INVITED SUBMISSION

Establishing a Quality Control System for Stem Cell-Based Medicinal Products in China

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Stem cell-based medicinal products (SCMPs) are emerging as novel therapeutic products. The success of its development depends on the existence of an effective quality control system, which is constituted by quality control technologies, standards, reference materials, guidelines, and the associated management system in accordance with regulatory requirements along product lifespan. However, a worldwide, effective quality control system specific for SCMPs is still far from established partially due to the limited understanding of stem cell sciences and lack of quality control technologies for accurately assessing the safety and biological effectiveness of SCMPs before clinical use. Even though, based on the existing regulations and current stem cell sciences and technologies, initial actions toward the goal of establishing such a system have been taken as exemplified by recent development of new “interim guidelines” for governing quality control along development of SCMPs and new development of the associated quality control technologies in China. In this review, we first briefly introduced the major institutions involved in the regulation of cell substrates and therapeutic cell products in China and the existing regulatory documents and technical guidelines used as critical references for developing the new interim guidelines. With focus only on nonhematopoietic stem cells, we then discussed the principal quality attributes of SCMPs as well as our thinking of proper testing approaches to be established with relevant evaluation technologies to ensure all quality requirements of SCMPs along different manufacturing processes and development stages. At the end, some regulatory and technical challenges were also discussed with the conclusion that combined efforts should be taken to promote stem cell regulatory sciences to establish the effective quality control system for SCMPs.

Introduction

STEM CELLS ARE featured by their abilities to differentiate into various cell lineages and capacity of self-renewal. The stem cell-based medicinal products (SCMPs), specifically, nonhematopoietic stem cell (HSC)-based products, have emerged as novel therapeutics in the human history of healthcare. They can be used in stem cell therapies (SCTs), in which the cells isolated or collected from autologous or allogeneic human tissues are *ex vivo* expanded, processed, and then administered to patients for treatments.¹

Stem cells used in SCTs can be categorized as somatic or adult stem cells (SSCs or ASCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). Among SSCs are HSCs, mesenchymal stem cells (MSCs), and various progenitor or precursor cells of fetal, adult, or birth-associated tissues.² MSCs of various origins represent the most frequent cell type utilized in clinical studies largely due to the ease of cell derivation, a relatively high safety profile, and unique immunomodulatory activities.^{3,4}

Over the past few years, a large number of clinical studies for SCMPs have been registered and/or conducted for treating various diseases in the world and many have generated very exciting outcomes. In China, by the end of 2011, there were ~300 ongoing clinical studies using stem cells to treat various diseases, such as cardiovascular diseases, diabetes, liver fibrosis, autoimmune diseases, Guest Versus Host Disease, osteoarthritis, spinal cord injuries, and degenerative disorders. About 50 of them have been registered in the National Institutes of Health (NIH) clinical trial website (<http://ClinicalTrials.gov>) and all of them were MSC-based studies with some being even advanced to phase III stage as claimed by the sponsors of these studies. However, only a handful of non-HSC studies were approved by SFDA (State FDA, currently known as China FDA, or CFDA), whereas the vast majority of the ongoing studies were approved under the category of the “Third Medical Innovative Technology” only by local medical institutions with or without institutional IRB review. To date, no SCMP has been approved by the CFDA.³

It has been realized that the big promises inspired by some exciting stem cell studies have inflated expectation of

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the public, thus resulting in a sharp increase of unapproved SCTs, which were practiced more often in unauthorized stem cell clinics in the world.¹ In China, a survey showed that, by the middle of 2012, ~700 unauthorized stem cell clinics were practicing the unapproved SCTs.^{3,4}

In many perspectives, SCMPs share characteristics with drugs, biological products, cell and tissue products, and even medical devices if used with scaffold materials.^{1,3} They represent the most complicated therapeutics in the human history of healthcare in terms of complexity of product characteristics and regulatory mechanisms to be developed. Given the extremely complicated biological features and, in most cases, very complex manufacturing process, all SCMPs should be clearly defined and rigorously regulated as novel biological products, and the development of all SCMPs should follow the new biologic development pathway, including preclinical studies and different phases of clinical studies with rigorous regulatory oversights to ensure product quality, safety, and effectiveness during the lifecycle of product development.^{1,3}

Proper regulations rely on an effective quality control system, which should be built on well-developed and validated quality control technologies, quality standards, reference materials, technical guidelines, and the associated management system. However, worldwide, the establishment of an effective quality control system for SCMPs is still in its infancy because of the still limited understanding of stem cell sciences, insufficient quality control technologies, lack of quality standards and reference materials, and also the lack of regulatory experiences. The situation is particularly true in China since the existing regulatory system can hardly provide sufficient legislative and regulatory support to the development of effective quality control system because the regulatory mechanism in the mode of “law/legislation-regulation-guidance” has not been established in general for biologics, and some critical issues regarding categorization and jurisdiction over the regulation of SCMPs still remain unsolved.³

As a measure of tackling unauthorized SCTs and ensuring the quality of SCMPs in China, a “stem cell steering group” was formed in early 2012 under the combined leadership of SFDA and MH (Ministry of Health, or currently known as MHFP, the Ministry of Health and Family Planning). One of the steering group’s missions was to promote quality control over SCMPs.^{5,5} In this review, the main institutions involved in the regulation of SCMPs in China are briefly introduced first, followed by the introduction of the interim guidelines, that is, the “Guidelines for quality control of SCMPs and their preclinical studies,”⁵ which represents a major commitment of the steering group. Introduced then are the quality requirements, quality control testing, as well as our current thinking of various testing approaches to be used along different manufacturing processes, and development stages. At the end, some key challenges limiting the establishment of effective quality control system are also discussed. It needs to be emphasized that this writing only represents the author’s current thinking about the topic, from which more discussions constructive to the establishment of the effective quality control system are encouraged.

Major Institutions Involved in Quality Control of Cell Substrates and Cell Products in China

The cell substrates used for the production of biologics, such as vaccines and recombinant proteins, are strictly

regulated by CFDA. The regulation is achieved, in part, through verification testing taken by the Cell Collection and Research Center (CCRC) of the National Institutes for Food and Drug Control (NIFDC) to ensure that all cell substrates meet the requirements of identity, purity, safety, and stability.^{6–8}

The institutions involved in regulation of cell substrates in China

In addition to NIFDC, other major institutions involved in the regulation of cell substrates within CFDA are the Center for Drug Evaluation (CDE), the Center for Certification of Drugs (CCD), and the Chinese Pharmacopoeia Commission (ChP). The NIFDC is the only laboratory-based institution or the National Control Laboratory (NCL) in CFDA. Under the NIFDC, there are 17 institutes, including the Institute of Biological Products Control (IBPC). The CCRC represents an integral part of the IBPC with a major responsibility of ensuring the quality of both cell substrates and therapeutic cell products.

In general, after the stage of cell derivation, manufacturers, developers, or investigators of biologics in China are encouraged to bank the cells to be used as cell substrates in a multi-tiered banking system in early product development. The products derived from the banked cells, such as vaccines and recombinant proteins, need then to go through preclinical and clinical studies before approval for marketing. During this stage, quality issues regarding process validation and bank characterization are generally addressed through the involvement of CCRC. In later stages, other quality issues, such as the scale-up-associated quality issues, may be raised by CDE or CCD, but in most cases addressed eventually through the technical support from CCRC. The relationship among different institutions in CFDA regarding the regulation of cell substrates is illustrated in Figure 1. The ChP is involved in the regulation through providing, and continuously updating, the regulatory basis, that is, the Chinese Pharmacopoeia.⁶ It is easy to understand that such a regulatory system for cell substrates should be applicable in many ways for the regulation of therapeutic cell products, including SCMPs.

The CCRC is actively involved in establishing the nation’s quality control system for both cell substrates and cell products

As a unique part of the NIFDC, the quality control responsibilities of CCRC focus mainly on cell substrates and therapeutic cell products. The CCRC takes its role by conducting verification or qualification testing for ensuring qualities of both the manufacturing process and the cells at different development stages. The CCRC is also critically involved in promoting regulations through revising relevant chapters of Chinese Pharmacopoeia and proposing or drafting new technical guidelines, such as the interim “Guidelines for quality control of stem cell-based medicinal products and their preclinical studies,” which has been accessible recently to the public.⁵ In addition, the CCRC also initiates and conducts various quality-associated research projects, including several pioneering projects, with objectives of developing standards, reference materials, and new quality control techniques for assessing the quality, safety,

FIG. 1. Major institutes involved in the quality control of cell substrates and cell products in China. It illustrates the relationship in quality control and regulation of cell substrates and cell products in China between different institutes within CFDA along the path of product development. CFDA, China Food and Drug Administration; CCRC, Cell Collection and Research Center; NIFDC, National Institutes for Food and Drug Control; CCD, Center for Certification of Drugs; CDE, Center for Drug Evaluation; IND, Investigational New Drug application; BLA, Biologics Licensure Application.

and biological effectiveness of different types of SCMPs. Over the past few years, through research projects, the CCRC has developed a large variety of new techniques, including a telomerase activity assay, which has been developed for assessing tumorigenicity and for determining completion of induced differentiation of ESCs, and a whole package of techniques for evaluating immunomodulatory activities of MSC-based products.^{3,7}

Types of SCMPs

SCMPs represent one of the three major categories of cellular therapy products, which are (1) primary or tissue cell products, such as chondrocytes, hepatocytes, epithelial cells, and fibroblasts, (2) stem cell or progenitor cell products, that is, the SCMPs, and (3) immunotherapeutic cell products.⁹ The SCMPs are derived from ESCs, SSCs or ASCs, or iPSCs. Among SSCs are MSCs and tissue-specific stem/progenitor cells, such as HSCs, neural stem cells, and endothelial progenitor cells.² Currently, due to the ease of cell derivation and a relatively high safety profile, the vast majority of SCMPs in China use MSCs, among all types of non-HSCs, as the sole cell type or the cell type in combination with other cell types.³

Regulatory Basis

The development of all cellular therapy products must be in compliance with current Good Manufacturing Practice (cGMP) requirements. In addition, all quality attributes of the products, that is, identity, purity, safety, biological effectiveness, and stability, must be strictly tested before using the products in preclinical and clinical studies.

Given that the SCMPs share similarities in many perspectives with drugs, biological products, and cell and tissue products, some principles and philosophies of the existing regulations for these products may be applicable to the regulation of SCMPs.^{1,3,5,10} In fact, over the past few years, several existing regulatory and guidance documents applicable to SCMPs, in terms of quality requirements and testing approaches from regulatory authorities in the world or international organizations, have been taken as important

references to guide the development of the interim guidelines and the associated quality control technologies for SCMPs in China.

The references used for addressing safety issues of SCMPs

The safety of SCMPs is determined all together by product identity, purity, and microbial and biological characteristics of the products. Thus, the references used for addressing safety issues included the guidelines for cell bank characterization in Chinese Pharmacopeia (Part III, 2010 Version)⁶ and European Pharmacopeia,¹¹ World Health Organization (WHO) Recommendations for evaluating animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (2010),⁸ FDA Guidance for Industry-Characterization and qualification of cell substrates (2010),¹² International Conference for Harmonization guidelines for evaluating cell lines of human or animal origin (Q5D, 1997),¹³ FDA Guidance-Current Good Tissue Practice and additional requirements for the manufacture of human cells, tissues, and Cellular and Tissue-Based Products.¹⁴ In addition, the principles from the General Chapter 1046 of US Pharmacopeia for cellular and tissue-based products and General Chapter 1043 for ancillary materials for cell, gene, and tissue-engineered products were taken into consideration of quality requirements for culture medium, serum, reagents, and supplements in preparation of the SCMPs.^{15,16}

The references regarding biological effectiveness issues

As a very important quality attribute, the biological effectiveness of SCMPs, which reflects the mechanism of action and predicts the therapeutic effectiveness of the products, should be properly evaluated through various *in vitro* and *in vivo* testings. The references used for this purpose include the Guidelines for development of human therapeutic somatic cell products in China,¹⁷ FDA Guidance for Human Somatic Cell Therapy and Gene Therapy,¹⁸ FDA Guidance-CMC Information for Human Somatic Cell

Therapy Investigational New Drug Applications,¹⁹ FDA Guidance-Potency Tests for Cellular and Gene Therapy,²⁰ and FDA Guidance-Draft Preclinical Assessment of Investigational Cellular and Gene Therapy Products.²¹ Included also are International Society for Stem Cell Research (ISSCR) Guidelines for clinical translation of stem cells,¹ the International Society for Cellular Therapy (ISCT) Position Statement-Minimum criteria for defining multipotent stem cells,²² and ISCT Working Proposal-Immunological characterization of multiple mesenchymal stromal cells, all of which are more relevant to SCMPs.²³

The interim guidelines serves as a very important guidance document for SCMPs in China

The adoption of the applicable principles from the aforementioned regulatory and guidance documents resulted in the generation of the interim “Guidelines for quality control of SCMPs and their preclinical studies,” which represented the first step of establishing the nation’s quality control system for SCMPs.^{3,5} The newly developed guidance document states that all SCMPs must meet key quality requirements through eligible cell derivation, cGMP-compliant manufacture, as well as different testing approaches.⁵

The interim guidance document also represented one of the most important achievements made by the stem cell steering group for tackling the problems associated with the unauthorized use of stem cells in China. The group was formed in early 2012 by top clinicians and research and regulatory scientists following a “stem cell regulation initiative” proposed by MH and SFDA together. Based on the initiative, all unapproved clinical studies using stem cells were mandated to halt for reassessment and all unauthorized stem cell clinics were asked to stop practicing all forms of nonhematopoietic SCTs by the end of 2011. In the meantime, the group was asked to develop new regulatory documents, one of which was the interim guidelines.³

However, due to the urgent need, the development of the guidelines did not follow the rout of “Legislation-Regulations-Guidelines,” a common mechanism employed by both the United States and Europe for generating technical guidelines.³ Rather, the guidance document was developed in a situation that the definition of SCMPs in terms of regulatory pathway and jurisdiction over the regulation of SCMPs were still not yet clear. Nevertheless, it did provide opportunities for both research scientists and regulatory scientists to seriously think about the ways of effective regulation of SCMPs. More importantly, the interim guidelines has now been well recognized as the first and very important guidance document by manufacturers, investigators, and developers for addressing both safety and effectiveness issues during the development of SCMPs in China.

Quality Requirements and Quality Control Testing for SCMPs

As stated in the interim guidelines, all SCMPs should meet the quality requirements for identity, purity, safety, biological effectiveness, and stability, all of which should be evaluated through quality control testing, which includes the tests for cell identity, freedom from contamination with microorganisms and endotoxins, residual ancillary materials, abnormal immune responses, tumorigenicity, and biological effectiveness.

The cell identity should be characterized according to the current understanding of cell biology, cytogenetics, biochemistry, flow cytometry profile of surface markers, and genetic polymorphism depending on cell types. Short Tandem Repeat (STR) profiling, especially the current 16-loci profiling, represents a very effective identity testing technique, which can not only provide the genetic polymorphism of each individual donor but can also serve as forensic evidence to demonstrate potential cross-contamination of the product with other human cells as a result of incompliance with Good Manufacturing Practice/Good Tissue Practice requirements, or to distinguish between host cells and administered stem cells in a later investigation of any adverse effect associated with the administered SCMPs. It is therefore strongly suggested that all therapeutic cell products, especially the allogeneic products, must have the STR-based cell identity.

Microbiological safety should be comprehensively evaluated for each batch of the product. It includes sterility tests for bacteria and fungi and the tests for mycoplasma, viruses, and endotoxins. For testing viruses, species-specific viruses of both human and animal origins, retroviruses of both endogenous and exogenous origins, and adventitious agents as well, must be included in the testing using *in vitro* and/or *in vivo* testing methods.

Abnormal immune responses should be assessed for all therapeutic cell products, including SCMPs. They can be assessed, in part, through testing potential stimulatory effects of the products on total lymphocytes. The enhanced lymphocyte proliferation indicates proinflammatory effects, while the reduced lymphocyte proliferation may suggest possible immunomodulatory activities, a common feature of the MSC-based products.^{23,24}

Tumorigenicity is a key biological safety issue pertaining to all therapeutic cell products. It should be evaluated through inoculation of the testing cells into immunodeficient animals and observation of tumor formation. However, this method is not appropriate for evaluating undifferentiated ESCs, which are already highly tumorigenic in forming teratoma.²⁵ Instead, the tumorigenicity of the terminally differentiated cells derived from ESCs should be evaluated using the inoculation method supplemented with telomerase activity assay, which may also serve as a very effective biomarker for tracking the status of induced differentiation.²⁵

In all quality control testing for SCMPs, the testing for biological effectiveness is the most difficult one in terms of specification selection, validation, and standardization.^{1,3,20,23} Generally, biological effectiveness should provide proof of concept and mechanisms of action contributing to the therapeutic efficacy of the product.^{1,20}

For all MSC-based products, biological effectiveness should be evaluated *in vitro* to demonstrate their abilities to differentiate into multiple cell lineages, such as osteocytes, chondrocytes, and adipocytes, and to modulate immune cells, such as inhibiting the proliferation of total lymphocytes, reducing the proliferation of proinflammatory CD4⁺ lymphocytes, like Th1 and Th17 lymphocytes, and inhibiting the release of proinflammatory cytokines, like TNF- α , from immune cells, while promoting maturation of regulatory T cells. All these abilities have been widely accepted as common critical properties contributing to the therapeutic efficacy of MSCs of different tissue origins.^{23,24}

Testing Approaches for SCMPs

To ensure the quality of SCMPs and the eventual therapeutic safety and efficacy of SCTs, different testing approaches, such as quality testing (QT), release testing, which should include cell substance release testing (CSRT) and final product release testing (FPRT), and verification/qualification testing, should be employed for addressing all quality issues along different manufacturing processes and development stages.³

In terms of readiness for use in humans, stem cells should be defined as either stem cell substance, which are cryopreserved cells that have finished all manufacturing processes but are not ready for use in humans, or final stem cell products, which are the cells formulated freshly from recovery of the cryopreserved cell substances with supporting materials, that is, excipients, and are ready for use in humans.

The QT approach

The QT approach represents a comprehensive testing approach, which should be designed based on the updated knowledge and techniques to address identity, purity, safety, potency, and stability of each SCMP. The specifications included in QT should be developed or selected for each SCMP according to the type, complexity of manufacturing process, and intended clinical use.

The specifications of QT for multipurpose MSCs and the associated testing methods should include the following: (1) cell identity, tested for cell morphology, biochemistry, for example, isoenzyme profiling, karyotyping, STR profiling, and expression of surface markers; (2) sterility, tested for possible contamination with bacteria and fungi; (3) mycoplasma, tested through cultivation, inoculation/DNA staining, as well as polymerase chain reaction (PCR)-based methods; (4) endogenous and adventitious agents, tested through both *in vitro* assays, such as PCR-based assays for testing species-specific pathogens and indicator cell-based assays for testing adventitious agents, and *in vivo* animal tests using suckling mice, adult mice, rats, guinea pigs, rabbits, and embryonated chicken eggs for covering a large variety of unspecified agents; (5) immunological activities, assayed for inhibition of proliferation of total lymphocytes, changes in different subsets of CD4⁺ lymphocytes and the ratio of CD4⁺/CD8⁺ populations, and inhibition of the release of proinflammatory cytokines, TNF- α in particular, from immune cells; (6) differentiation potentials, assayed for the abilities to differentiate into adipocytes, osteocytes, and chondrocytes; (7) cell viability, tested for viability, preferably using new sensitive cell death determination techniques in place of conventional Trypan blue exclusion staining, supplemented with growth curve description and cell cycle progression; and (8) tumorigenicity, tested mainly using nude mice tumor formation assay, supplemented by soft-agar colony formation assay and/or telomerase activity assay. The specifications selected in the QT approach for evaluating qualities of MSCs of different tissue origins are summarized in Table 1.

Considerations and strategies for the QT approach. In most situations, the QT is employed to evaluate stem cell substances rather than final stem cell products to reflect the quality of both the manufacturing process and the tested cells. Therefore, the QT should be conducted either at each key

TABLE 1. SPECIFICATIONS SELECTED IN THE QT APPROACH FOR EVALUATING QUALITIES OF MSC-BASED PRODUCTS

Specifications	Each batch of MSC-based products		
	P-1 ^a	P-2 ^a	P-3 ^a
Identity			
Morphology	★	★	★
Biochemistry-isoenzyme profiling	★		★
Genetic polymorphism-STR profiling	★		★
Cell surface markers	★	★	★
Cytogenetics karyotyping			★
Cell viability			
Viability	★	★	★
Growth rate	★		★
Cell cycle	★		★
Microbiology			
Sterility-fungi and bacterial	★	★	★
Mycoplasma	★	★	★
Endogenous and adventitious agents			
Adventitious agents <i>in vitro</i> assays	★		★
Adventitious agents <i>in vivo</i> assays	★		★
Human-specific viruses	★		
Animal-specific viruses	★		
Retroviruses	★		
Tumorigenicity			
Colony formation in soft agar	★		★
Tumor formation in nude mice			★
Telomerase activity	★		★
Biological effectiveness			
Cell differentiation			
Three lineage differentiation	★	★	★
Immunomodulation			
Inhibition of PBMC proliferation	★	★	★
Modulation of lymphocyte subpopulations	★	★	★
Inhibition of proinflammatory cytokines	★	★	★

★ represents the test selected under each specification category for certain passage number of each batch of the testing products.

^aP-1, P-2, and P-3 represent early, middle, and late passages, respectively, of each MSC-based product.

MSC, mesenchymal stem cell; PBMC, peripheral blood mononuclear cells; QT, quality testing; STR, short tandem repeat.

manufacturing process with the selection of the most relevant specifications or at the end of the entire manufacturing process by assessing all preselected specifications.

The stringency of specification selection and the amount of the associated testing methods for QT should be adjusted case by case according to the type, complexity of manufacturing process, and intended clinical use of each product. For autologous MSCs without complex processing, the relatively less stringent specifications, such as cell identity, tested only for fewer surface markers and STR profiling, viability, adventitious agents, induced differentiation only for one representative cell lineage, immunomodulation only for one representative immunological activity, maybe appropriate. The representative immunological activity should be determined in a case-by-case manner for each individual MSC product. However, for allogeneic MSCs or autologous MSCs with complex processing,

and for the banked cells, the intense testing with more stringent specifications with each being tested by at least two mutually supportive methods should be employed, including cell identity, tested by more than two testing methods; viability, assayed by growth curve description and cell cycle progression; microbial contaminations of both endogenous and exogenous origins, viruses of both human and animal origins; purity; tumorigenicity or tumor-modulatory activities; multifaceted immunomodulatory activities; differentiation potentials for at least three lineages; and ideally, product-specific or clinical indication-specific potency assay(s).

Different passages of the cells, including the early passage(s), middle passage(s) and late passage(s), should be evaluated with specification selection varying in different passages for determining the quality of manufacturing process, the trend of the changes in critical quality attributes, and potential adverse effects associated with cell passaging. The passages to be evaluated should be determined on case-by-case manner. The selection of specifications for revealing the passaging-associated differences through the QT approach is summarized in Table 1.

To minimize variations of qualities among different batches, a minimum batch number for each type of SCMP should be required when taking the QT approach. For SSC/ASC-based products, the batch should be clearly defined as the same preparation of both the substance and final product derived from the same tissue/organ of the same donor at the same time following the identical derivation and manufacturing protocol(s). For ESC-based products, each batch should be defined as one ESC line established from one individual embryo or one individually differentiated cell from one ESC line. Arbitrarily, at least three randomly selected batches of the MSC-based products, or three ESC lines established from three individual embryos, or three independently derived cell products from the same ESC line following identical differentiation protocol should be evaluated through the QT approach to meet the minimum batch number requirements.

To minimize variations of qualities among final products of each batch, a multi-tiered cell banking system in the form of Master Cell Bank (MCB) and Working Cell Bank (WCB) should be established. Cell banking is especially encouraged for cells of large quantity from each batch as well as the established cell lines. The banked cells must demonstrate a clear uniform identity and freedom from microbial contamination.^{5,6,8,13} In the MCB-WCB mode, at least MCB cells need to be fully characterized through the QT approach.

Given that cryopreservation can significantly compromise qualities of SCMPs, it is suggested to set up relevant assays to evaluate the cryopreservation-related stability for each type of SCMP.²⁶

The QT should be reconducted whenever the changes with potential effects on product qualities occur, such as the significant changes in derivation protocol, manufacturing process, critical ancillary materials, or even location of manufacturing and/or storage facility.⁵

The CSRT approach

Considering the efficiency of quality control testing and variations among different batches of the product, an abbreviated QT approach or CSRT approach should be

conducted to ensure the quality of each individual batch of the same type of SCMPs meeting all key quality requirements. To fulfill this purpose, the CSRT should be conducted mainly for stem cell substances of each individual batch at the end of the entire manufacturing process, provided that the QT has been conducted for a minimum batch number of the same product with all specifications meeting the acceptable criteria. The specification selection for CSRT should be consistent with, but less stringent than that for QT and, considering the workload, all specifications should be assessed using the validated time-saving testing methods.

The specifications selected for CSRT of a multipurpose allogeneic MSC product should include the following: (1) identity, tested only by STR profiling and fewer surface markers; (2) sterility; (3) mycoplasma; (4) adventitious agents, tested only with *in vitro* methods; (5) induced differentiation only for the most relevant cell lineage based on tissue origin; and (6) a representative immunomodulation, such as the inhibition of Th1 lymphocyte proliferation or inhibition of TNF- α release.

The FPRT approach

The use of QT and CSRT in combination should serve as a comprehensive quality control strategy for ensuring the quality of both the manufacturing process and all batches of cell substances. However, there is still lack of assurance for the quality of final cell products. Given the risks of introducing microbiological agents and reducing cell viability during the preparation of final cell products, a prior-to-use testing, or FPRT, serving as the final quality assurance should be established to ensure the viability and microbiological safety of the products.

Due to the features of a short shelf-life and no final sterility or filtration for all cell products, the FPRT has to be finished in a very short time frame, ideally less than 24–48 h before use in humans. Therefore, only the quick testing methods are feasible to meet this need. The specifications of a typical FPRT for multipurpose MSCs should include (1) sterility, tested by Giemsa staining or other quick sterility testing method; (2) viability, tested by the sensitive quick cell death identification assay; and ideally (3) one relevant quick potency assay. IDO1, a catabolic enzyme of tryptophan, may serve as a surrogate marker for immunomodulation of MSCs and its activity can be quickly tested in several hours by sampling the supernatant of the tested cells.^{23,24,27} In addition, it is only feasible to conduct FPRT by the authorized medical institutions, where SCMPs are administered to patients.

The verification or qualification testing approach

The verification/qualification testing conducted by the third independent party reflects the general quality control philosophy in China for ensuring qualities of therapeutics without bias. It is required by CFDA to ensure the quality of cell substrates used for production of biologicals. Based on the risk analysis approach, the verification testing for cell substrates include the verification for cell bank characterization, testing of end of production cells (EOPCs), and testing of supernatants of EOPCs.⁶ Given that SCMPs represent a novel biological product with an extremely complex nature, the verification testing for SCMPs should be equally

important as, or even more comprehensive and vigorous than, that for cell substrates.

In principle, it can be understood that the objectives of QT, CSRT, and FPRT for SCMPs are in parallel with that of the bank characterization for MCB/WCB, testing of EOPCs, and testing for supernatants of EOPCs for cell substrates, respectively. However, different from the testing for cell substrates, to fulfill the objectives of verifications for SCMPs, the minimum batch number, for example, at least three batches, should be required for verification testing for both QT and CSRT. The qualification testing instead of verification testing should be conducted for FPRT by the third independent party to qualify the medical institutions, where SCMPs are administered to humans.

As described in duty statements in the NIFDC website and the newly developed interim guidelines,^{5,7} the CCRC of NIFDC/CFDA serves as the authorized independent third party in China to conduct the verification testing for both cell substrates and SCMPs.

Challenges of Establishing the Effective Quality Control System for SCMPs

Over the past few years, although a variety of significant achievements have been made from stem cell studies in the world, there are still many challenges limiting the development of effective quality control system for SCMPs, especially in China, where several critical issues regarding regulation of SCMPs still remain unsolved. Up to this writing, there is still no legislative support available in China for defining SCMPs as novel therapeutics and no consensus about product categorization in terms of regulatory mechanisms has been fully reached among industries and medical and regulatory institutions. Moreover, the jurisdiction over the regulation of SCMPs has not been clearly defined. Within this context, the existing regulation systems and experiences for drugs and biologics can hardly provide useful inputs for generating new regulations and guidelines for SCMPs. Therefore, a supportive regulation environment should be generated before being able to establish a truly effective quality control system for SCMPs.

Besides the regulation environment, the current quality control technologies are also not fully sufficient to meet all needs of effective quality control. For example, many aspects of safety and biological effectiveness of almost all SCMPs cannot be accurately evaluated based on current technologies, and thus, new assessment techniques need to be developed; a variety of bioassays commonly selected for evaluating biological effectiveness need to be further validated by new preclinical and clinical data before establishing their predictive functions to therapeutic efficacies. More urgently, quick testing methods for assessing both safety and efficacy are needed for SCMPs due to the aforementioned reasons. Among the most urgent needs is the development of quick sterility testing because the very short shelf-life of the final products makes the conventional sterility tests described in the existing regulations/guidance not feasible to meet the clinical needs for SCMPs.^{1,3,5}

Moreover, there is lack of standards and reference materials used to facilitate quality control of SCMPs and comparisons among different laboratories or institutions. Given the extremely complex nature of SCMPs, the devel-

opment of standards and reference materials for SCMPs is much more difficult than that for small chemical or even recombinant protein-based therapeutics, which can be manufactured to high degrees of homogeneity. Manufactured cells or those harvested and processed from different anatomic sites or different individuals are of significant variations in product qualities. Pure small-molecule or protein drugs can be developed as reference materials to facilitate the assessment of almost all quality attributes of the testing materials in various assays.^{1,3} However, developing a standard MSC line for assessing all quality aspects of biological effectiveness is almost impossible mainly because MSCs represent a huge heterogeneity in terms of tissue origin, passage number, expression of specific surface markers, differentiation potential, and immunoregulatory and tissue repair activities. No single one-fit-all MSC line can be identified as a reference cell line meeting all the needs for quality control of all MSC products. It is, however, realistic to establish a group of standard MSC lines with each being able to represent one critical characteristic of MSCs.

With the limitation of current technologies and lack of reference materials, it is extremely difficult to establish quality standards for SCMPs. However, following a previous publication²³ and continuously increased understanding of MSC biology, it is now more feasible to set up a roadmap for establishing a minimum set of standards for the MSC-based products during cell derivation, processing, characterization, and before release for clinical use.

All these challenges emphasize the urgent need of broad in-depth quality control-related studies and enhanced communication and collaboration within national and international stem cell communities.

Conclusions

Over the past decade, although many exciting achievements have been made in stem cell studies, there are still many challenges limiting the successful translation of very promising stem cell studies into clinical use and healthy development of the relevant industries. It appears that all issues converge to the lack of an effective quality control system. The establishment of such a system needs an increased recognition of the importance of stem cell regulatory sciences, through which the broad in-depth quality control-related studies should be strongly encouraged and continuous efforts from regulators, NCL, researchers, clinicians, and industries all together. It is believed that through the continuous endeavor for improving the regulatory system, increasing investment in quality control-related studies, and enhancing national and international communications and collaborations, an effective quality control system for SCMPs will be eventually established for safeguarding translation of stem cell studies and the development of SCMPs and relevant industries.

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