Technical Guidance for Research and Evaluation on Chemistry, Manufacturing, and Control (CMC) of Human-derived Stem Cell Products

(Draft for comment)

Center for Drug Evaluation

National Medical Products Administration

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Preface

In 2017, the former State Food and Drug Administration issued the ***Technical Guidance for the Research and Evaluation of Cell Therapy Products (for Trial Implementation)***, which provides a general description of the technical requirements for cell therapy products when they are developed in accordance with the relevant laws and regulations on drug administration. On the basis of that, this technical guidance is to promote the development of the stem cell industry by further regulating and guiding the industry and reviewers on research, development, application and evaluation of stem cell products from CMC perspective.

The purpose of this guideline is to provide advice on the technical aspects of CMC research from the development to the marketing stage of stem cell products developed as drugs based on existing current knowledge.

Stem cell products that are developed and registered as pharmaceutical products are subject to the supervision of the State Drug Administration (NMPA) and comply with the requirements of the Drug Administration Law of the People's Republic of China, the Administrative Measures for Drug Registration, the Pharmacopoeia of the People's Republic of China and other relevant laws and regulations. The whole production process of stem cell products for human use should comply with the basic principles and relevant requirements of the Good Manufacturing Practice (GMP).

Scope

"Stem cell products" in this guidance include stem cells and their derivatives obtained from human-derived adult or somatic stem cells (ASCs/SSCs), human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), expanded, induced to differentiate, or transformed (differentiated) from mature somatic cells, mixed into appropriate formula, dispensed into specific containers, in line with specific drug release standards, can be applied directly to patients, and can also be used in combination with tissue engineering materials for patient therapeutic products.

General principles

Stem cell products are characterized by complex origin and composition, diverse cell types, the ability of the cells themselves to survive in vivo, proliferate or/and differentiate on their own, large variability and complexity in production scale and processes, and complex mechanisms of product action, etc. Therefore, the design and validation of production processes, quality research and control of stem cell products should fully consider the above basic characteristics, such as having a clear and reasonable source of donor cells and screening criteria, selection of packaging systems, storage and transportation conditions that can effectively maintain cell viability and activity.

Stem cell products should follow step-by-step and phase appropriate drug development rules. In principle, in the clinical trial application phase, the files package should meet the requirements of clinical trials to carry out with the convincing safety of participants. In clinical trials period, the

production process and product quality and other related research should continue to be improved. In the marketing authorization application, the thorough research data should be provided to support the safety, effectiveness and quality control of the final product.

The development and production process of stem cell products includes the whole process from the acquisition, transportation, receipt of donor materials, product production and inspection to the release, storage and transportation of finished products, in line with the general rules of cell therapy products, while applying this guideline, other relevant general guidelines for biological products [1~6] are also applicable.

Risk assessment and Management

In addition to the common risks of biologics in general, the risks of stem cell products include the risk of contamination and cross-contamination (donor, raw materials, operational processes), the risk of residues of high-risk starting raw materials (ESCs/iPSCs, gene delivery and modification systems), the risk of impurities such as non-target cells and unintended changes during processing, the risk of changes in production processes and other quality of stem cell products risk factors. Risk factors affecting the quality of stem cell products specifically include, but are not limited to: (1) donor age, health status, etc. (2) cell source (autologous, allogeneic, iPSC source, etc.), biological characteristics (proliferation ability, differentiation ability, migration ability, genealogical proximity to the therapeutic target tissue), and genetic mutations. (3) cell production process operations (in vitro culture/expansion/induction/differentiation/ genetic manipulation/ cryopreservation/recovery, etc.) and the effect of the complexity of the operation on cell characteristics, such as the effect of genetic modification on the cell genome. (4) the duration of in vitro exposure of cells to specific culture substances, cell culture time, cell survival and cell generation, etc. (5) induction materials and dose, induction time, and the order of adding treatment, etc. (6) the mode of administration (topical application, intravenous infusion, or surgical application), whether the recipient needs to be pretreated, whether to form a combination with non-cellular products (bioactive molecules or structural materials), etc.

Based on the comprehensive consideration of various factors, the overall risk of products for different types of product characteristics and the whole life cycle process should be accessed., and the corresponding risk control strategies should be developed. At least the following aspects should probably be considered to develop stem cell product risk control strategies:

(1) Establishing operational specifications for clearing and isolation, focusing on strict quality control of raw materials of animal or human origin, analytical inspection of cell banks and harvesting fluids, and detection and control of sterility, mycoplasma, and endogenous viruses at key production steps.

(2) To manage upstream and downstream material batch numbers and labels, establish a perfect traceability system, and pay attention to cell identification testing during the production process to ensure that the quality attributes of stem cell products are qualified.

(3) Try to use continuous, closed production process to reduce the environmental exposure link in the production process to avoid the risk of pollution.

(4) Set up process control indicators and waste indicators to check or monitor the quality of intermediates, such as the transduction efficiency of exogenous genes or cell differentiation, cell

phenotype and genotype of genetically modified cells or cytokine-induced differentiated cells.
(5) Conduct comprehensive production process development and validation, carry out systematic comparability studies to access the change impact on qualities, and continuously conduct quality characterization studies in conjunction with product characteristics and process development to fully characterize cell morphology, viability, genetic stability, tumorigenicity/oncogenicity, cell phenotype characteristics (including expected and unintended cell populations) and function, with particular attention to impurity levels and safety effects that affect product quality.

Materials for production

Materials for production include all raw materials and excipients used in the production of stem cell products. Raw materials include starting materials (production cells, helper cells, in vitro gene delivery and modification systems) and other raw materials (such as culture media and biochemical reagents such as cytokines, cell separation and combination devices and other consumables). The use of excipients should comply with the requirements of the pharmacopoeia and the ***Rule of Joint Review and Approval Combining Excipients and Package Materials with Drug*** requirements. Materials used for production are directly related to product quality and are subject to risk assessment and quality control with reference to the relevant requirements of the Chinese Pharmacopoeia [7,8].

Raw materials

Starting materials

Cells for production

Stem cell products developed in accordance with pharmaceuticals are generally derived from donors (autologous or allogeneic) and may also be derived from cell banks. The source of stem cells and related operations should comply with relevant national laws and regulations and ethical requirements, and their use should obtain informed consent and authorization from the owner. The production of stem cell products should establish a stable source of cells for production and ensure consistent quality. If human embryonic stem cells are involved, appropriate donor screening procedures and criteria should be established according to the characteristics of the product, and the safety and reasonableness of the production cell application should be comprehensively assessed.

Donor screening

Donor screening is an important approach for risk control of stem cell products. Proper donor screening procedures and standards are necessary to prevent risks such as viral contamination, tissue rejection, and genetic diseases. For cell bank-derived stem cell products, cell donors for production

should be donors of somatic cells/gametes. Regarding the screening of pathogenic microorganisms in donors, especially for donors used for clinical research of allogeneic stem cells, the relevant requirements for blood collection could be as a reference [9], such as screening donors for the presence of HBV, HCV, HIV, syphilis spirochetes and other infections. In actual development and application, corresponding screening items should also be included in the acceptance criteria, like the donor's health/disease history or living stay in regional epidemic areas, tissue cell-specific susceptible pathogens and other specific circumstances. For stem cell donors of autologous origin, the quality requirements, screening items and criteria for the donor may be appropriately adjusted according to the source tissue or organ, the characteristics of the stem cell preparation, and the clinical indications, etc. It should be determined whether the production procedures increase the risk of possible pathogen transmission in the donor and indicate the precautions to prevent the transmission of viruses or other exogenous factors to others than the autologous recipients. In order to ensure the reliability of the pathogen screening results, the organization screening the donor should have the relevant qualifications, and the donor screening process should use blood-borne screening kits approved by regulatory agencies to detect pathogens if possible, and the test method should be validated for suitability, with attention to the sensitivity of the test method to detect pathogenic microorganisms. The impact of the window period on pathogenic microbial screening must be considered when preparing universal stem cell products. The risk in terms of tissue rejection can be considered with reference to the requirements in blood collection, such as ABO blood type, HLA-I and II typing information of the donor. A tissue typing match between donor and recipient (patient) needs to be demonstrated, along with the method and basis for the assay [8]. For patients using stem cell products, the risk of introducing genetic disorders should be adequately assessed in conjunction with advanced analytical testing techniques.

Donor cells

The steps of donor cell acquisition, preservation, transportation and incoming inspection should be comprehensively studied. Standard operating procedures and key quality control parameters should be clearly defined. The well-regulated homogeneity traceability system and stem cell supply quality assurance system should be developed. The necessary validation should be completed.

Information on the procedures used to obtain donor cells (e.g., surgery and, if possible, the instruments used), the name and location of the collection site, and the conditions of transport (e.g., transport to a processing site for further production) should be specified. The donor cell isolation process, which may have an impact on the quality of stem cell products. The variation in isolation process should be evaluated. In the process variation evaluation studies should use representative donor-derived cells as sample, with attention to cell identification, viability and growth activity, exogenous pathogenic microorganisms and basic stem cell characteristics testing (if applicable) during processing. Donor cell storage and transport stability should be studied, and the performance of transport containers should be verified and regularly confirmed. When the collected cells are admitted to the plant, according to the process requirements and product characteristics, corresponding tests for cell type, quantity, phenotype, viability, microorganisms, etc. should be performed, e.g. Cell type identification can be identified and confirmed by relevant genotypic and/or phenotypic markers, and the proportion of cells with positive markers can be used as a basis for the assessment of expected cell population indicators. Appropriate quality-related criteria should

be selected and incorporated into the collection cell quality release specification.
The entire donor cell obtained procedure should be well designed, studied and validated as necessary to ensure process stability and consistent quality of donor cell.

Cell seeds and cell banks

The technical requirements for cell seeds are as described in the Pharmacopoeia General Rules, which should be of clear origin, legal compliance and comprehensive testing. Human embryonic stem cells and induced pluripotent stem cells should be used as cell seeds after the establishment of cell line/strain (cell line), which can refer to ICH Q5D and the ***Protocol for the Preparation and Testing of Animal Cell Substrates for the Production and Testing of Biological Products"*** in CP for the establishment of suitable donor cells and cell seeds for production, cell banking and testing, etc. The frequency of banking for commercial production scale is based on the characteristics of the product itself and batch production, etc. The history, origin, derivation, and characteristics of each cell bank (MCB and WCB), as well as the frequency of testing performed, should be described. MCB testing generally involves microbiological characterization of the product, including in vivo and in vitro testing for sterility, mycoplasma, exogenous virulence factors, including CMV, HIV-1 and 2, HTLV-1 and 2, EBV, B19, HBV, and HCV testing (if applicable), or other specific pathogens. For cell lines exposed to animal derived components (e.g., serum, serum fractions, trypsin), Testing for exogenous factors of bovine and/or porcine origin should be included. Cell identification includes assays to distinguish specific cells by physical or chemical characteristics of the cell line (i.e., phenotype, genotype, or other markers). The purity of the cell bank should include identification and quantification of any contaminating cells, growth activity (including resuscitation activity after freezing), proliferation capacity, etc. Cell activity (e.g., iPSC pluripotency) and cell differentiation relevant to the therapeutic properties of the product should also be tested. For stem cell products that have been genetically edited, the transduction efficiency of the genetic material, the integration of the gene into the cell, the regulatory elements, the phenotype and genotype of the cell, the genetic stability of the target gene, the residual amount of genetic material for transduction, and the ability of viral replication to reply to mutations should be concerned. There is other information that can be helpful in determining product safety, such as: the culture conditions used, including records of all media and reagents/components used during production, and copies of relevant test reports (COA); cryopreservation, storage and recovery of MCB, including information on cell density, number of frozen vials, storage temperature and cell bank location; genetic and phenotypic stability of MCB after multiple passages, and genetic and phenotypic stability, and cell viability after cryopreservation. If a two-tier cell banking system is employed (MCB and WCB), the following tests are recommended for WCB: in vitro exogenous virulence factor assay; bacterial and fungal sterilization; mycoplasma; and representative identification tests (e.g., Southern blot, flow cytometry). For stem cell samples being used in clinical trials, the samples should be evaluated for phenotype and genotype stability from a cell differentiation perspective upon completion of preparation.
When more advanced methods and technological improvements are used in cell matrix identification and detection, a comprehensive comparative validation and assay bridging study of the old and new methods should be conducted to demonstrate that the specificity, sensitivity and precision of the new methods are at least equivalent to the existing methods.

Genetic stability

Stem cells may be unstable and generate cell heterogeneity during the passaging process, therefore, both banked and non-banked stem cells need to be adequately studied for genetic stability in a standardized passaging manner. The passaging conditions should be representative of the actual clinical/commercial production process. In genetic stability study, the following elements should be concerned, like changes in endogenous factor contamination, cell stemness, pluripotency, tumorigenicity/toncogenicity, etc..A clear definition of the generations of in vitro production and clinical use should be employed. The research items of genetic stability often include the stability of growth characteristics such as cell STR identification, number, morphology, viability, population ploidy time, purity, telomerase activity, etc., as well as the genetic stability of karyotype and whole gene sequencing, alkaline phosphatase staining/mouse teratoma test, immunofluorescence method or PCR method to detect pluripotency gene expression, etc.

Auxiliary cells

Once auxiliary cells are used in the stem cell products production process, they should also conform to the basic principles of clear and traceable source, controllable safety risk and establishment of cell bank hierarchy management. These auxiliary cells may remain in the stem cell final product and should be treated as impurities. Their risk should be studied and controlled. For example, for the feeder layer cells derived from human or animal to support human embryonic stem cells vitro culture and induced pluripotent cells, endoderm-like cells for induced differentiation, etc., the basis and rationality of their selection should need to be clarified. According to the possible risk factors associated with the use of exogenous cells in humans, the donor of the cell source, cell isolation, culture and lineage building process should be clearly traceable. The risk of introducing exogenous pathogenic microorganisms, etc. should be analyzed, assessed and tested for control. The process that may involve cell deactivation treatment, such as irradiation or addition of drugs, as well as the amount of auxiliary cells added and residual, should be studied and verified to prove that it will not The safety and functional impact on the product. Auxiliary cells can be managed by banking and fully tested according to the pharmacopoeia cell bank testing requirements, especially for human or animal source specific viruses. The stability of different generations of auxiliary cell bank cells should be examined in conjunction with the performance of the auxiliary cells, etc.

In Vitro Gene Delivery and Modification Systems

The upstream production of stem cell products obtained by gene modification and reprogramming technology involves the participation of in vitro gene delivery and modification systems. The CMC requirements for gene delivery and modification systems such as material selection (including virus packaging cells), vector design, process and quality control are recommended to refer to the relevant technical guidelines.

In vitro gene delivery and modification systems often employ both integrative and unintegrative vectors. Integrative vectors, such as lentiviruses and retroviruses, are widely used, but their random

integration phenomenon may lead to insertional mutations that interfere with the regulation of endogenous gene expression, and these viruses may be aberrantly reactivated in terminally differentiated cells, with a potentially higher risk of tumorigenesis. Unintegration reprogramming methods, such as adenovirus, adeno-associated virus and Sendai virus, can mediate transient expression of transcription factor genes in the host and induce multi-potential by affecting cell morphology, with a lower risk of genome integration. In addition, the risk of stem cell tumorigenesis may be higher compared to terminally differentiated immune cells. The impact of the application of in vitro gene delivery and modification systems in stem cell products on stem cell end-products and clinical safety, etc., needs to be fully evaluated.

Other raw materials

Raw materials for stem cell products can refer to the relevant requirements of the Chinese Pharmacopoeia [7] for raw material selection and supplier audit. Full consideration should be given to the necessity, safety and reasonableness of the use of raw materials, as well as the continuity of supply when large-scale product production. Some raw materials are in the process of continuous updating and optimization, giving priority to low risk level, such as the use of pharmaceutical grade raw materials, clear information on the source, composition, use, dosage and quality control of raw materials, with proof of raw material sources, inspection reports, packaging instructions, TSE/ BSE analysis statement and other documents. If research grade reagents (such as culture media, transcription factors, chemical small molecules, etc.) are used in the production process, information on the source, process and performance of the reagents should be clarified, and quality identification procedures should be established, which include safety testing (sterility, endotoxin, mycoplasma and exogenous factors), functional analysis, purity testing and content analysis (e.g. residual solvent testing) to prove that the reagents are free of potentially harmful substances. These reagents should meet the requirements related to sterility, mycoplasma-free, and low endotoxin when they are used to manufacture stem cell samples for clinical use. The scope of testing will depend on how the particular reagent is used in the process and will also take into account technological developments in the knowledge of new exogenous factors. For all raw materials that may affect product quality and safety (e.g., mutagenic), residues need to be evaluated and controlled at a reasonable stage of the final product or in appropriate stage of process, and the residue limits that need to be controlled need to be determined according to the clinical dose. Some high-risk raw materials may require in vivo safety assessment in animals. The use of β-lactam antibiotics should be avoided in production, and if the use of antibiotics is unavoidable in other parts of the process, information on the basis of antibiotic selection, residue control and safety assessment should be clarified.

The use of human- or animal-derived materials should be as much as possible avoided in the production of stem cell products. should be screened in early R&D. If human or animal-derived materials (such as autologous serum or autologous plasma) are necessary, they should be screened in early R&D and information on the source organism, distributor/supplier, country of origin, production process and quality standards should be fully described, and reasonable internal control tests and acceptable standards should be set to strictly control the risk of contamination by exogenous factors (such as exogenous viruses and prions) and immunogenicity. Always be alert to the potential risk of introducing TSE/BSE into the final formulation due to the use of human or animal-derived materials.

Some stem cell products may form combination products with other medical devices, active implantable medical devices, matrices, microcapsules and other materials, and should meet the relevant requirements for pharmaceutical and medical device combination products, and require detailed description and justification of the components, indicating the approval status of each component and evaluating its safety and suitability for its intended use, as well as the need to conduct appropriate types of biocompatibility studies and to assess whether testing of other devices adequacy of testing of other devices and adequacy of testing of any hardware and software controlling the device components.

Containers and containment systems (culture flasks, disposable lines, bioreactor bags, filters, etc.) that come into direct contact with intermediate samples during production should undergo rigorous screening, with attention to preventing microbial contamination and batch-to-batch cross-contamination, carrying out assessments of the suitability and biosafety of consumables, and conducting adequate studies of the compatibility and airtightness of consumables based on risk assessment.

Excipients

The source, dosage and quality control of excipients used in stem cell products developed in accordance with the drug should be based on the principle of risk assessment, full research and validation by prescription screening, etc., to prove the necessity, safety and reasonableness of their use, and should comply with relevant requirements of ***'General Rules for the quality control of raw materials and excipients used in the manufacture of biological products'*** *in* the current version of the Chinese Pharmacopoeia, and should try to choose low-risk excipients, giving priority to pharmaceutical-grade excipients. For excipients with high risk level, the necessity of using these excipients should be evaluated in the early stage of product development, and other alternatives or alternative sources should be sought.

Process

The process of stem cell products is complex. The batch-to-batch differences may be different from those of general drugs. For stem cell products developed as drugs, the research of their production process should follow the general rules of drug production process as far as possible, The relationship between production process and product quality, residue removal, and establishment of a robust and reliable production process should be comprehensively studied.

Process development

Since the early stage of R&D, it is necessary to gradually define the production process steps and operating parameters, as well as the production process control, acceptable ranges and abandon limits, to keep the process relatively stable and reliable, and gradually determine the product target product quality profile (QTPP) and critical quality attributes (CQA). The process of stem cell

products generally includes the upstream cell culture process and the downstream formulation process. The process flow may include several process steps in cell recovery, expansion, induction of differentiation, gene delivery and modification, harvesting, canning, freezing, storage, transportation, etc. The production scale and the definition of batches and lots should be clarified. Upstream and downstream process scales should be compatible. If there are combined batches or split batches in the process, adequate studies and corresponding principles need to be conducted.

Cell Culture

The quality characteristics of stem cell products are mainly developed at the cell culture stage, in addition, both the cell culture system and the process parameters at each step may have an impact on the growth and differentiation characteristics, biological functions and epigenetic properties of cells from different origins, and therefore a comprehensive process study should be performed. Cell culture process risk variables factors include seed cell banks, cell separation (mechanical, enzymatic), culture vessels (cell confluence and timing of passaging/harvesting for the apposed flat layer culture process, micro-carrier selection, reactor parameters for the bioreactor culture process), culture systems, inoculation/passaging densities and growth kinetic conditions, and also include starting cells, infection complexes, induction systems and differentiation systems, and each amount of the key production materials/components added, addition conditions (time, temperature), centrifugal washing conditions and number of times in each process stage. The quality of the cell culture process is examined in terms of cell viability (viable cell rate, generation, doubling time), cell characteristics (morphology, phenotype and genotype, differentiation, biological function), purity, transduction efficiency, reprogramming efficiency, viral vector reversion mutation, pluripotency, tumorigenicity, unintended cells/impact, genetic and epigenetic monitoring, etc. The risk of cancer and genetic disease-related mutations accumulated in the genome of stem cell products can be analyzed with the aid of sequencing methods.

Drug Product Process

The formulation and process should be determined after thorough research based on the clinical use requirements and combined with the product's own characteristics. The target cell component is a determining component to the effectiveness of the product. The formulation study should specify the composition of the prescription, clarify the CQA such as cell density or cell concentration, clarify whether the final preparation is fresh or frozen cells, whether the preparation is in free cell form or bound to the matrix, or whether the frozen cells are recovered and then given to patients after washing and other processes, etc. If the final product is transported to the clinical research center in a frozen state, it should be described in a way that includes a description of how the product was transported and provides data showing that the product can be thawed with consistent results, and encourages the use of advanced procedural cooling equipment with online monitoring for studies with different cooling procedures.

The reasonable filling process of the preparation can be selected according to the production batch and the effect of the canning operation process on cell viability. If the preparation needs to be frozen, research on the recovery process of the preparation should be carried out. If the frozen cells are

recovered and then given to patients after washing and other processes, research on the washing process should also be carried out and the safety risks of the operation process and the tolerance of the freshly recovered cells to the washing process should be evaluated. For new drug delivery devices and drug delivery methods, the accuracy of drug delivery needs to be ensured. The conditions for handling time and storage of cell preparations in the hospital prior to inject into humans should also be clearly defined. If autologous or allogeneic cell products are irradiated prior to injection to destroy replicative capacity to reduce the risk of cancer, data should be provided to demonstrate that the cells lose replicative capacity after irradiation but retain their desired properties. In clinical protocols for stem cell products to be infused, the risk of the different cell types themselves and the risk of contamination, infection and pathogen transmission during clinical use should be highly concerned.

Process control

The entire process of stem cell product should be monitored by reasonable and feasible process control parameters and intermediate control limits. These process control parameters and intermediates control limits include a clear definition of the culture duration of the process steps, the production cycle and the number of cell-limited generations, the time control range of each step in the production, etc. Since stem cell products generally cannot be subjected to terminal decontamination and de-viralization processes, the risk of contamination by exogenous factors is high, and microbiological safety indicators (including sterility, mycoplasma, endotoxin, etc.) should be controlled at appropriate steps in the production process. In addition, effective isolation measures are important to prevent confusion and errors in products from different donor sources or different batches. Considering the dynamic variability of cells during the production process, for quality parameters that can characterize cell morphology, viability, tumorigenicity, phenotypic features (including expected and unintended cell populations), function, genetic stability, and product stability, intermediate process control at key production stages or intermediate products is recommended to set acceptable ranges and provide a basis for correction limits.

The risk of using gene transduction and modification operations is higher than those without. The strict risk control strategies should be established. The transduction mode, genetic manipulation methods and conditions, target gene transduction efficiency, and integration in chromosomes should be clarified and rationalization should be discussed. If viral vectors are used, viral replication, reverse mutations and insertion mutations should also be studied and verified. The cell phenotype, genotype, function, and properties before and after in vitro gene transduction and modification, as well as the trend of process-related impurities and non-target cell populations, should be studied, characterized and/or validated with emphasis on the robustness of the process.

Process change

Like other pharmaceutical products, the preparation process of stem cell products is subject to changes during the life cycle, and these changes are optimizing the process or improving the quality of the product. The impact of these changes on the safety, characteristics, purity and biological activity of stem cell products should be thoroughly studied and comparability studies should be

conducted to the impact from process changes on stem cell product qualities. For stem cell products, the comparability study should cover the comparison of raw materials, process comparison and changes in quality attributes of intermediate/final products before and after the process changes, focusing on the content of the changes, the capacities of analytical method testing that can distinguish the changes in product quality attributes, and the development stage of the product, etc. Reasonable comparability study can follow the guidance of ICH Q5E [10]. The representative batches at each stage are included in the study. The testing items should be comprehensiveness. The reasonableness of the pre-set comparative study criteria, the validity of the testing methods and the objectivity of the analysis of the testing results could be the key points to be concerned. For production processes that may exist in multiple stages during the development, such as non-registered clinical sample preparation process, non-clinical sample preparation process, sample preparation process for clinical trials, and commercial production process, the differences between the processes in each stage should be specified, and comparability studies should be conducted to evaluate the processes changes impact on product quality. In some case, animal studies or human trial studies should be provided if necessary. In general, all expected changes should be completed before the pivotal clinical trials are conducted.

Process verification

Compared with general pharmaceuticals, the batch-to-batch variation of stem cell products may be larger and process validation is more difficult, but it is still necessary to ensure product consistency and quality controllability. At the market authorization application stage, the production of multiple consecutive batches of stem cell products at commercial production scale should be conducted, and the process and product quality of each batch should be fully characterized to confirm the robustness of each process and the consistency of product quality. The process validation process is recommended to focus on the in vitro assessment of genotypic instability, tumorigenicity and phenotypic characteristics (including expected and unintended cell populations) of the product at key production stages. The process validation should ensure the safety of the product. In addition, validation of the production process of key raw materials, storage conditions and time of intermediates, medium/buffer preparation and placement conditions, filter validation, transport validation, aseptic mock filling validation, cleaning validation and container seal integrity validation should be completed.

As with general pharmaceuticals, stem cell products in early clinical trials have generally not been validated for production processes, but the quality control standards for raw and auxiliary materials used in production should be clarified prior to clinical trials, while taking appropriate process monitoring and control measures to ensure that the process meets the safety requirements of clinical trial samples. Samples used in pivotal clinical trial should be representative of the marketed product, and their processes must be validated to demonstrate that the production process can ensure consistency of production. Commercial scale process validation should focus on the challenge study of the actual simultaneous production of the maximum capacity, considering the ability of the overall operational capacity of personnel, equipment, materials, environment, testing, etc. to support the maximum capacity. Trends of data on process performance and product quality from batch to batch should be documented in detail to carry out continuous process validation throughout the life cycle.

quality research and quality control

Quality research

As stem cell products are diverse, variable and complex, quality studies should be conducted on representative production batches and appropriate production stage samples (including initial isolated cells or cell seeds, cell banks, intermediate products, stock solutions and finished preparations). If possible, a series of state of the art and orthogonal analytical techniques should be used, and the analytical methods should be confirmed by research to ensure that the methods are applicable and reliable.

Cell characterization

Cell morphology: Morphological analysis of cells is indicative of cells at different stages of growth and development, and light microscopy (which can be combined with specific staining) is a common method for observing cell morphology.

Cell activity: Stem cell products are usually live cell therapy products, and the cell activity can be evaluated comprehensively by cell viability, number of live cells, population multiplication time, cell cycle, etc.

Cell identification: Identification of cell populations is an important way to ensure the purity of stem cell products, generally through short tandem repeats (STR) mapping, mid-term karyotype analysis and isozyme profiling or genetic polymorphism gene analysis to evaluate cross-contamination of different cells, and also through surface markers and specific genes. The analysis can also be done by surface markers and specific genes.

Cell marker assays: Cell expression of specific markers is part of cellular characterization, and characterization of stem cell products is often performed by selecting multiple surface markers for cell type, pluripotency, lineage, terminal differentiation, and/or functional assays, and relevant analytical assays should be validated by specification. Valid correlation of mRNA markers with protein marker expression can be used for cell characterization aided by mRNA-based markers if verified.

Physical and chemical characterization

The general physical and chemical characterization needs to be studied in the context of product type and formulation characteristics, and may include items such as appearance, color, clarity, pH, visible foreign matter, molar concentration of osmolality, loading capacity, etc.

Cell purity analysis

Stem cell products may introduce non-cellular impurities (e.g., physicochemical impurities), cellular debris, or non-functionally required cells during the manufacturing process, which may affect the purity and homogeneity of the product in terms of biological properties and may pose safety risks. These non-purpose cellular components should be removed in the process, detected in quality studies, and controlled qualitatively/quantitatively.

Typical purity analysis studies may include: ratio of live cells, ratio of cell subpopulations, ratio of functional cells, ratio of non-intended cell populations, etc. For some cellular and non-cellular component formulation combination type products, which contain non-cellular components (e.g. matrix, scaffold, etc.) in addition to cells, it is necessary to focus on assessing impurities such as unintended cells and non-cellular components.

Biological safety: It refers to the safety issues that are intrinsic to the cells and determined by their biological properties, and extrinsic to induce changes in their biological properties. When evaluating the biological safety of stem cells, the clinical indications, routes of administration, doses and other factors directly related to clinical treatment of the relevant cells should be considered as much as possible, and the relevant biological safety should be effectively evaluated using appropriate in vivo and in vitro test models. The biological safety of stem cells includes tumorigenicity and oncogenicity, abnormal immune response and abnormal differentiation, etc.

Tumourigencity, Oncogenicity: The risk of tumourigenicity and oncogenicity of stem cell products should be considered, especially for stem cell products of high generation, or autologous origin with complex in vitro processing and modifications, and various allogeneic sources. For stem cell-derived products derived from human embryonic stem cells and induced pluripotent stem cells, end-product oncogenicity and tumorigenicity assays, analyzing the relationship with the removal of undifferentiated multi-potential cells and the residual levels of ESCs/ iPSCs in the end-product, should be highly concerned.

Abnormal immune responses: For stem cell preparations, especially allogeneic-derived, autologous or allogeneic-derived preparations that have been cultured and specially treated in vitro, abnormal immune responses should be evaluated by in vitro and animal tests in combination with product characteristics. For example, the expression of cellular autoimmune compatible antigens HLAs molecules (ES and iPSC differentiated to re-express donor HLA), immune co-stimulatory molecules, pro-inflammatory factors (IFN, TNF), etc., and the detection of mesenchymal proliferation/activation of allogeneic immune cells (e.g. total lymphocyte proliferation).

Unintended differentiation: This includes non-target cell differentiation or non-target site differentiation. It is recommended to develop specific assay technologies (e.g. Biocore assay markers) to study, evaluate and monitor the possibility and impact of unintended differentiation of stem cell products, which can be specifically analyzed in conjunction with the purity of target cell differentiation.

Microbiological safety: It refers to that stem cell products should meet the requirements of no microbial contamination such as bacteria, fungi, mycoplasma and viruses, and no contamination of microbial metabolites (represented by endotoxin produced by Gram-negative

bacteria). Monitoring contamination by endogenous and exogenous factors can be achieved by direct detection of relevant pathogens (e.g. nucleic acids, proteins, polysaccharides) or microbial metabolites, or indirect analysis of the presence and effects of relevant microorganisms by in vitro cell models, sensitive animal inoculation and chicken embryo inoculation.

Aseptic testing - bacteria, fungi: Use of the current version of the compendial method for aseptic testing is encouraged. Based on the process stage, management needs, clinical application needs, etc. Different detection methods can be employed at the cell samples in different process stages at different time., such as the use of fully automated bacterial / Mycobacterium detection system or Gram stain microscopy, or rapid detection methods based on nucleic acids or specific metabolites. The selection of rapid detection methods should be assessed for suitability and progressively complete a comprehensive methodological validation and comparison with compendial methods to demonstrate comparable detection capabilities.

Mycoplasma testing: A combination of culture and indicator cell infection methods (or DNA staining) in the current pharmacopoeia is encouraged for confirmatory mycoplasma testing. Mycoplasma nucleic acid amplification methods can also be applied as rapid detection methods when needed, or a combination of confirmatory and rapid methods for mycoplasma detection for different process stages of detection needs. Common human-derived mycoplasmas are usually introduced by laboratory personnel, such as oral mycoplasma, human mycoplasma, and salivary mycoplasma; animal-derived mycoplasmas are usually introduced via bovine serum, such as Mycoplasma fermentum, Mycoplasma argentum, and Mycoplasma leprae; and mycoplasma porcine nasalis may be introduced when trypsin is used during cell isolation and digestion. Possible sources of contamination should be taken into account during method selection and validation.

Virus-specific and non-specific virus testing: Virus contamination refers to genus-specific viruses (e.g., human- and animal-derived viruses), endogenous and exogenous retroviruses, and all non-specific viral factors that originate from raw materials such as starting cells or are introduced during the manufacturing process. The risk of viral contamination of products should be evaluated at the appropriate stage according to the product characteristics, using in vivo and in vitro methods, in conjunction with the entire production process. Specific viruses of human origin may include HIV, HBV, HCV, HTLV, TB, B19, etc. The relevant tests are based on pathogenic nucleic acid/antibody tests, using approved in vitro diagnostic kits for testing if possible, and the relevant testing methods should be validated. If bovine serum is used, specific viruses of bovine origin shall be tested, which may include bovine parainfluenza virus, bovine adenovirus, bovine microvirus, bovine diarrhea virus and arc enterovirus, etc. If pig-derived materials such as pancreatic enzymes are used, porcine microvirus, porcine circovirus and porcine circovirus may be tested. if use animal-derived feeder layer cells (Feeder layer) have been used in embryonic stem cells and iPS cells preparation process, cell origin-related tests shall be performed. If embryonic stem cells and iPS cells are prepared using an animal-derived feeder layer, comprehensive testing for specific animal-derived viruses associated with the cell source is required. For the detection of retroviruses and non-specific viral factors, reference can be made to the ***Chinese Pharmacopoeia"Part 3 Animal cell matrix preparation and quality control for the production and testing of biological products"*** related requirements.

Replication competent virus (RCV): For stem cell products obtained by in vitro transduction using replication-deficient virus, there is a possibility of reverse mutation of the viral vector, which is one of the major risks affecting product safety and should be fully concerned during product design and

quality studies. In general, in addition to the standard RCV assay completed in the virus process (supernatant, end cells in virus production) using the validated indicator cell culture method, a validated rapid method should be used for RCV detection to the final stem cell product at the release inspection. Samples should also be retained and the final product retrieval assay analysis could employ the indicator cell culture method if necessary, and RCV should be continuously monitored in later studies.

Bacterial endotoxin detection: The gel method and photometric method in the current version of the pharmacopoeia are recommended, and a better method can be used if validated, such as the modified gel method (also known as recombinant C-factor method).

Biological activity analysis

Stem cells are living cellular drugs and their biological effects are multi-target and multi-pathway. The biological effectiveness of various types of stem cells can be basically categorized as induced differentiation ability, immunomodulatory ability and tissue regeneration ability. Stem cell biological potency assays include: secretion of relevant bioactive substances (e.g. recombinant proteins, glycol-proteins or lipoproteins, growth factors, enzymes and cytokines), formation of cells and extracellular matrix/structures, cellular interactions (e.g. immune activation or inhibition), and migration differentiation or self-renewal potential of cells.

The biological effects and mechanisms of action of the product relevant to clinical treatment should be defined. The quantitative/semi-quantitative biological activity assays that are representative of the mechanism of action of the product [(11)] should be developed. If possible, multiple complementary analytical assays for the study should be adopted and complete methodological validation should be conducted whenever possible。For example, in cases where mixed cell populations with functional and phenotypic plasticity may be required, potency tests should complement the phenotypic profiles of different cell populations data on the phenotypic profile of different cell populations. In some special cases, surrogate assay of biological activity can be developed, such as abiotic analytical assays performed outside the alive system, which could provide extensive product characterization data by assessing the immunochemical, biochemical, and/or molecular properties of the product. The analytical assay used should perform alternative method and potency correlation studies and should also demonstrate that the assay can distinguish between active cells and inactive material or dead cells, among other forms of products, with adequate control studies and methodological validation. Some stem cells and their derived products have complex and/or incompletely defined mechanisms of action or multiple biological activities, and the characterization of cell biological functions shall be evaluated in combination with in vitro biological effects and in vivo animal models.

Quality standards

The development of quality standards for human-derived stem cell products should be determined based on product characteristics, production processes, quality studies and risk assessment, and generally include quality standards for stock solutions (if any), quality standards for semi-finished products (if any) and quality standards for preparations. With the concern with the limited batch size

and short validity period of some stem cell products, on the basis of adequate research, release testing can be simplified by improving process control and testing of intermediates (simplified release inspection must include at least identification and biological potency testing), while new release testing methods are used to supplement traditional testing methods, but the testing methods should be fully validated.

The quality standard testing program for stem cell products should be based on product quality studies and a thorough understanding of the production process and manufacturing process, while taking into account the characteristics of the product and the current scientific knowledge and common understanding. In addition to general tests for appearance, loading, pH, sterility, endotoxin, and mycoplasma, release tests should include cell number/dose (cell count, functional cell count, etc.), cell viability, identification, purity, biological potency (quantitative/semi-quantitative functional assays, molecular markers, etc.), product and process-related impurities, and abnormal immune responses. In the case of genetic modification, the percentage of genetically modified cells per batch of final product, and the number of vector/plasmid copies per cell should be tested. If exogenous genetic material is removed from the final product, this can be demonstrated by relevant sensitive assays at the time of release. For cells transduced with replication-deficient viruses, the absence of replication vectors (RCV) should be demonstrated. Product-related and process-related impurities can be analyzed and controlled in combination with stable and reasonable process clearance verification. For process-related impurities that have certain residues in the end product and may affect the quality, safety and efficacy of stem cell products (such as BSA, digestive enzymes, magnetic beads, microcarriers and other related impurities (such as harmful cell residues), the applicable method should be selected for quality release control. In principle, final product release testing should be based on the biological products sterility and mycoplasma inspection method in the current version of the pharmacopoeia, if necessary, the use of rapid methods for release should be confirmed methodologically, before fully verify the new method can completely replace the traditional method of the pharmacopoeia, it is recommended that the use of new detection methods for release testing at the same time, as well as leaving samples using the traditional method of the pharmacopoeia for parallel testing, while a backup protocol should be available when the postpone tests possibly give out unintended results that may impact the certainty of the qualities of the products . For stem cell products that are transported to the hospital and need to be re-operated before administration (e.g., container conversion, physical state transformation, association with other structural materials, filtration and cleaning, etc.), it is recommended that quality criteria such as cell morphology, color, number and ratio of viable cells, exogenous foreign matter other than cells, etc., as well as review of operational steps and label verification be set again. Acceptable specification need to be based on data from batches of samples at the time of preclinical and/or clinical studies, data used to demonstrate manufacturing consistency batches, stability study data, and R&D-related data, etc.

Reference products established after quality characterization should be representative and traceable, and should be calibrated using validated analytical assays (content calibration and activity calibration), and stability studies should be conducted on reference products used in all phases of product development to determine the retest period and expiration date.

The development and validation of analytical assays should follow the general methodological rules of drug development, including release testing methods and process control testing methods. Analytical methods should be gradually improved and validated as the research progresses to meet

the quality control requirements at all stages. New methods based on their own products should be fully validated. The applicability of methods using the pharmacopoeia should be confirmed, and if the pharmacopoeia methods are revised or replaced, comparative studies should be conducted to confirm their reasonableness. For example, some new rapid and highly sensitive testing methods for trace amount sample of products with short expiration dates and small sample sizes should be compared with the old methods and evaluated. Both the old and new methods should be used in the product release for mutual verification.

Stability study

The stability study can be carried out with reference to the general requirements of the Chinese Pharmacopoeia Guidelines for Stability Testing of Biological Products [12] and ICH Q5C [13].

The purpose of stability studies is to support the storage, transportation and use of stem cell products. Stability studies generally include impact factor tests (temperature, light, mechanical force, etc.), acceleration tests, long-term tests, transportation tests and stability tests in clinical use. The test conditions should fully consider the difference between fresh and frozen cells, the special requirements of stem cell product preservation, packaging, transportation, clinical compounding and actual drug administration, and the influence of the cumulative preservation time of each link of sample storage on the stability of the final product.

Test samples: include representative stock solutions (if any) in general, finished products and intermediate cell products that require temporary or staged freezing.

Test items: normally include biological potency, cell purity, cell characteristics, number of live cells, cell viability, number of functional cells, microbial safety indicators and excipient content, etc. The test time should be able to support the formulation of the expiration date, taking into account the complexity and variability of stem cell products, generally do not support the extrapolation of the expiration date.

The stability data at the clinical trial application point should support the clinical trial.

The stability data of the proposed representative batches should be provided to support the determination of storage conditions, conditions of use and expiration date, and the sensitivity conditions, inactivation pathway and inactivation rate of the product should be clarified.

Packaging and closed container system

The object of the suitability assessment of packaging and containment container system refers to the packaging container and containment system that are in direct contact with the product. Should be combined with the route of product administration (intravenous administration, local administration, ophthalmic preparations), the nature of the preparation (fresh cells, frozen preparations), etc. to select the appropriate inner packaging materials (celine bottles, soft bags, etc.). Refer to the relevant guidelines [14], compatibility studies of samples (collected tissues or cells, cells in the preparation process) and finished products in the process of stem cell products and direct contact packaging materials should be carried out, with using small size packaging containers of the

same material, but should cover the density and volume range of the products to be packaged. Extractable/leachables studies should be carried out and safety assessments should be conducted. Toxicological assessment of extracts should be based on good scientific principles and consider specific container sealing systems, drug prescriptions, dosage forms, routes of administration and dosing regimens (chronic or short-term dosing), etc.

Terminology

Adult stem cells: mesenchymal stem cells (MSC), hematopoietic stem cells and various types of precursor cells (progenitor cells) with multidirectional differentiation potential in developed embryonic or adult tissues and accessory tissues (e.g. umbilical cord, placenta) accompanying birth, such as neural stem cell (NSC), etc.

Human embryonic stem cells: Stem cells with the potential to differentiate to the triterminal layer, isolated and established from the cell mass within the blastocyst (blastocyst) at the early stage of in vitro development, can self-renew indefinitely in vitro and have pluripotency.

Induced pluripotent stem cells (iPSC): are stem cells with multi-directional differentiation potential obtained by reprogramming differentiated adult cells using viral or non-viral vector technology, with pluripotency similar to that of human embryonic stem cells.

Teratoma: A multilayered benign tumor that arises when pluripotent stem cells are injected into immunocompromised mice. The scientific community has tested whether a human embryonic stem cell line (hESC) has been established by injecting stem cells into mice to verify that the resulting teratoma contains three germ layers of cells.

Feeder layer: Cells that do not have the ability to proliferate on their own, but are used to support the growth and expansion of other cells through cell-cell interactions or secretion of certain nutrients.

Batch: refers to a certain number of products prepared from the same source and the same process in the same production cycle, within the specified limits, the batch has quantitative and qualitative homogeneity.

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