, the same approach can be applied to any of the sorbents. It can be beneficial to take the DoE approach, as we did with Mustang Q membranes,

because it allows developers to better understand the design space. We found that there is a good range in which we can operate, typically over 1 mS/cm or more. While it can still be challenging to prepare those buffers, we found that we can do so reliably and reproducibly, achieving repeatable performance.

Q Most process development for the two-step elution strategy focused on optimizing the first elution step. How was the second elution step selected?

MS We focused a lot of our work on the first elution step because it is generally the most challenging part when eluting the empty capsids. However, when we dived deeper into the full capsid peak, we noticed that there were different populations of material present within that 'full' peak. It is still not entirely clear what some of these additional peaks represent. Some hypotheses suggest they could be misfolded or aggregated virus particles that bind more strongly and elute very late in the salt gradient. Therefore, conducting 1 mS/cm conductivity tests is important to gain a clearer understanding and to accurately identify the different populations, some of which contain DNA, while others do not. These non-DNA peaks are also worth removing, and they can be effectively separated through this process.

Q What about the small pI difference between empty and full capsids, which can be as little as 0.1? What is an effective ion exchange strategy to separate them, and how can we accurately detect the pI of both empty and full particles?

MS The amount of published data on the pI of AAV capsids is very limited. Some studies suggest that empty capsids have a pI of approximately 6.3 and full capsids have a pI of 5.9 [2]. We also hear from customers who have only 0.1 pI difference between empty and full capsids. This small difference highlights the challenge of separating them effectively. Additional complexities include capsid heterogeneity. For example, different capsids within the same batch can have varying VP1, VP2, and VP3 ratios that may have formed randomly. Charged variants of capsids may also be detected, which adds further challenges to the process.

Due to these complexities, the strategy comes down to finding conditions that allow for the separation of these various particles. Techniques such as AUC may be utilized, but it may not be the fastest and simplest method. Therefore, AIEX chromatography with small conductivity steps is the optimal strategy for process development. By using increments as small as 0.5 mS/cm or 1 mS/cm, slight pI differences between the two populations can be resolved.

Finally, detecting the precise pI of capsids, while academically interesting, may not always be necessary for practical purposes. While methods such as capillary electrophoresis may be used to assess pI, separation efficiency matters the most, and it can be achieved with AIEX chromatography with small conductivity steps.

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Q What is the performance of Capto Q resin and Mustang Q membranes when separating partially filled capsids?

MS We have not directly investigated partially filled capsids with Mustang Q membranes. Instead, we focused on comparing ddPCR, ELISA, and mass photometry data, and none of these techniques provide information on partially filled capsids. However, our sister group in Uppsala, Sweden, led by Åsa Hagner-McWhirter obtained some AUC data showing some removal of partially filled capsids with Capto Q resin [3]. It is unclear whether the data completely resolves the mass balance issue though, which is a significant challenge in analytics.

The discussion around partial capsids may be less prominent now than it has been in the past, but I believe the future focus will shift toward understanding the heterogeneity and variations within capsids themselves. One of the key future challenges will be to better understand these differences and work towards a more homogeneous product.

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BIOGRAPHIES

Julio Huato Hernandez is a bioprocess R&D engineer at Cytiva, Boston, MA, USA. He holds a Bachelor's degree in Chemical Engineering from Northeastern University, Boston, MA, USA. He has been working at Cytiva for 5 years centering on viral vector and mAb downstream applications. His work has a focus on membrane-based separations.

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Mark Schofield earned his degrees in Scotland, he received his Bachelor's degree from the University of Edinburgh, Edinburgh, Scotland, UK, and his Molecular Biology PhD from the University of Dundee, Dundee, Scotland, UK. For the last 12 years he has been an employee of Pall and now Cytiva, Marlborough, MA, US, focusing on chromatography applications. Currently he holds the position of Senior R&D Manager, his team works on bioprocess intensification solutions and chromatographic separations for gene therapy modalities.

Mark Schofield, Senior R&D Manager, Cytiva, Marlborough, MA, USA

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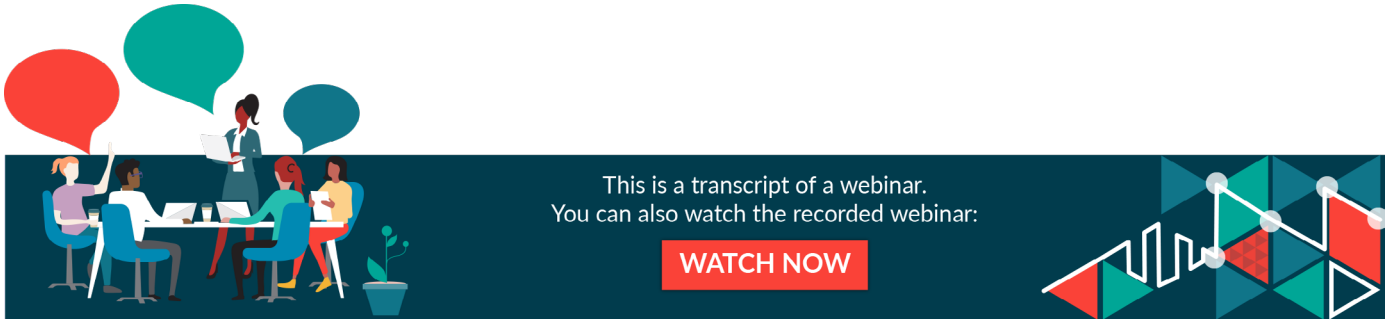

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Robotic gloveless isolator technology enhances regional production of advanced therapeutics

Zach Hartman and Peter Boman

To meet growing demands of advanced therapy manufacturing, production architectures must shift towards agile, efficient manufacturing systems. Robotic gloveless isolator (RGI) technology addresses the challenges faced by CDMOs and other manufacturers, particularly in aseptic filling, a critical step in advanced therapy production. By integrating RGI technology into bioproduction, manufacturers can meet the needs of plasmid DNA, viral vector, and mRNA-based medicines while maintaining compliance with stringent regulatory standards.

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INTRODUCTION

The complexity of developing and manufacturing biologics has not deterred their strong growth, and regional manufacturing must keep pace to ensure these modern medicines are available where and when they're needed. In 2022, the number of approvals of biologics outpaced small molecules for the first time ever, accompanied by a 12–13% increase in outsourcing to CDMOs [1,2].

To meet the demands of manufacturing these advanced therapeutics—smaller batch sizes, higher drug substance cost, stricter regulatory guidelines, aseptic fill-finish processes, among others—CDMOs must reframe their production architectures to overcome challenges, guide sponsors through the process, and ensure sufficient supply for patients.

Aseptic filling is a critical make-or-break step in bioproduction, and gloveless robotic isolator filling technology can anchor built-for-purpose biomanufacturing architectures designed to support the production of cutting-edge treatment modalities. Comprehensive manufacturing solutions for advanced therapeutic medicinal products (ATMPs) must support seed-to-fill manufacturing capabilities that meet EU Annex 1 GMP guidelines and overcome the hurdles inherent to the production of advanced therapeutics.

WHY ROBOTIC GLOVELESS VIAL FILLING?

Traditional manual vial filling with restricted access barrier systems and gloved isolators still faces challenges for ensuring

the level of aseptic assurance required for ATMP production. Robotic gloveless isolators provide the aseptic consistency and operational precision necessary in the production of therapies like plasmid DNA, viral vectors, mRNA, and recombinant proteins. As an example, after assessing various aseptic isolator options, NorthX Biologics of Matfors, Sweden (Figure 1) selected Cytiva's Microcell™ vial filler, a first-in-industry robotic gloveless isolator (RGI) (Figure 2). GMP-ready design, user-friendly operation, and flexibility in meeting production demands at various scales enable RGI technology to meet the emerging needs of ATMPs as they increasingly enter production. RGIs ensure reliable and consistent manufacturing parameters including:

- Minimized product loss, crucial for clinical trial materials and personalized therapies, where high drug substance manufacturing costs make losses in tubing, filters, and quality control especially expensive.
- Efficiency for small-batch production—e.g., batch sizes between 100–1200 vials with an average batch time of 8 h for 1200 unit batches—and ongoing optimization for larger batch sizes.
- Accessible and timely maintenance and support, along with consistent availability of consumables and technical support within Europe.

'Our mission at NorthX Biologics is to bridge the gap between groundbreaking research and scalable clinical solutions. Robotic gloveless isolator technology is a cornerstone of that vision,' says Peter Boman, Chief Operations Officer at NorthX Biologics.

MOVING FROM RESEARCH TO THE CLINIC

Therapeutic innovation has historically faced a significant challenge in the

FIGURE 1

NorthX Biologics manufacturing site located in Matfors, Sweden.

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FIGURE 2

Cytiva's Microcell™ vial filler, a first-in-industry RGI. RGI: robotic gloveless isolator.

biopharmaceutical field: translating academic research into clinical products. Expanding drug substance manufacturing to include the critical final step of drug product simplifies development and production processes for advanced therapeutics, adapting to the growing demands of personalized medicines. These hurdles, especially acute during clinical trials, include technology transfer, engineering fully aseptic workflows, and shifting clinical trial phase needs.

The translation of academic research to clinical-scale production still faces many barriers. ATMPs are fragile and often must use processes that are aseptic from pooling through to filling to maintain drug product integrity. Also, efficacy of ATMPs at smaller effective concentrations means that wasted residual volumes represent lost therapeutic potential. As such, minimizing loss is critical to therapeutic success.

Considering the increasing need for small-to-mid-batch production driven by personalized medicines and single-patient batches, aseptic filling with robotic gloveless isolator technology is an optimal solution that helps de-risk ATMP bioproduction and facilitates more precise and efficient manufacturing processes.

FIT-FOR-PURPOSE IMPLEMENTATION AND OPERATIONS

Incorporation of RGI technology into aseptic bioproduction is a straightforward process that can reach key milestones within 15 months. An example for NorthX Biologics:

- Purchase and installation—6 months from acquisition to site acceptance testing.
- Media fill completion—5 months after installation.
- GMP certification (by the Swedish Medicinal Products Agency)—3 months after media fill completion.

- Initiation of first GMP batch production—1 month after GMP certification.

While NorthX Biologics required some room venting modifications for H_2O_2 cycle development and humidity control, the installation process was predominantly straightforward. Manufacturers benefit from the support of vendors like Cytiva through internal risk assessments and training, to ensure successful integration.

A FUTURE-READY APPROACH

RGI technology, paired with strategic vision, can help transform biopharmaceutical manufacturing. By focusing on personalized medicine and maintaining an agile, fit-for-purpose approach, manufacturers can be prepared and enabled to meet the high demands of the dynamic landscape of small to mid-batch production. RGIs are a key technology designed to safeguard the final critical step in manufacturing modern therapeutics.

Peter Boman is the Chief Operations Officer at NorthX Biologics, responsible for the manufacturing facilities in Matfors, Sweden. He holds a master's degree in engineering biotechnology and business administration. Peter has a background in research and development, working primarily with analytical and process development. He joined NorthX Biologics in 2013 and has continuously improved his talented team, resulting in the production of many biological drugs for clinical trials.

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CELL & GENE THERAPY INSIGHTS

EXPERT ROUNDTABLE

Producing MSC-derived cell therapies: workflows, technologies, and case studies

Irana Coletti Malaspina, Shirley Mei, Tony Ting, and Whitney Cary Wilson

PANEL

"The key point about using MSCs and exosomes is the possibility of treating diseases that currently have no treatments available..."

In this expert roundtable, four highly experienced industry professionals discuss key considerations in mesenchymal stem (or stromal) cell (MSC) manufacturing, including reagent selection, dosing, delivery methods, and final product formulation. The panelists also explore emerging modalities such as induced pluripotent stem cell (iPSC)-derived MSCs, gene-engineered MSCs, and exosome-based therapies, and emphasize the need for cost reduction and scalable manufacturing to improve accessibility and clinical success.

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Q What makes MSCs attractive for developers of stem cell therapeutics?

SM MSCs became prominent because of their ability to differentiate into bones, cartilage, and fat, as well as their use in various regenerative purposes. For example, there is a lot of potential in orthopedics and other fields. Over time, people have realized that MSCs can not only regenerate tissue but also modulate the host immune system. When MSCs are used in different disease environments, such as sepsis or acute respiratory distress syndrome (ARDS), they can interact with the immune cells and secrete molecules such as microRNAs, exosomes, and proteins, which can influence the immune system's response.

For instance, in sepsis, it is not just that patients have hyperinflammation—many also develop immune suppression. This means that if you only give a drug to suppress inflammation, it will not be beneficial for patients who later enter the immune-suppressive stage. Fortunately, MSCs can adapt to the conditions and influence the body either to boost or dampen the immune system.

The key factor that attracts developers, academics, as well as big companies in the industry to MSCs is their ability to be used in an allogeneic manner, meaning it is not necessary to match the donor and recipient. This opens the potential for MSC-based cell therapies to be developed into commercially viable products. For example, you can isolate cells from a single donor or a pool of donors and produce large quantities of MSCs. These cells can then be packaged in various doses and given to unrelated recipients, potentially reducing the cost of goods (COGs).

Q What are the main disease indications and applications where MSCs show promise?

TT Given their properties and strong safety record, there have over a thousand studies using MSCs, covering many different indications. As mentioned earlier, orthopedics was one of the initial areas where MSCs were explored. Graft-versus-host disease (GVHD) is another area, and we have recently witnessed the first approval of an MSC product in the US for the treatment of pediatric steroid-resistant GVHD. Additionally, MSCs are being used for various respiratory indications, such as COVID-19-related ARDS. MSCs are also being studied for neurological conditions, with research in stroke and other CNS disorders. Autoimmune diseases, such as multiple sclerosis, are yet another area that has been explored.

SM As mentioned earlier, MSCs can be used for sepsis and septic shock, which currently do not have an effective treatment—we have been developing a modified MSC product aimed at solving these issues.

ICM There are many neurodegenerative applications, such as Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

WCW There are also many topical applications for MSCs, such as for conditions like rosacea and basic wound healing. There is also a very interesting study going on at UC Davis, using an MSC-based patch to help infants with the spina bifida condition [1]. The surgery is performed *in utero* by applying the patch to the fetus.

Q What are the key challenges in working with MSCs?

ICM From my perspective, the major challenge in using MSCs as a medicinal product is establishing a standard protocol. As the production is scaled up and the developers move from small batches in academic settings to larger batches, it is crucial to be cautious with the isolation step. The isolation process can introduce contamination from other cell types or even microbiological contaminants, which can depend on the tissue source.

Another challenge is related to phenotype and potency, particularly with genetic markers, which makes it difficult to establish a standard quality control. In the beginning, we may have many MSCs, but we need to select a small number of batches to move forward into production. Additionally, MSCs can undergo senescence during extended culture periods, leading to more challenges.

We also need to consider good proliferation rates when working with MSCs. It is essential to limit the number of passages the cells undergo during cultivation and be mindful of their phenotype and differentiation potency. Strong quality control and quality processes throughout production are crucial.

Scalability is another significant challenge. Therefore, when working with MSCs, we must carefully plan how to scale up the process and ensure that quality control is in place to support production.

SM The tissue source and regulatory compliance are also crucial considerations. For example, if the cells are isolated from bone marrow, adipose tissue, or cord blood, you not only have to go through an ethics board but also ensure proper donor screening. Furthermore, the tissue source must be tested, and once it is made into a master cell bank, that also needs to be tested.

Different regions have varying regulatory requirements that must be satisfied. As an academic, you might primarily focus on isolating cells and scaling them up for a clinical trial. However, if you are in a company, you also need to consider different types of regulatory requirements, especially if you plan to market or sell the product across different jurisdictions.

From a more scientific point of view, potency is another critical factor. The type of potency assay you develop must be indication-specific. Many researchers use a T cell inhibition assay because MSCs naturally inhibit T cell proliferation, making this approach commonly used for conditions like GVHD. However, for diseases such as sepsis, the potency assay must be carefully designed. During product manufacturing and process development, it is essential to ensure that the cells remain potent as they are scaled up, and regulatory agencies increasingly require this data. In the beginning, they may accept early-stage assay data, but later on, these assays could become part of the release specifications. Therefore, any MSC developer must carefully consider potency assays when developing a scale-up strategy to produce clinical-grade cells.

TT It is also important to understand the mechanism of action and ensure that you have control over the manufacturing process. Developing a robust potency assay is critical, and it should be one that can be used routinely.

Another important point to mention is the media. In the early days of MSCs, fetal bovine serum (FBS) was commonly used, but it is not an ideal ingredient for MSC manufacturing. Most commercial companies have now moved to xeno-free media formulations, either completely chemically defined or containing human platelet lysate. However, even with human platelet lysate, there are concerns about batch-to-batch variability, which requires careful management.

Q How are scientists addressing those challenges?

SM In my laboratory, we work on different ways to scale up the production of these cells for clinical use, which is very different from working at the research laboratory level. For instance, when working with mice, you only need around 1 million MSCs per animal. But when thinking about using MSCs to treat patients, the required dose can range from 30 million to 1 billion cells per dose. How do you scale up to produce so many MSCs while also complying with clinical-grade and regulatory requirements?

We also work on optimizing expansion protocols by collaborating with vendors and exploring strategies such as microcarriers and bioreactors. The tricky aspect with MSCs is that they are adherent cells, meaning they need a surface to grow on. MSCs need something to adhere to, which is why microcarriers are used. However, there are many different types of microcarriers, and some are good for MSC growth but may also prevent the cells from coming off, which is another challenge we actively work on solving.

Additionally, there are many media choices available, and each of them can grow MSCs differently in 3D or bioreactor-based cultures. We also carry out 2D expansion because it has a lower barrier to technology transfer. Moving a protocol from an R&D product development setting to a CDMO, which will manufacture GMP-grade MSCs, provides more surface area for the cells to grow, but it also introduces new challenges. Typically, it is required to have a larger facility, incubators, and more people to manage the cell cultures and harvest the cells. You also need a large amount of media, which will impact the COGs. For early trials or cell therapies that do not require large doses, 2D expansion is usually easier. However, after scaling up the cells, we must also consider whether they can still maintain their potency. Genetic stability, which I mentioned earlier, is also very important.

TT One of the biggest challenges is the variability of the starting material. As we discussed, MSCs can be isolated from a variety of tissue sources. While there have been over 1,000 clinical studies using MSCs, very few products have been approved. One of the considerations is that while MSCs are effective, they may not always be potent enough. This has led to various techniques aimed at enhancing the potency of the cells. This can include preconditioning MSCs with different cocktails of cytokines. There are also people working on genetically engineered MSCs to enhance their properties. One of the more interesting technologies I have seen is the development of iPSC-derived MSCs. These offer several advantages, such as a consistent donor source (the original iPSC line), as opposed to standard MSCs, which require multiple donors. Overall, I think these approaches will help overcome some of the challenges.

Furthermore, it is important to think about the commercialization plan. If your program is successful, how many cells will you need for your product? The sooner you think about a scalable manufacturing process, the easier it will be down the manufacturing path. I would even suggest that, before starting a clinical trial, if you can move to a bioreactor system, you will be set for the long term. It is not the cheapest approach, but if you have the resources and capabilities, it will make further development much easier.

WCW It is crucial to identify the critical quality attributes of the MSC therapeutic early in the process, as well as develop potency assays. Developers must also account for how they will scale the cells and develop potency assays that work at scale. The earlier one can start thinking about these factors, the better off they will be in the long run.

ICM From my perspective, it is not just the MSC scale-up we need to be preoccupied with. We also must figure out how to produce the large amounts of media required for large-scale production. We must also be mindful of the downstream process, ensuring it is gentle on the cells, as well as the fill-and-finish steps. With MSCs, there are a lot of steps in the process that we need to handle carefully.

Q What are some of the technologies available for scaling up cell expansion?

WCW There are many ways to expand MSCs. As mentioned earlier, MSCs are adherent cells, meaning they prefer to attach to a substrate. Some of the earlier technologies, which are still widely used, include stackable cell culture vessels. There have been improvements in closing these systems, such as adding closed system caps with tubing for the fill and harvest process steps.

There are also hollow fiber bioreactors that have been used for expanding MSCs, as well as some fixed-bed bioreactors on the market, which are part of the early adoption of MSC scale-up. Additionally, as already mentioned, a lot of work has been done with microcarriers in what we would call a pseudo-suspension, which allows for much more efficient use of media and reagents when scaling up.

TT The technologies for cell culture and bioreactors are improving. In particular, more companies are developing technologies that allow us to monitor the process in real time. For example, now we can measure lactate and glucose in real time, which was impossible 10 years ago. It is encouraging to see that companies are developing these tools for large-scale manufacturing of MSCs.

Q What are the specific reagent and material considerations when manufacturing for patients?

WCW To emphasize again, it is crucial to ensure that the media and reagents are all GMP-compliant. Historically, MSCs have been grown

with FBS. However, FBS is not the optimal product for manufacturing, as there is significant lot-to-lot inconsistency with it. Therefore, using more defined media and products can help reduce batch-to-batch variability as you scale up manufacturing, which is very important.

SM FBS also poses regulatory concerns: for example, if the MSCs grown in these media are given to the patient, you will have to justify the source of the serum. Some sources of FBS cannot even be used because regulatory agencies will reject them due to safety concerns, such as those related to mad cow disease. For this reason, many media companies are developing serum-free or chemically defined media.

From a developer's point of view, you do have to test these media because MSCs are isolated from different tissue sources and have different isolation protocols. They might not work well in all media types, so you must test which one suits your needs. Additionally, you also want to work with a reliable vendor that has experience producing a certificate of analysis and can justify the sourcing of the raw materials used to produce the media. Working with good vendors who can help ensure the media are GMP-grade, or at least closer to GMP-grade for early-phase trials, is crucial. They should have a track record and specifications that can address regulatory concerns. These are important considerations when moving into clinical applications because you will need to address each of them in your CMC documents when submitting them to regulatory agencies.

Another factor to consider is cost. Some of these media and reagents can be quite expensive, which will factor into the COGs, so you will need to address this and find ways to minimize it for successful commercialization. This is especially important if you aim to treat a widespread disease, like COVID-19, where many people would need access to the treatment.

TT As you move toward commercialization, it is crucial to ensure that you have multiple vendors for each key ingredient in the media, if possible. It is also important to think about how the product will be stored, whether in a bag or a vial.

ICM During MSC production, we do not only have the MSCs themselves but also the media for culture and preservation. We must be careful about residual products in the final product. Using GMP-compliant, specialized products will be better in terms of residual considerations. This is important for ensuring the safety of the product during clinical trials.

Q What are the critical considerations when it comes to dosing, method of delivery, and site of delivery?

TT Given all the different indications that have been explored with MSCs, one can imagine there have been many different delivery processes. In essence, it is about understanding the biology of the MSCs in relation to the specific disease indication. For example, in a variety of CNS indications, researchers have tried direct injection into the brain, intrathecal delivery, or intraspinal delivery. However, intravenous delivery is most commonly used.

Regarding dosage, it is a huge challenge in the MSC space. Most studies have been done on small animals, such as mice or rats, and it is very difficult to scale dosing to humans.

I was fortunate enough to conduct cardiac studies in pigs, whose hearts are roughly the same size as a human heart, which made it much easier to work on the dosing strategy. However, if you are working with mice or rats and then transitioning to humans, your first human clinical studies must evaluate a range of doses to establish safety and determine the optimal dose for efficacy.

ICM Since MSCs are a treatment, we need to determine the optimal dose for each patient. At the beginning of your clinical trials, it is important to consider various doses to find the best one for treating the specific disease.

SM Cell therapy developers must communicate with the people who will eventually deliver the therapy because it will affect how the final cell product is packaged and delivered. It is not just about injecting the treatment into the patient. In fact, there are many questions to address: Is it in a bag? Is it in a vial that needs to be washed or diluted? Will it be gravity-fed, or will it go through an infusion pump?

Due to these complexities, there has been a lot of movement away from using fresh cells, as was common in the past. If you are thinking about commercialization, treating more patients, and addressing urgent diseases such as sepsis, ARDS, or COVID-19, you typically do not have time to grow, process, cryopreserve, and thaw the cells for weeks. Instead, you need a standardized protocol that allows for readiness.

In the hospital setting, there are different clinicians or coordinators you can work with, thereby the protocol must be adaptable to most of them. When running a clinical trial, you do not want issues such as non-compliance or loss of cell viability or potency. These factors could affect trial outcomes and, ultimately, the product's progression to the next stage. We have encountered this challenge both in academic and industry trials, and it is important to approach the problem from different angles and work closely with the clinical teams.

WCW Going back a little bit to the biology of the MSC itself and its mechanism of action in eliciting a therapeutic effect, when we consider how to deliver the MSC, we also need to understand whether we are aiming for a transient effect or a longer-term therapeutic effect.

For example, if you have engineered the MSC to secrete a growth factor, such as brain-derived neurotrophic factor for Huntington's disease, you might want the MSC to survive longer in the location where you are injecting it. In this case, you could think of the MSC as a biofactory. On the other hand, it is different if you are using the cell to provide a transient effect, such as an immunomodulatory effect. This is an important aspect to consider when deciding between an IV injection or a direct injection.

Additionally, it is crucial to consider scaffolds. If you are seeding the MSC onto a scaffold and want the cell to persist for a while, but not necessarily proliferate, you need to choose a scaffold that supports the cell and allows it to receive nutrients from the body.

Lastly, another consideration is the environment into which you are injecting the cells. Will they be exposed to a hypoxic environment, such as the brain, or will they have access to nutrients and oxygen?

Fundamentally, it all comes back to understanding the mechanism of action and how you expect the MSC to elicit the therapeutic effect.

Q What are the critical factors regarding final fill/finish and storage, and CQA and assay development?

ICM MSC products are fresh, meaning we must be careful not only in the fill/finish model but in all the steps—both upstream and downstream—to ensure a sterile process. We must maintain the sterility of the entire process, and the fill/finish is the last step. The best option, of course, is utilizing closed systems, but there are currently limited machines available that can perform the fill/finish in these systems. Time is also critical in these steps because we are using dimethylsulfoxide, a cryoprotectant, to preserve the cells.

Consistency between different bags or vials, especially regarding the volume concentrate, is another important consideration during fill/finish. For this, a proper formulation for cryopreservation media, as well as a well-defined standard protocol and standard operating procedure for this step, is crucial. After fill/finish, cryopreservation is the next critical step because the cells must be cryopreserved in nitrogen. Ice formation during this process is one of the most challenging aspects. All things considered, from my perspective, the fill/finish process is the major challenge in the final production of MSCs.

For storage, we need the MSCs to be a long-term product, so we must ensure the stability of the process. Stability studies are essential to ensure consistency in cell viability and recovery after cryopreservation. It is important to be mindful of the cryopreservation solution and the temperature ratio, as these factors are critical during the fill/finish steps.

WCW Another significant consideration in the final fill/finish is handling volume. Depending on the vessel used for scaling the product, the physical volume that comes out of the technology will vary. It is crucial to consider this aspect, especially when planning for volume reduction while maintaining an aseptic environment and performing the process in a closed system.

SM It is crucial to establish a stability program when developing therapies because regulators will ask about it. The specifications and functional, identity, and other (FIO) assays to evaluate the stability of your product are crucial. In the laboratory, you might think about using a complicated co-culture assay to measure various factors, but it can be very difficult and expensive to tech transfer that assay to a CDMO or CRO.

The key is developing assays that provide quantitative measurements that can give outputs equivalent to the potency or specifications of the product. You want to satisfy the regulatory requirements, but you also want to ensure that the assay is highly reproducible and standardized. This assay will likely be required not only for your product release but also for the stability program.

TT It is also important to retain enough product so that, if additional testing is needed, you have enough on hand. I would recommend keeping more than you think you need.

Q What are you most excited about in this space over the next few years?

TT I am thrilled about the clinical studies that are currently ongoing with iPSC-derived MSCs. I think it is going to be a very exciting space to watch. Gene-engineered MSCs will also play a crucial role in improving therapeutic potency.

WCW I am very excited to see these therapies becoming more available. From an accessibility perspective, it is important to drive down the cost of manufacturing. I see a lot of efforts in that space, aiming to make cell manufacturing more affordable. It is truly amazing to develop these different therapeutics and see the biology behind them, but if we can only administer them to a small group of people who can afford them, that is not a good solution for humanity.

ICM I am also very excited about the potential of having a product based on exosomes. Often, exosomes were considered a residual byproduct, but now we can use both MSC products and exosomes from a single production process. The key point about using MSCs and exosomes is the possibility of treating diseases that currently have no treatments available, giving hope to thousands of patients.

SM With the approval of the first MSC therapy in the USA, there is a lot to be excited about. When I first started in the MSC field 20 years ago, there were barely any MSC-specific media. Now, there is a repertoire of options for various purposes, and many companies are willing to invest in different systems to scale up MSC production and support clinical translation. The monoclonal bioreactor developments will help MSC technologies address challenges and further our understanding.

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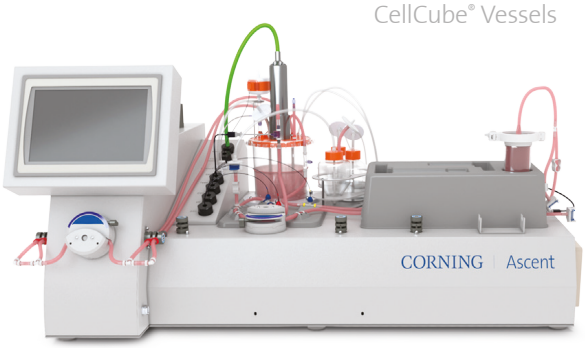

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CAR-T manufacturing intensification: the next wave of CAR-T commercialization

Alexey Bersenev, Emilie Kleiren, John Knighton, and Paul Bowles

Since the commercialization of the first CAR-T therapies, these revolutionary treatments have reshaped the landscape of cancer therapy, offering unprecedented hope to patients with hematologic malignancies. Here, a panel of industry and healthcare sector experts to discuss the critical intersection of commercialization strategies and manufacturing technology in advancing the next wave of CAR-T therapies to market. They explore the current industry status and the latest innovations that have the potential to optimize manufacturing processes for scale-up or scale-out, impacting cost of goods (CoGs) and manufacturing success to meet the growing demand and ensure broader patient access.

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Q How have market dynamics evolved since the commercialization of the first CAR-T therapies? What strategies have been most effective in overcoming these obstacles from manufacture to final product delivery?

AB I would like to focus on the hospital side of commercialization and widespread adoption, particularly hospital readiness and market dynamics and trends. We are now living in a post-approval world with multiple CAR-T products available worldwide. One of the key issues is hospital readiness. Some hospitals are better prepared than others to get certified and offer these new therapies to patient populations. Academic medical centers, which have historically been involved in clinical trials, are generally more ready than mid-size hospitals or non-academic medical centers.

Hospital readiness depends on both management and administration, as well as the hospital's willingness to act quickly and allocate resources. One issue here is the need to train large numbers of people. For example, for the first two CAR-T products we delivered, we trained about 400 people from five or six different departments, which was challenging

"Market dynamics have evolved in a positive direction for patients in recent years."

to conduct. The first certification process for Kymriah® took us almost a year. Now, we have learnt from the process and are able to train and certify faster.

I also want to address several products currently approved on the market, specifically in the USA. Hospitals often need to choose which CAR-T product to certify for. For example, if a hospital is deciding what CAR-T to be certified for to treat adult B-cell lymphoma, there is a choice of three right now: Yescarta®, Kymriah, and Breyanzi®. Each hospital will look at data and decide what is optimal for them, considering efficacy, toxicity profile, and reimbursement. Reimbursement plays a significant role in these decisions.

We became certified for Kymriah, as this was the first available CAR-T therapy on the market for the adult lymphoma population. Since Breyanzi entering the market, we have seen more prescriptions for Breyanzi and a rapid decline in Kymriah prescriptions. The market dynamics in hospitals are clearly shifting in favor of Breyanzi.

Next, I want to discuss the manufacturing capacity within hospitals. For some CAR-T therapies, specifically for multiple myeloma anti-BCMA CAR-Ts like Abecma® and Carvykti®, we have so many patients, but are limited by production slots. When we got certified for Abecma, we had a significant number of patients eligible, but we were limited to a few production slots per month. Our need was about four or five slots per month, leading to a waitlist for the therapy. Unfortunately, at the 2023 American Society of Hematology (ASH) meeting, data indicated that one-third of patients on the waitlist might die before a production slot becomes available. However, the situation has improved significantly with the approval of Carvykti. We no longer face the same production slot limitations for Abecma, and Carvykti has been very responsive to our needs. For our hospital, manufacturing capacity is no longer a significant obstacle.

Right now, to be a CAR-T certified hospital in the USA, FACT accreditation is required. There is ongoing discussion about how to extend access beyond these specialized centers, in order to make these treatments available to more patients worldwide. Steps are being taken by manufacturers, regulators, and hospitals alike. One move is that some hospitals are offering some CAR-T products in outpatient settings. Improvements in toxicity profile could drive the market dynamic towards mid-size hospitals and even community centers.

JK I agree with the points that you raised. Market dynamics have evolved in a positive direction for patients in recent years. There are more options now, alongside the ability to move into earlier lines of treatment for some indications. This move into earlier lines allows the patients to receive these treatments sooner, so even if there is a supply constraint or production slot utilization, the patient will be healthier and better able to tolerate treatment.

As these products have been commercialized, we have learned how to work better with physicians and patients in terms of planning, pre-treatment, and optimizing vein-to-vein time. As more options become available and we continue to learn from our experiences, we are improving.

It was great to hear from your perspective, Alexey, that production is no longer a concern for Abecma and Carvykti. We are working to optimize our cell therapy slots and

lentivirus capacities to ensure we can meet growing demands as we move into earlier lines of therapy.

Patient communication is also very important. We have learned how to communicate effectively with both physicians and patients. As the technology expands beyond clinical immuno-oncology, we will take the learnings we have gathered from clinical sites to work with different physicians in the immunology space.

Q With advancements in regulations and health authority engagement, how has the landscape of CAR-T commercialization shifted, and what implications does this have for industry stakeholders?

EK There has been tremendous effort made by the health authorities, especially the US FDA and the EMA, to keep pace with the rapidly growing field of CAR-T and cell and gene therapy in general. They have generated a multitude of guidance directly applicable to CAR-T therapies.

For example, in Europe, the EudraLex Volume 4, part 4 provides guidelines on GMP for cell and gene therapies. Recently, the FDA released updated guidance on the development of CAR-T cell products, which has further clarified regulatory expectations and requirements for manufacturers.

We've seen several examples of companies that have engaged open discussions with the health authorities early in their development, allowing them to benefit from the support of the authorities to achieve accelerated development and successful commercial approval. While health authorities are providing clear framework and guidance for filing and have developed programs for accelerated approval, their expectations for quality and control of CAR-T products are equally increasing. As such, commercial CAR-T manufacturing processes must comply with the latest cGMP requirements, as any other sterile injectable products.

In Europe, compliance has become even more challenging since the revision of the new Annex 1. This emphasized the importance of qualification and training of personnel and the implementation of a robust contamination control strategy. Annex 1 states that operators involved in manual filling activities must be requalified every 6 months through aseptic process simulations (APS). This can be a significant challenge, especially for CAR-T processes that involve many manual and open steps. This can result in needing to execute a substantial amount of APS runs, which must be accounted for in production planning. There is an increasing need at the commercial level to work towards closing systems and automating CAR-T manufacturing processes, while maintaining the same quality yield and the same or lower COGS.

These new guidelines highlight the importance of having a strong control strategy by design, not only for CAR-T manufacturing processes directly but also the whole supply chain. It is likely that efforts towards successful commercialization will increase in the future and should not be underestimated.

It is important that suppliers and service providers involved in the CAR-T cell therapy field follow the same trends to support industry, and that marketing authorization holders and regulatory bodies remain engaged and open to innovation to refine the quality and control of CAR-T products. Evolving standards bring some challenges for industry stakeholders, whilst also generating unique opportunities for CAR-T cell products to be considered as

trusted alternatives to current standards of care. For example, the approval of products for earlier lines of treatment is a positive development for the field and ultimately for patients.

Q What advancements in CAR-T manufacturing technology have shown the most promise in intensifying manufacturing to meet both scale and quality demands while addressing cost and time considerations?

EK There is a profusion of new technologies being developed, and it can be difficult to keep track of all the progress. For me, the most promising developments are ones that contribute to reducing human interventions and thus risk of errors during manufacturing or testing. Some technologies can increase process yield, reduce process failure, reduce process timing, and reduce COGS. Technologies with some predictive modeling can help also predict the outcome of the product behavior to provide better product and process knowledge.

For instance, we can consider non-viral gene delivery methods and technologies which are working towards closing systems and automating manufacturing processes. Examples include Miltenyi Biotec's CliniMACS Prodigy® Platform, Lonza's Cocoon® Platform, and Cellares' Cell Shuttle. Decentralized manufacturing solutions are also something to consider for the future.

Another promising area is in systems to help reduce complexity in the supply chain, which can integrate several software solutions, like enterprise resource planning (ERP), manufacturing execution systems (MES), and laboratory information management systems (LIMS). An example includes Hypertrust's X-Chain, amongst other computational approaches that aim to enhance CAR-T cell therapy manufacturing, like digital twins and predictive modeling.

PB Thinking back to the start of CAR-T therapies, many of the processes being developed were open, manual processes, often using technology that was not designed for cell and gene therapy. Now, with many vendors understanding the importance of cell and gene therapy, custom products have been developed. These have typically gone down two routes.

First, the all-in-one route, such as the Prodigy and Cocoon, which have the benefit of reducing associated training and labor costs. The challenge with these types of bioreactors is that there is less flexibility in process design. These can also have the potential of higher upfront capital costs, and lead to reliance on a single vendor for materials, consumables, and equipment.

Second, modular unit-based systems have their own benefits and drawbacks. These allow developers the ability to optimize their processes, select the best unit operation for each manufacturing workflow, and optimize equipment utilization. For example, for a 7-day process, you may only need one cell isolation device rather than seven expansion devices. There is potential for more manual manipulations, moving from one unit to another. This can require more product development, as each particular unit must be understood and optimized. This could also lead to a more complex digitization strategy; instead of linking one vendor's system to your LIMS and MES, you may need to integrate several different systems from multiple vendors.

"...all current commercial CAR-T cell therapies are viral-based. With the FDA putting the secondary lymphoma warning on any viral-based CAR-T product, there could be a push towards non-viral ways of gene modifying cells."

I am interested to see how non-viral technology progresses over the next 5–10 years because all current commercial CAR-T cell therapies are viral-based. With the FDA putting the secondary lymphoma warning on any viral-based CAR-T product, there could be a push towards non-viral ways of gene modifying cells. This could potentially reduce CoGs, as we know viral manufacturing and QC testing is expensive, and even enable better product efficacy and potency. However, there are licensing issues with non-viral gene modification that need to be overcome.

Q How are emerging technologies such as AI, robotics, and process analytical technology being leveraged to streamline CAR-T production processes and enhance efficiency and supply chain logistics? What's still missing?

PB The industry is in an exciting phase of rapid development, with both therapeutic providers and technology companies working to advance CAR-T manufacturing, particularly in automation. We are seeing the development of many devices aiming to remove the human component from CAR-T manufacturing.

These methods of automation typically follow two approaches: either developing independent solutions for the entire manufacturing process, including consumables, or optimizing the transfer between the different units of operations using already established technology, removing the human linking component. It will be interesting to see how these technologies evolve over the next 5–10 years.

Another aspect to consider revolves around the supply chain and clean room optimization. Many clean rooms are not operating at full capacity due to challenges in scheduling patients and variability in manufacturing processes. For example, if you have a variable length culture in a process, that could harvest on day 7, 8, or 9, you have to block that clean room for all those days. As we understand more about the process, we should gain confidence in predicting when cell harvest will be and only book slots that will be used. Improving our understanding of the biology, as well as improving how we manage the supply chain and logistics, can lead to big increases in efficiencies.

AI also holds significant potential, both in CAR-T drug discovery in finding receptors, and also patient stratification. Right now, we're not always matching patients with the right therapies, and AI could help optimize this process. How can we manufacture cells so that they always achieve a phenotype designed to give the best clinical efficacy? By improving our understanding of the biology behind CAR-T therapies and using digital tools like digital twins, we can enhance manufacturing and bring CAR-T therapies to the next level.

Q What are the critical considerations and challenges in scaling out autologous CAR-T manufacturing processes to meet growing demand while maintaining product quality and consistency? What are the additional considerations when scaling up allogeneic CAR-T manufacturing processes?

EK I'll start by discussing the scaling out of autologous processes. At the commercial scale, the focus is on maximizing production, and there are several key aspects that need to be considered to achieve this. The first one, which may not be immediately obvious, is the management of the supply of critical materials and consumables. It's important to secure your supply chain to ensure there are no risks, delays, or interruptions in the production process. This is applicable to allogeneic processes as well, but the needs and constraints are even more significant in autologous manufacturing at the commercial scale. Additionally, precautions should be taken to control the risk associated with suppliers who have a monopoly on certain consumables, critical reagents, or equipment.

The second consideration is the design of the facility and clean room, along with the equipment footprint. This can drastically impact capacity when increasing production of autologous processes. Third, for the process itself, I would make a distinction between manual and automated processes.

With manual processes, there is a significant need, as you scale out, for many new hires and timely training and qualification. This reflects what Alexey said; there is tremendous effort involved in bringing people up to speed. Depending on where your CAR-T manufacturing site is located, talent acquisition can become a bottleneck. Furthermore, as mentioned previously, according to Annex 1 regulations in Europe, operators must undergo aseptic requalification every six months, taking up considerable production slots. These are important considerations when scaling out manual manufacturing processes.

For automated processes, the main concern is equipment management, including troubleshooting, maintenance, and repair or replacement. These elements must be well-controlled to minimize turnaround time and to avoid losing production slots in the production planning. It is also important to take into account the release process. Achieving prompt release and delivery of commercial batches is crucial for patients. Several aspects must be carefully managed to reduce the turnaround time between apheresis collection and product infusion, such as release testing, batch record review, and deviation management. The first two factors can be supported by validated computerized systems like LIMS and MES, but deviation management still demands a unique set of skills from experienced investigators.

When it comes to scaling up allogeneic processes, the main focus is on maximizing yield per batch. Designing processes that can achieve significantly higher yield per batch while alleviating graft-versus-host disease (GvHD) effects remains a major challenge in CAR-T manufacturing. Additionally, the supply and qualification of healthy donors need to be well-controlled to sustain manufacturing.

Another aspect to consider in allogeneic processes is the opportunity to explore different formulations, filling solutions, and other final containers. For example, while bags are mostly used for autologous products, vials can be envisioned for allogeneic manufacturing, bringing advantages in terms of filling, integrity, testing, storage, and eventual infusion to patients. The stability program and storage capacity must also be adapted accordingly for allogeneic products.

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"...companies should consider production and process technologies that are easily scalable within their development strategy."

In both autologous and allogeneic manufacturing cases, companies should consider production and process technologies that are easily scalable within their development strategy. As mentioned, increasing capacity for commercial manufacturing can present significant challenges in the process.

Q Where are rapid-release testing methodologies being utilized to expedite the delivery of CAR-T therapies to patients whilst ensuring their safety and efficacy? What is still needed here?

JK This ties in with what Emilie said in terms of the challenges of scaling out and up. Rapid release is especially critical for autologous CAR-T and certain disease states, where even a day can make a huge difference for our patients. QC release can become a bottleneck quickly during manufacturing. Waiting, in particular, for the sterility test is usually on the critical path. There are newer technologies that are shortening the manufacturing time to 1–2 days, but you still need to complete QC release on the back end. This is where rapid release becomes so critical.

New technologies, particularly in sterility testing like qPCR tests, including BioFire panels and automated systems, are helping to limit the time needed for QC release, especially in sterility. However, there's still more work to do to shorten manufacturing times even further and get patient material out faster. There are other advancements being made in sterility testing, such as nanopore technology. Companies are also exploring technologies such as next-generation sequencing (NGS) that could impact sterility testing and cell release, which I am excited about.

As a collective industry, we are working with the agencies to talk through moving away from traditional sterility tests into rapid release, as they see the advantage for patients. This area requires continued work with the regulators as well as with the software and equipment manufacturers to refine sterility testing technologies. As we reduce the time for sterility testing, the next step is to look at potency testing and explore newer technologies that can identify potency without relying on biological cells.

AB Rapid release testing is evolving. As manufacturing timelines shorten, from 10–12 days to 2–4 days, release assays still need to be done. The major bottleneck, as John mentioned, is microbiology, because most assays for release typically take 1–2 weeks. Even rapid methods like BacT/Alert and BACTEC, based on CO_2 detection, still take 14 days according to the manufacturer's recommendations. Many developers have validated that these assays can be done in 7 days, but with a fast CAR-T process, where you have just 2–3 days of manufacturing time, a 7-day wait for release is still too long.

Other recent advances here surround the detection of nucleic acids and PCR-based methods. There are several kits developed by different companies, including the MycoTOOL by Roche and the Microsart by Sartorius. These kits can detect microorganisms in just a few hours. From my perspective, there has been relatively slow adoption of these due to a

lack of familiarity, unlike the methods based on CO_2 detection that regulators are familiar with. For methods based on detection of nucleic acids, the regulators are looking for comprehensive validation from developers to prove that results are similar or better than the compendial method.

Another method to mention is the new endotoxin detection kit from bioMérieux, which uses Recombinant Factor C. This kit can detect toxins in just 3 minutes and 40 seconds, which is a huge improvement over existing methods.

Q How do evolving collaborations between industry healthcare sectors and regulatory bodies continue to facilitate the acceleration of CAR-T commercialization, and improve patient access to these lifesaving therapies?

EK There has been increased engagement and collaboration between industry stakeholders and health authorities. For example, the FDA Center for Biologics Evaluation and Research (CBER) and the EMA's Committee for Advanced Therapies were created to support the assessment and certification of advanced therapy products. Interest groups focused on ATMPs are also emerging as privileged forums to bring stakeholders together. This engagement has facilitated open communication on specific product development challenges, regulatory pathways, and post-approval monitoring strategies. Stakeholders should keep engaging in discussions with a focus on even more global harmonization and international cooperation.

Good steps have already been made in Europe and the USA, but there is still a lack of supranational harmonization at the global level. I would like to see similar guidelines across China, Canada, and other countries involved in the CAR-T field. It's also important to focus on streamlining regulatory processes and providing more regulatory flexibility, creating clearer frameworks and accelerated pathways.

As all the panelists have mentioned, embracing technology and innovation is crucial. We need to increase the penetration of new technologies in the field. Most innovation currently comes from the USA and China—how can we make these technologies more accessible worldwide? As Alexey pointed out, how can we make sure these innovations reach manufacturers faster and, ultimately, benefit patients sooner?

AB I want to highlight a collaboration between industry and academia. This field is very collaborative, and CAR-T commercialization was born out of the collaboration and tech transfer from industry to academia. A well-known example is the commercialization of Kymriah. Many innovations in academia are licensed out to the industry, often through spin-outs. Academic facilities still play a critical role in conducting the proof of principle studies and first in human/Phase 1 clinical trials, before handing over to industry CDMOs or company-owned facilities. We are now seeing more dynamics where the industry is taking over with their own innovations, and sometimes no longer need academia as much.

From the healthcare perspective, we can provide feedback to industry on how things could be done better or faster, especially when it comes to patient access. Without reimbursement, there can be no patient access. The widespread adoption of CAR-T therapies would be accelerated if reimbursement issues were worked out before or immediately

"We are seeing a call for collaboration with industry. We would like to see standardization between commercial CAR-T therapy developers..."

after approval. Waiting 1–2 years to finalize reimbursement is a big delay, especially when patient populations are waiting for these products.

We're seeing that new developers are anticipating regulatory approval and are already engaging with players to discuss reimbursement strategies. They are beginning these discussions well before approval, sometimes 6 months ahead of time, to avoid delays in finalizing agreements with hospitals. Hospitals can take several months to establish these agreements, so developers want to start early to speed up the process.

These dynamics show how the industry can learn and improve based on past experiences. For example, we collect real-world data and report it at conferences, such as statistics for out-of-spec situations. Clinical outcomes are also reported to Center for International Blood and Marrow Transplant Research (CIBMTR), a national registry with publicly available data. We work with several companies with commercial CAR-T therapies, providing feedback on logistics and other aspects. For example, we have given feedback on how to improve shipment security, and developers have made adjustments to help streamline logistics.

Finally, at some point we may reach a situation when we have too many CAR-T cells and we cannot keep onboarding due to limited resources in hospitals. Building the required infrastructure on many levels is not fast, including hiring more staff. We are seeing a call for collaboration with industry. We would like to see standardization between commercial CAR-T therapy developers, such as on the level of software. We currently have six commercial CAR-Ts on the US market, each using different software that requires training and/or installation. This makes the whole process slower and more inefficient. Many other things could be standardized, including shipment, labelling, and logistics. This is a call for future collaboration that will make things much easier and more efficient to accelerate patient access.

Q What key lessons from the industry will inform future advancements in CAR-T manufacturing technology and commercialization strategies?

PB Recently, Legend Biotech and Johnson & Johnson announced that Carvykti has moved to second-line treatment, meaning that there will be many more patients eligible for CAR-T therapies. The challenge now is how to deliver those. This involves challenges within the hospital in terms of administration, but also challenges in manufacturing. With enhanced automation, we could hopefully solve some of these challenges and make a more consistent product, reducing human error.

Reducing vein-to-vein time is another important aspect. We talked about rapid release testing and getting it down from 14 days to 5, but can we get it down to a day? In terms of reducing manufacturing timelines, the T-Charge from Novartis uses a 2-day process and has also shown some better product efficacy and hopefully will reduce some of the CoGs associated with the clean room.

Cost reduction in general is important. Currently, these first line of treatments are around $400,000–500,000, which is too expensive, especially in areas with a National Health Service. I'm British and I'm proud of our NHS, but they will not be able to fund thousands of treatments a year. We have to work on reducing the CoGs so that these therapies can be delivered in all areas. Hopefully, CoGs will also go down in terms of materials and consumables. Higher clean room utilization will hopefully reduce the cost of manufacturing, removing labor components.

If we can standardize more within the process, that will help everywhere within the manufacturing workflow and the delivery of the final product to the patient. Standardization in regulatory bodies around the world is also key. It is challenging if we have to do something different in China than in the USA or Europe, for example. This would add to the cost of the overall development of those products.

AB One last promising trend is decentralization. This is decentralization not to the degree of point-of-care, because situations where the CAR-T cells are directly produced in hospitals can be controversial and difficult to achieve with high efficiency, quality, and regulatory support. Instead, decentralization at the level of smaller countries/economies developing their own indigenous CAR-T cell manufacturing and CAR-T products can be explored.

India recently received approval for two indigenous CAR-T products. These are available at a fraction of price—as low as one-tenth of the price of CAR-T products approved in the USA and Europe. China already has three approved indigenous CAR-T cells products on their market, independently of the USA and Europe. These are also available at a fraction of the price.

Another example is Spain, where one product is approved for adult B-cell acute lymphoblastic leukemia (B-ALL) by a Spanish regulatory agency as hospital exemption because there was nothing available offered by big pharmaceutical companies. This product was manufactured using CliniMACS Prodigy Systems. There will be more and more examples like this, which is ultimately good for patient access and driving costs down.

BIOGRAPHIES

Alexey Bersenev received his medical education and certification as a general surgeon in Russia. He holds a PhD in Transplantation/Pathology and completed his post-doctoral training in the USA at Thomas Jefferson University and at the Children's Hospital of Philadelphia. He worked as a cell manufacturing specialist at the University of Pennsylvania, trained in clinical cell processing in a GMP cell manufacturing facility, and was involved in the manufacture of CAR-T cell products for clinical trials and technology transfer to industry. He has expertise in the manufacturing of cellular products for clinical trials and regulatory submissions. In addition to his position as Director of the Advanced Cell Therapy Lab at Yale-New Haven Hospital, he is an Assistant Professor of Clinical Laboratory Medicine at the Department of Laboratory Medicine at Yale University.

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Emilie Kleiren is Process Engineering and Validation Senior Manager at Legend Biotech Europe, overseeing tech transfer, manufacturing support and CAR-T process lifecycle management initiatives. She holds a PhD in Bioengineering from the ULB, Brussels, Belgium. Emilie has been working in the CAR-T field since 2018. Before joining Legend, Emilie held several positions in Quality and Technical Operations in GMP certified companies at clinical and commercial level, including in CAR-T GMP manufacturing.

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John Knighton is the Head of Cell & Gene Therapy API at Johnson & Johnson and has been with Janssen for over 18 years. With 30 years of biopharmaceutical development experience, John leads a diverse and dedicated global team of scientists and engineers in biopharmaceutical viral vector & gene therapy drug substance and CAR-T process development as well as global technology transfer.

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Paul Bowles has spent more than a decade in cell and gene therapy process development since obtaining his PhD in Immunology. Paul started his industrial career working first at GE Healthcare and then part of a collaboration between Cytiva and CCRM, where he worked on process development of various immunotherapy platforms. Paul developed several closed, semi-automated processes, a CAR-T cell, and an antigen-specific T cell process, that went into Phase 1/2 clinical trials. He then spent time working at Treadwell Therapeutics, leading the process and analytical team focused on TCR-T therapies. Paul currently works as an Associate Principal Scientist and is a co-lead of the Immuno Cell Therapy platform, responsible for developing robust platform processes that OmniaBio can offer to benefit therapeutic sponsors.

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AUTHORSHIP & CONFLICT OF INTEREST

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

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CELL & GENE THERAPY INSIGHTS

CORRIGENDUM

Corrigendum to: Efficient AAV purification with AAVX and AAV9 magnetic beads

Shu Uin Gan and Kian Chuan Sia

In the version of this poster initially published, the caption of **Figure 2** read:

Figure 2. Purification steps using AAVX Magnetic Beads.

However, this should read:

Figure 2. Purification steps using AAV9 Magnetic Beads.

This error has been corrected in the HTML and PDF versions of this article as of March 18, 2025. The amended article can be accessed **here**.

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CELL & GENE THERAPY INSIGHTS

ERRATUM

Erratum to—Therapeutic epigenome editing: safety and quality considerations of a new class of gene-targeted medicines

Houria Bachtarzi and Tim Farries

This erratum contains corrections to the article: Bachtarzi H, Farries T. Therapeutic epigenome editing: safety and quality considerations of a new class of gene-targeted medicines. *Cell & Gene Therapy Insights* 2024; 10(9), 1257–1272.

In the version of this article initially published, there was an error in the reference numbers. The correction is listed in full below. The corrections were made to the PDF version of this article as of April 10, 2025; the amended article may be accessed **here**.

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The corrected references are as follows:

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