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PERSPECTIVE

Quality cell therapy manufacturing by design

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Transplantation of live cells as therapeutic agents is poised to offer new treatment options for a wide range of acute and chronic diseases. However, the biological complexity of cells has hampered the translation of laboratory-scale experiments into industrial processes for reliable, cost-effective manufacturing of cell-based therapies. We argue here that a solution to this challenge is to design cell manufacturing processes according to quality-by-design (QbD) principles. QbD integrates scientific knowledge and risk analysis into manufacturing process development and is already being adopted by the biopharmaceutical industry. Many opportunities to incorporate QbD into cell therapy manufacturing exist, although further technology development is required for full implementation. Linking measurable molecular and cellular characteristics of a cell population to final product quality through QbD is a crucial step in realizing the potential for cell therapies to transform healthcare.

The past decade has seen unparalleled growth in the field of cell therapy, which uses cells as therapeutic agents to treat and cure disease. Although the first transplants of blood stem cells date back to the late 1950s, cell therapies for diverse medical indications have begun to emerge only recently with the advent of reliable methods to cultivate clinically desirable cell types. Manufacturing of cell therapy products (CTPs) for clinical application typically requires several of the following steps: acquisition or generation of the starting cell type; cultivation; modification; harvest; concentration; purification; formulation, fill and finish (preparing the CTP at the correct concentration and composition, dispensing into the final product 'container', and any post-fill processing); storage; and shipping of the product. Although these processes are similar to those used to produce therapeutic proteins from mammalian cells, CTP manufacturing is considerably more challenging because of the much greater complexity of live cell products, incomplete understanding of their mechanisms of action, difficulties in product characterization and variability of starting materials[1]. We propose here that these daunting challenges can be addressed by taking advantage of QbD, a product development and life cycle management framework promoted by regulatory agencies, and, where necessary, by investing in new technological solutions to enable its implementation.

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In 2002, the US Food and Drug Administration (FDA) introduced the Pharmaceutical current good manufacturing practice (cGMP) Initiative to address inefficiencies and challenges in identifying the underlying cause of drug manufacturing failures, and in 2006, the International Conference on Harmonisation[2] outlined the QbD framework, a systematic approach to process and product management based on scientific knowledge and risk assessment. Today, following the widespread adoption of QbD by manufacturers of small-molecule pharmaceuticals, biopharmaceutical manufacturers are now increasingly implementing QbD principles in their processes[3]. Although some regulatory agencies, such as Health Canada, currently accept QbD-based submissions only for well-characterized chemical and biological drugs, application of QbD to cell-based therapies would greatly improve process and product understanding and outcomes. Indeed, the biological complexity of CTPs is what makes the iterative philosophy of QbD especially relevant to this therapeutic modality and indeed critical to the success of the CTP industry.

Here we review key QbD concepts (**Box 1**) and discuss their application to four investigational CTPs: pluripotent stem cell (PSC)-derived cardiomyocytes, hematopoietic stem and progenitor cells (HSPCs), mesenchymal stromal cells (MSCs) and chimeric antigen receptor (CAR)-T cells (**Table 1**). The promise of these four CTPs is underscored by the investment they have attracted from pharmaceutical companies. In the emerging space of PSC-derived therapies, Ocata Therapeutics (Marlborough, MA, USA), whose PSC-derived retinal cell therapies are in clinical trials for blinding diseases, was recently acquired by Astellas Pharma (Tokyo) for $379 million, Novartis (Basel, Switzerland) invested $35-million in Gamida Cell's (Jerusalem) cord blood expansion technology and agreed to exclusive licensing of and collaboration on Regenerex's (Louisville, KY, USA) HSPC product. In 2010 Mesoblast (New York) struck a $2-billion deal with Cephalon (now a subsidiary of Teva (Petach Tikva, Israel)) over its MSC technology. CAR-T cell therapies for malignant diseases are the subject of acquisitions and partnerships by Novartis and the University of Pennsylvania (Philadelphia); Pfizer (New York) and Cellectis (Paris); Amgen (Thousand Oaks, CA, USA) and Kite (Santa Monica, CA, USA); GlaxoSmithKline (London) and Adaptimmune (Abingdon, UK); Celgene (Summit, NJ, USA) and Bluebird (Cambridge, MA, USA); and Celgene and Juno Therapeutics (Seattle). However, exciting clinical progress in these and other cell therapies is tempered by manufacturing challenges that must be overcome. QbD provides a rational framework by which to tackle such challenges and facilitates the emergence of cellular therapies as a routine therapeutic option. Not addressing these challenges will lead to an industry-wide inability to reliably produce high-quality products at an acceptable cost, affecting products that emerge from the >600 regenerative medicine clinical trials underway worldwide[4].

Box 1 Quality-by-design terminology

Quality-by-design. A scientific, risk-based framework for process design based on relating product and process attributes to product quality.
Quality target product profile. The properties of the end product desired for clinical use.
Critical quality attribute. Properties that ensure product quality.
Critical process parameter. Variable elements of the production process that influence critical quality attributes.
Material attribute. Variable elements of the input materials that influence critical quality attributes.
Design of experiments. Controlled experiments to identify system response to inputs.
Design space. Range of variability in critical process parameters and material attributes to achieve critical quality attributes.
Control strategy. Method of maintaining critical process parameters and material attributes within design space.
Process analytical technology. A system to design, measure, monitor and control critical process parameters.

Aligning cell manufacture with QbD

Bioreactor systems are the method of choice for culturing cells to produce protein therapeutics, as they are scalable, amenable to integration of measurement technologies and capable of automated responses to maintain environmental homogeneity. However, these systems were designed to obtain high protein yields and do not prioritize the quality of the cells themselves. In contrast, the output of CTP manufacturing is healthy, functioning cells that are highly sensitive to environmental factors during manufacturing. Many bioreactor types, cell growth formats, scales and control parameters are now available for CTP manufacturing (**Fig. 1**). Understanding the proprieties of these different 'modules' and how they can be applied to CTP manufacturing is both critical to, and facilitated by, QbD.

QbD encompasses product and process description, characterization, design, monitoring and continuous improvement (**Fig. 2**). It is guided by a thorough understanding of the fundamental biology and engineering underlying a product and its production. QbD begins by describing the desired product quality characteristics (quality target product profile; QTPP), identifying attributes that directly influence the safety and efficacy of the product (critical quality attributes), identifying the parameters that influence these attributes (critical process parameters) and developing a design space that quantifies how parameter variability affects the quality attributes. Next, a control strategy is developed to maintain process parameters within a range that ensures product quality, and the process is validated at scale. In contrast to traditional control strategies based on discrete set points, QbD identifies acceptable operating envelopes, or multivariate design spaces, within which a process will produce high-quality products. Once implemented, the production process is monitored and modified iteratively as process knowledge increases (**Fig. 3**).

Quality target product profile (QTPP)

The QTPP describes properties of the desired end product—such as identity, potency and purity—and is the starting point for QbD[2,5]. We show example QTPPs for our four chosen CTPs (**Table 2**). Importantly, the definition of identity, potency and purity of each CTP is therapy dependent. Identifying stringent, easily measurable criteria for these is critical for establishing a QTPP.

Identity. For protein or small-molecule drugs, identity is defined by molecular composition. However, cell identity (i.e., phenotype) is a complex continuum, that is often poorly understood. Identity is typically demonstrated through the presence or absence of cell surface marker proteins that correlate with functional activity and are identified using flow cytometry. However, this approach can miss other informative markers whose expression varies during the manufacturing process or as a result of changes to the process. Robust, rapid biological assays for cell potency (discussed below) can mitigate these concerns, but more attention to standardizing identity assays is needed. Extensive study of the hematopoietic system has identified cell surface marker profiles that correlate with reconstitution of the blood system *in vivo*[6]. In some cases, the presence of multiple cell types contributes to clinical benefit, and this should be reflected in the identity attributes. When minimal

Table 1 Production requirements for four experimental cell therapies

Indication	Relapsed chronic lymphoid leukemia immunotherapy	Umbilical cord blood transplantation for acute myeloid leukemia	Immunomodulatory MSC therapy for Crohn's disease	Cardiomyocyte replacement therapy after chronic heart failure
Therapeutic cell type	Autologous CAR-engineered T cells	Allogeneic umbilical cord blood	Allogeneic MSC transplant	Allogeneic cardiomyocyte therapy
Cell source	Autologous T cells	Fresh allogeneic umbilical cord blood	Allogeneic MSC from bone marrow	Allogeneic transplant from differentiated PSCs
Therapy type	Patient specific		Bulk manufacturing	
Scaling production	Scale out		Scale up	
Patients per year	1.6×10^4 (USA)	8×10^3 (worldwide)	2.8×10^5 (worldwide)	6×10^6 (USA)
Cells per dose	3×10^7 CAR-T cells/kg	2.5×10^7 cells/kg, or 2×10^5 $CD34^+$ cells/kg	1×10^8 MSCs	10^9 cardiomyocytes
Doses per batch	1	1	>100	>1,000
Number of annual batches	16,000 (USA)	8,000	280	6,000 (USA)
Delivery attributes	Fresh or frozen product	Fresh or frozen product	Frozen product	Frozen product
Anticipated time to industrialization (years)	2–4	5–7	2–4	7–10
References	Based on CAR-T process validation[9], and incidence from the American Cancer Society (http://cancer.org)	Based on worldwide HSPC incidence and current manufacturing strategies[43]	Based on Crohn's incidence estimates[44] and previously used dose sizes for Prochymal therapy[45]	Prowse *et al.*[46]. Different manufacturing strategy would be used for autologous iPSCs

Bulk-manufactured allogeneic CTPs require 'scale up' to produce large, multi-dose batches of cells, whereas patient-specific autologous CTPs are 'scaled out' to efficiently manufacture many lots of cells. Patients per year is estimated from the average disease incidence in the world or USA, as noted. Doses and batches are calculated assuming a cell density of 10^5 cells/ml for MSCs and 10^6 cells/ml for PSC derived cardiomyocytes. In this example, batch sizes have been limited to 1,000 liters. Umbilical cord HSPCs, MSCs, and CAR-T cells are already used in the clinic, whereas PSC-derived cardiomyocytes are not. The anticipated time frame to industrialization estimates when well-designed bioreactor-based manufacturing processes will be implemented.

identity criteria for MSCs were first proposed in 2006 (ref. 7), these cells were being developed primarily as a multipotent cell-replacement therapy. Today, the focus on immune modulation combined with a greater range of source tissues containing MSCs of varying properties has necessitated new identity criteria to specify the therapeutically relevant subpopulations[8]. In CAR-T cell manufacturing, the target cell population must express both the chimeric antigen receptor and the correct subset of T-cell markers. This enables positive identification of the product and discrimination against untransduced T-cells and undesirable cell types that have been transduced with the CAR[9]. As more specific CARs are developed, reliable assays that can identify unwanted cross-reactivity of the CTP must be created as well.

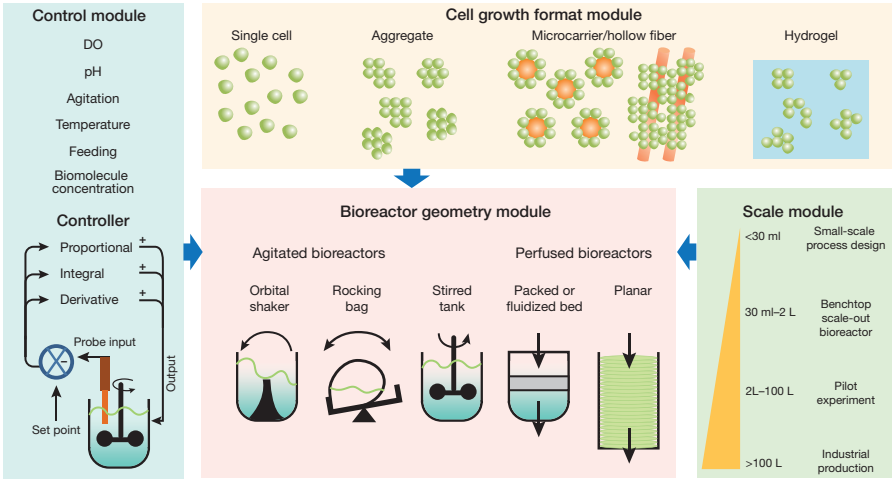

Figure 1 Modular bioreactor design facilitates QbD. Combining standardized, well-characterized modules reduces the process development required for each new cell type, although not all modules are compatible with each other. Top: cells can be grown in various formats. Lower right: bioreactor size ranges from small-scale screening devices, to benchtop, pilot, and industrial-scale devices. Larger stirred tanks have not yet been used for stem cell applications. Small-scale bioreactors are critical for manufacturing patient-specific products such as HSPCs and CAR-Ts. Ideally, these systems are disposable, integrate isolation and selection with cell growth and formulation operations, and minimize cell manipulations. They also provide a basis for developing strategies to manufacture allogeneic cells in small lots, which is useful for early and mid-stage clinical programs or for rare indications. Lower center: bioreactors use agitated or perfused geometries. Left: parameters currently controllable in bioreactors include agitation, pH, dissolved oxygen, temperature, feeding, and biomolecule concentration, using proportional-integral-derivative controllers. DO, dissolved oxygen.

Potency. CTPs must not only have the correct identity, they must also function appropriately as demonstrated by *in vitro* functional assays[10]. A crucial element of these functional assays is potency, which provides a measure of drug activity and is an important means for evaluating the impact of process changes[10]. For example, evaluating the impact of cryopreservation on a CTP requires a baseline assay for cell potency[10,11].

PSC-derived cardiomyocyte potency is currently assessed through electrophysiology assays that do not capture the key functional characteristic of heart tissue—the ability to rhythmically contract. The development of force-of-contraction assays could be beneficial for cardiomyocyte therapies intended to act primarily through cell integration and replacement of contractile tissue mass. It is common to see assays of differentiation potential described as a measure of MSC potency in academic research articles, which is appropriate where engraftment and tissue formation are the intended mechanism of action. However, many MSC therapies act through the secretion of paracrine factors, and differentiation assays that do not directly relate to this mechanism of action are inappropriate. Testing the bioactivity of secreted cytokines would be a more appropriate measure of potency. In contrast to the previous examples, CAR-T cells destroy target cells as a consequence of cellular engineering, and the mechanism of action is thus better understood. Incubation of CAR-T cells with cells expressing the appropriate target antigen can be used to quantify T-cell degranulation, release of cytokines, proliferation and cytotoxicity *in vitro*[9]. As with identity, the multimodal potency of CAR-T cells may not be sufficiently characterized with a single assay.

Potency is not the same as strength, the quantity of active ingredient in a dose. Of two drugs with equivalent potency (effect), the lower-strength drug is preferred. Similarly, from a risk and cost perspective, the CTP with high potency at the lowest strength, or viable cell density, is preferred. Many methods are available to determine cell density and viability, each with advantages and disadvantages. Manufacturers should carefully consider which approach is most appropriate for the product and ensure that the data obtained reflect the intended use. For example, cell density and viability are often assessed indirectly by measuring metabolic activity, which can be affected by other factors including changing population composition, mitochondrial content or activity, substrate abundance, redox state of growth medium or temperature.

Purity. Impurities in CTPs can include undesirable cell types, culture contamination, ancillary materials and particulates. Thresholds for cell purity vary between therapies. In cardiomyocyte therapy, transplantation of nodal cardiomyocytes transiently induce arrhythmias, whereas noncardiac cell types will not electromagnetically couple to heart tissue, also potentially leading to arrhythmias[12]. In PSC-derived cell therapies, as few as 1 in 4,000 residual undifferentiated PSCs can lead to teratoma formation[13]. Although this threshold is below the level of detection for flow cytometry–based phenotypic assays, recently described approaches such as metabolic selection for cardiomyocytes[14] or selection against PSCs[15] could substantially reduce this risk. In double umbilical cord transplants of HSPCs, T-cells impurities can cause immune complications, though T-cells and other cells may also serve accessory functions during engraftment[6]. MSCs are known to be functionally heterogeneous[16], whereas in CAR-T cell therapies, the proportion of proliferative naive or central-memory T cells in the final CTP may affect efficacy. For genetically engineered CTPs, purity analysis must identify the frequency of the desired modified cell type, other cell types, and any off-target effects of genetic engineering.

Cell cultures are susceptible to many types of contamination, notably microorganisms (bacterial, fungal and mycoplasma), endotoxin and cell line cross-contamination, which make them unfit for use. Furthermore, non-cell particulates (including plastic fragments, residual microcarriers and fibers) arising from manufacturing equipment and materials must be controlled[17]. Production of CTPs generally requires a cocktail of bioactive chemicals, including cytokines, small molecules, serum and vectors. These ancillary materials must be removed sufficiently to avoid being regarded as drugs themselves. They can also alter other process variables[18]. For example, dimethyl sulfoxide, a common cryoprotectant, can increase plasticizer leaching from bioreactor tubing, and pH indicators can interfere with cell differentiation. Thus, the quality target product profile must describe the maximum residual levels of these ancillary materials in the final

product to ensure its safety[19], and may necessitate additional processing that could affect final cell yield.

Critical quality attributes and critical process parameters

In developing a robust manufacturing process, it is essential to identify the attributes that are critical to ensuring product quality. Such critical quality attributes are "… physical, chemical, biological, or microbiological propert[ies] or characteristic[s] that should be within an appropriate limit, range, or distribution to ensure the desired product quality"[2]. In other words, critical quality attributes are the QTPP attributes that are directly linked to product safety and efficacy, including those listed in **Table 2**.

Critical quality attributes are influenced by variability in critical process parameters and raw materials attributes, which must therefore be controlled. Critical process parameters may include cellular features such as growth kinetics, cell age, cell secreted factors or expression of specific genes, and noncellular features such as raw material attributes, physicochemical parameters (pH, dissolved oxygen, temperature) or concentrations of exogenous factors. Elucidation of relevant critical quality attributes, critical process parameters and material attributes involves performing experimentation, mechanistic modeling, use of prior knowledge and application of a broad array of analytical techniques.

Once these parameters and attributes are identified, a risk assessment is conducted to prioritize the study of the most influential critical process parameters and material attributes (**Fig. 2**). Critical quality attributes directly determine product quality, whereas critical process parameters and material attributes influence it indirectly by affecting critical quality attributes[2]. Some critical quality attributes may be independent of identifiable critical process parameters and material attributes (**Fig. 3**).

Critical quality attributes guide process and product development, and should be continually refined as process knowledge develops. During early process development, a broad-based multiparametric analysis of cell attributes can be useful for identifying a more limited set of critical parameters to focus on during later development stages. It is worth noting that identifying and measuring critical quality attributes can be challenging. Without first obtaining some understanding of what the critical quality attributes are, complex multiparametric studies (discussed in "Design space" below) may be of limited value as these studies identify impacts of parameters on critical quality attributes. Thus, early CTP development should focus on identifying the critical quality attributes, and as this knowledge base grows, increasingly sophisticated empirical or mechanistic experimental models can be used to explore the influence of critical process parameters and material attributes.

Product safety and efficacy can be affected by many process parameters including operational features of bioreactor systems. For example, agitation can not only ensure homogeneous environmental conditions but also change the phenotype of shear-sensitive cells. Dissolved oxygen affects PSCs, HSPCs and MSCs, potentially affecting both potency and purity. Even subtle changes in pH can have a major impact, and if not controlled, cellular metabolism will decrease the pH. Metabolic by-products can also directly affect outcomes. For example, lactate accumulation and pH decrease are linked to inhibition of cell growth and loss of PSC phenotype[20]. Media exchange rate and strategy (e.g., batch, fed-batch or perfusion) can be used to control accumulation of waste products and endogenous secreted factor buildup[21].

The reagents used in CTP manufacturing are continually being improved. Many candidate CTPs, originally cultured in complex serum-based media on fibroblasts, are increasingly being cultured with chemically defined, xeno-free, cGMP formulations, reducing process variability. However, variability in starting reagents (basal media, recombinant proteins, small molecules and starting cell source) still exists and must be quantified. Strategies to reduce the number of media components will reduce the number of required cGMP-grade, lot-validated reagents. Growth factors and small molecules are often

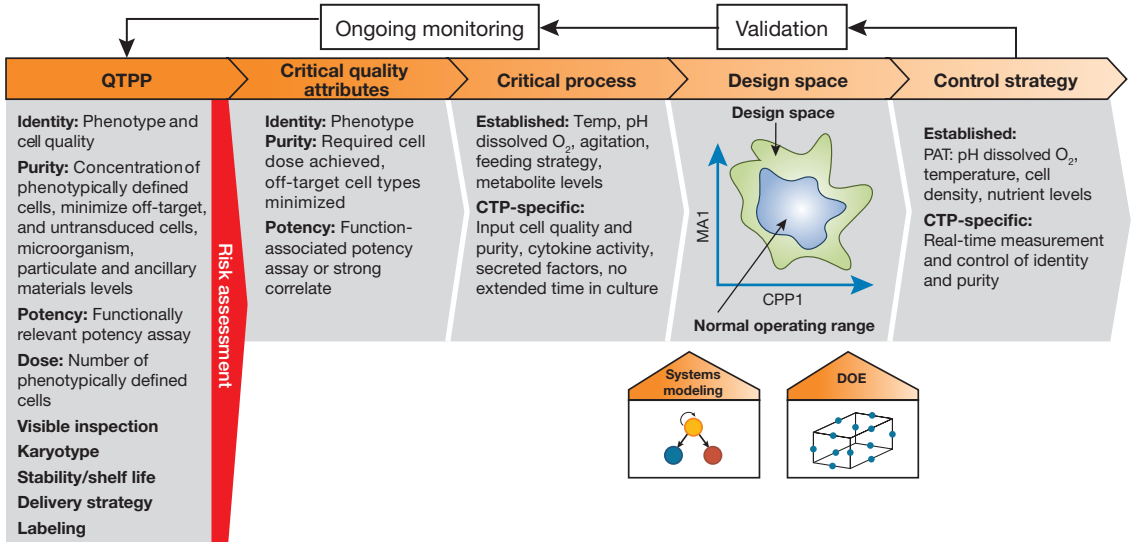

Figure 2 The QbD process. The first step in applying QbD is to define the quality target product profile—the characteristics of the CTP that assure its quality, safety, and efficacy. Second, the quality attributes that are critical for meeting the Quality Target Product Profile are determined by a risk assessment. Third, the critical process parameters and materials attributes that affect critical quality attributes are identified, and their effects on critical quality attributes are quantified in a design space. Fourth, a control strategy is developed to ensure that critical process parameters remain within the 'normal operating range' that ensures the production of quality product. Finally, the process is validated in the manufacturing facility at scale, and is continually monitored during manufacturing runs and improved as knowledge about the process increases. This figure includes both established analyses common to QbD processes for any therapeutic agent and analyses specific to cell therapy processes. Specific products may have parameters not represented here. MA, material attribute.

Table 2 Quality target product profile considerations for four CTPs

Property	CAR-T cells immunotherapy (T-cell derived)	Umbilical cord blood expansion (HSPC derived)	Crohn's disease therapy (MSC derived)	Myocardial infarction (PSC-derived cardiomyocytes)
Therapy type (patient specific or off-the-shelf)	Autologous	Allogeneic, matched	Allogeneic, unmatched	Allogeneic, unmatched
Identity (cell phenotype, morphology)	$CD3^+$ Average vector copy number	$CD34^+$ $CD133^+CD90^+$ CD45RA	$CD105^+CD166^+CD45^-$ $CD73^+CD90^+CD80^-$ $HLA\text{-}DR^-$	$cTNT^+$ $VCAM^+$ $SIRP\alpha^+$
Viability	High cell viability (typically >70–80%)			
Potency (ability of cells to perform desired action)	*In vitro* anti-tumor activity	Identity-based surrogate assay (high correlation between identity and potency in xenograft model)	Secreted factor profile	Force-of-contraction and electrophysiology measurement
Cell expansion (quantity of desired cells)	Sufficient cell expansion to meet target dosage		Meets number of doses needed	
Cellular impurity to minimize (cells in final product to minimize)	Residual $CD5^+CD19^+$ tumor cells & retrovirus	$CD3^+$ T cells $CD19^+$ B cells	Unknown	Residual pluripotent cells
Impurities and microbiology (below threshold)	Endotoxin, mycoplasma, bacteria, viruses, particulates, ancillary materials			
Karyotype (as expected)	CAR present	Normal		
Storage and stability (assessed by potency assay)	Fresh product, stable >24 h	Frozen product, stable >6 months		
Reference	Hollyman *et al.*[9]	Reviewed in ref. 43	Reviewed in ref. 47	Reviewed in ref. 46

Although target levels for many elements of the QTPP are essential, these evolve with the progression of clinical trials and corresponding clinical data. Targets are provided here as examples.

not readily available and very expensive for small-lot-sized clinical development, and growth factor activity varies widely from lot to lot, which can have major influences on process outcome. Standardized and consistent cell-based activity assays, or preferably, structure-sensitive biochemical assays, are required to mitigate the associated risks.

Cell expansion is usually an economic consideration but may be a critical quality attribute if it affects CTP quality. For patient-specific CTPs, the degree of cell expansion achieved may affect dose. Manufacturing HSPC and CAR-T therapies with cells sourced from a single donor requires sufficient cell expansion to reach the target dosage. In contrast, for allogeneic CTPs, cell aging and stability concerns take prominence. Extended cell expansion and enzymatic dissociation can also lead to the accumulation of genetic abnormalities. Population doubling can thus affect both potency (impairing differentiation potential) and purity (promoting genetically abnormal cells).

Secretion of signaling factors by cells may be a potency attribute or a critical process parameter. In the PSC system, autocrine and paracrine signals are required for maintenance of a pluripotent phenotype. Similarly, during differentiation to cardiomyocytes, cyclic perfusion feeding can alleviate a buildup of bone morphogenetic protein (BMP) agonists and antagonists, which normally decrease the purity of ventricular-like cell populations[22]. This is also the case for cultivation of HSPCs, where secreted cytokine accumulation is a major determinant of CTP quality[23] and therefore a critical process parameter. In contrast, MSCs derive their therapeutic effect from secretion of immunomodulatory factors, thus representing a critical quality attribute. Continued development of in-line sensor systems that can measure secreted factors will improve our understanding and ability to control secreted factors in cell culture. Cell expansion processes are dependent on the quality of both the starting cell population and the reagents used. HSPC expansion yield varies nonlinearly with phenotype ($CD34^+$ fraction) of the starting cell population[23]. Screening systems have been developed to quantify the variability in differentiation potential observed between PSC lines and passages[24]; CAR-T cell therapies have high variability in input cell number and composition arising from differences between donors. The potency of these therapies correlates best with cell proliferation, and efficacy may be controllable by selecting for specific cell subtypes[25]. Donor-to-donor variability in patient-specific therapies as well as run-to-run variability in allogeneic therapies may be addressable using QbD to systematically investigate and control or mitigate the sources of this variability.

The product design space

The design space describes the interactive effects of the critical process parameters and material attributes on the critical quality attributes[2] and the range of variability, or 'normal operating range', that is compatible with maintaining product quality. Variability in critical process parameters is particularly relevant to CTP manufacturing because of the variability in the input cells and the complex interactions between critical process parameters, which must be adapted accordingly. Changes within the design space are not considered changes to the process as their impact on critical quality attributes has already been studied and determined to be acceptable. This feature of QbD reduces the burden of regulatory oversight considerably. From the perspective of the FDA, changing operating parameters within a design space built on reliable scientific data and a robust quality system does not require notification. Furthermore, well-defined and validated design spaces do not require extensive in-process testing because the system's performance has already been characterized (leaving only release testing as a major testing step).

The design space is developed from multifactorial process understanding, experiments, and systems modeling. We illustrate the design

Box 2 Small-scale bioreactors for process development

Several small-scale (2–15 ml) bioreactor systems (Micro-24 Microreactor, Pall; ambr, Sartorius; microMatrix, Applikon) could enable cost-effective development of design spaces for CTPs. Although these systems support cell growth[48,49], many challenges exist in transferring results to larger-scale bioreactors, including the following:

- identification of the relevant scaling parameters
- conversion from small-scale impeller geometry or mixing patterns to large-scale geometries
- conversion from small scale liquid handling protocols to large-scale handling
- development of bioreactors and impellers that maintain appropriate shear
- development of appropriate small scale sensors
- resolution of mass transfer issues (including those relating to sparging, which is avoided in small-scale manufacturing)

space for one critical process parameter and one material attribute (**Fig. 2**). Although it is challenging to develop a high level of confidence in a design space for complex CTP systems, two complementary tools exist to guide design space development: design of experiments (DOE) and systems modeling.

Design of experiments

DOE describes a collection of approaches for organized, structured investigation of the relationship between factors and outcomes. The control afforded by bioreactors enables a systematic evaluation of cell behavior in response to changes in critical process parameters, identification of optimal operating conditions and elucidation of the design space in production processes[1]. Established methods for statistically meaningful exploration and characterization of multiparameter processes involve a series of tests to determine the effects of input variables (singularly and combinatorially) on outcomes[2]. DOE can be used to develop a high degree of process understanding unattainable by traditional stepwise optimization, leading to increased process robustness and cost efficiencies, and it can additionally integrate cost-of-goods analyses[26]. Multiparameter investigations will benefit from the use of new small-scale bioreactor systems to develop response surfaces for critical process parameters (**Box 2**).

DOE-based investigation of the effects of two interacting critical process parameters (seeding density and agitation rate) for PSC cultivation identified a process optimum that stepwise experimentation may have missed[27]. A similar study of seeding density, media volume and feeding time using response surface methodology identified conditions for optimal cell yield after cell dissociation[28]. In HSPC studies, beginning with our early investigation into the cytokines necessary for cell expansion[29], multiple studies have used DOE methods to explore and optimize cell culture[4,30,31]. For neutrophil production from HSPCs, one study identified four-factor interaction effects between cytokines that could not have been identified without DOE methods or with a reduced model of cytokine effects[32]. In MSC studies, preliminary design spaces have identified the effects of varying microcarrier density, cell seeding density and impeller geometry on final cell expansion[26,33].

DOE must link critical process parameters not only to critical quality attributes related to cell expansion but also to identity, potency and purity attributes. We emphasize that multiparameter investigation should be conducted only on the most important critical quality attributes; overanalysis risks identifying irrelevant characteristics that may result in misleading information about the state of a manufacturing process. As process knowledge develops, iterative evaluation of critical quality attributes and critical process parameters can help eliminate the investigation of irrelevant parameters.

Systems modeling

Given the complexity of interactions between cells and their environment, the development of design spaces for CTPs would benefit greatly from systems modeling. Large omics data sets provide a starting point for building reduced-parameter mechanistic models that link critical process parameters with critical quality attributes (**Fig. 3**). Although few such models have been generated to date, various techniques have been used to integrate cellular and bioprocess signals to predict critical quality attributes relevant to CTP manufacturing. Compartmentalized models of the transition between stem cell states have guided microenvironmental manipulation[34] and culture feeding strategies[21]. Metabolic pathway analysis and reconstruction based on omics data have been applied to optimize biopharmaceutical production[35] and are applicable to CTP manufacture. Such analyses have described the criticality of specific amino acids for PSC culture[36,37], whereas metabolic flux analysis has been used to describe the metabolic pathways active in exponential growth of PSCs at varying oxygen levels and to inform media formulation[38]. Recently, the distinct lactate metabolism patterns of PSC-derived cardiomyocytes has been exploited to achieve high cardiomyocyte purity[14], indicating that metabolic modeling–based optimization of CTP production may soon be feasible.

As a product advances through development, a large amount of valuable data accumulates. Techniques for correlating these data to critical quality attributes to enable predictive network models are being developed. For example, statistical tools such as multivariate analysis can identify subsets of genes (or other elements) in omics data that are associated with a desired response. Such a method identified the starting and final HSPC population composition, a critical process parameter, in heterogeneous samples[39], and could be used to account for variability in the starting material of other patient-specific therapies. Extending model-based identification of critical process parameters based on gene-expression data sets to model-based design space development would require the predication and validation of gene regulatory networks linked to identified critical process parameters. Recent computational efforts to relate input cytokines to changing cell states in PSCs[40] have demonstrated that gene regulatory networks can link critical process parameters to critical quality attributes. A valuable next step in the CTP field would be to use modeling techniques to connect phenotype to potency.

Control strategy

Process analytical technology is an approach to design, measure, monitor and control critical process parameters, including pH, dis-

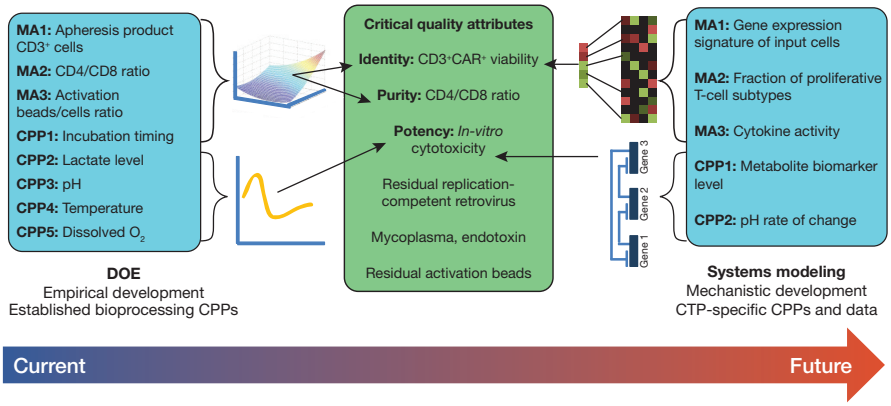

Figure 3 Design space development for CAR-T therapies. Traditionally, design spaces are created by using empirical design-of-experiments methods to test the influence of material attributes and critical process parameters (CPP) on critical quality attributes. As understanding of cell manufacturing processes increases, this approach will be augmented with systems modeling and advanced analytical methods. For example, CAR-T cell manufacturing stands to benefit from systems modeling of the mechanisms of T-cell activation and CAR function. However, certain critical quality attributes, such as microbiology, cannot be predicted by design of experiments or systems modeling and must still be tested in the final product. Identified CPPs (e.g., dissolved oxygen in this DOE example) that are found not to influence any critical quality attributes are removed from the list of CPPs. Critical quality attributes that are most influenced by variability in CPPs and material attributes are prioritized when developing control strategies. The examples used were drawn from the text of the review with specific examples from a related process (ref. 11). MA, material attribute.

solved oxygen, temperature, cell density and nutrient levels, and is an important element of QbD[41]. Production processes for biopharmaceuticals use advanced control strategies with online monitoring and control of process variables[42], and these same technologies are applicable to CTP manufacturing. Indeed, the increased complexity of CTPs compared with other biologics creates a great opportunity for more advanced monitoring and control strategies. Recently, our group has demonstrated real-time monitoring and control of soluble signaling factors to enhance cell outputs in a human cord blood expansion process[23]. The importance of measuring and controlling secreted factors, population composition and other advanced parameters is increasingly being recognized in the context of cell therapies.

Future applications of QbD

Although many challenges lie ahead in the industrialization of CTP manufacturing, there is much reason for optimism. Decades of experience with industrial cell culture process design provide grounding in the engineering aspects of bioreactor scale-up and intensification, cellular metabolism, medium design and optimization of feed strategies, and process control. Our understanding of how cells interact with their environment is improving, and bioreactor systems that can control the cellular environment are generating data sets that are increasingly focused on molecular and cellular information. In parallel, our understanding of the molecular basis of cell states, including adhesion dependence, metabolic network state, clonogenicity and proliferation control, are improving. Although we have focused here on upstream cell manufacturing processes, QbD is equally applicable to downstream operations. Similarly, process design is only one part of the product lifecycle, and using the QbD tools described here creates flexible processes that are amenable to changes over the CTP lifecycle.

The future of CTPs rests on the development of cost-effective technologies for cell manufacturing. Given the inherent complexity of CTPs and their production processes, QbD approaches will be essential in transforming today's experimental CTPs into widely available medicines.

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The authors declare no competing financial interests.

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