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Research on Quality Control Strategy of CAR-T Cell Therapy Products based on QbD

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Abstract

Objective: This paper aims to provide reference for how to use the concept and method of QbD to control the quality of CAR-T cell therapy products in the commercial production of CAR-T cell therapy products in China, and how to formulate reasonable and effective quality control strategies.

Methods: This paper systematically analyzes the industrial development of CAR-T cell therapy products and the concept, application methods and implementation process of QbD in China through literature research and case analysis theory

Results: Cell therapy products are a hot area in the research and development of new drugs, but at present, there are no specific regulatory rules in the commercial production of CAR-T cell therapy products in China, the awareness of risk control is weak, and the quality control is unstable, making patients face many risks.

Conclusion: Based on the concept of QbD, the quality control strategy of CAR-T cell therapy products was proposed from three dimensions.

Keywords: QbD; CAR-T cell therapy; Critical quality attributes; Critical process parameters; NMPA; quality target product profile (QTPP); Kymarih

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1. Introduction

In recent years, cell therapy has become a research hotspot in the field of medicine, providing new therapeutic methods for major and refractory diseases such as cancer. Chimeric antigen receptor T cell (CAR-T) is one of the hottest cell therapy products studied in recent years. The United States is the first country in the world to approve the launch of CAR-T cell therapy products, and is in a leading position in the research and application of CAR-T cells. In 2017, the US FDA took the lead in approving the launch of two CAR-T cell therapy products, namely Kymriah from Novartis and Yescarta from Kite Pharma, which opened a new chapter in the clinical application of CAR-T cell therapy products. The successful practice of the United States in this field has also led to global clinical research. In recent years, the clinical research of CAR-T cells in China has shown an explosive growth. In June 2021, a CAR-T cell therapy product in China was approved for listing by the National Medical Products Administration (NMPA), which is also the first CAR-T cell therapy product approved for listing in China.

With the increase of clinical research on CAR-T cell therapy in China year by year, new research results have been obtained continuously, and the voice for the industrialization and clinical application of related products is also getting higher and higher. (1) However, as a special drug, CAR-T cell therapy product has its particularity in product characteristics and production process, which is not only reflected in individualization, small output, limited batch and other aspects, but also has problems such as large differences in starting materials, immature preparation process, complex biological efficacy and safety evaluation, (2) which limits the expansion of cell therapy products from laboratory scale to reliable and economic commercial production scale. Therefore, how to make use of the modern drug development strategy of "Quality by Design (QbD)" to formulate a reasonable and effective quality control strategy for CAR-T cell therapy products in view of their particularity, so as to ensure the safety,

effectiveness and quality controllability of CAR-T cell therapy products is a problem that must be faced and solved in the current commercial production.

2. Industrial development of CAR-T cell therapy products in China

2.1 Industrial development history of CAR-T cell therapy products in China

In 2009, the clinical research on immunocyte therapy was just starting in China. The Ministry of Health included immunocyte therapy technology in the third category of medical technology. Since the safety and effectiveness of immunocyte therapy at that time still needed to be further verified through standardized clinical trial research, the clinical trial could only be carried out after the technical review of the third category of medical technology audit institution of the Ministry of Health. In 2015, the former National Health and Family Planning Commission cancelled the access approval for clinical application of Category III medical technology and changed it to record management. In this period, the technology and management level of various enterprises in the immune cell therapy industry were mixed, and the lack of supervision led to some confusion in the immune cell therapy industry.

In March 2016, the "Wei Zexi Incident" hit hard the development of immunocellular therapy technology in China. In May of the same year, the former National Health and Family Planning Commission urgently stopped all clinical applications of cell therapy in violation of regulations, and required that the projects that have been carried out must be reported, emphasizing that immunocellular therapy is limited to clinical research.

However, in 2017, FDA approved the listing of two CAR-T cell therapy products, which once again aroused domestic attention to immunocyte therapy, and the regulatory authorities also realized that scientific guidance is needed. In December 2017, the former State Food and Drug Administration issued the *Guiding Principles for Research and Evaluation Technology of Cell Therapy Products (Trial)*, which clarified the drug attributes and regulatory approaches of cell therapy products, interpreted relevant issues, and made detailed regulations on pharmaceutical research, non-clinical research and clinical research involved in the research and development of cell therapy products, On the basis of confirming the principle of drug declaration, we also understood the uniqueness of cell therapy. (3) Since then, China's cell therapy products have achieved an important transformation from medical technology to drugs, which officially kicked off the standardized and industrialized production of cell therapy products. Under the correct guidance of the regulatory authorities, which give consideration to both safety and innovation, CAR-T cell therapy products also ushered in new development in China.

In 2018, the China Institute for Food and Drug Control released the *Consideration Points of CAR-T Cell Therapy Product Quality Control Testing Research and Non clinical Research*, which clarified the scope of application of CAR-T cell therapy products and the provisions on quality control testing. At the end of 2019, the NMPA issued the *GMP Appendix - Cell Therapy Products (Draft for Comments)*, seeking comments from the society on various aspects of the GMP requirements for cell therapy product production, in order to fill the gaps in China's production and quality control of cell therapy products at the regulatory and technical levels. (4) In 2021, the NMPA issued the *Guiding Principles for Clinical Trial Technology of Immune Cell Therapy Products (Trial)* to guide how to carry out clinical trials of immune cell therapy products. In 2022, the Center for Drug Evaluation, NMPA also released the *Guiding Principles for Pharmaceutical Research and Evaluation Technology of Immune Cell Therapy Products (Trial)*, which further standardized the pharmaceutical research, development, production and registration of immune cell therapy products. It can be seen that the regulatory authorities in China are gradually improving the regulatory system of cell therapy products, and at the same time, they are also guiding the cell therapy industry in China to develop more healthily and rapidly. Table 1 summarizes the policy documents related to CAR-T cell therapy products issued in China.

Table 1. Policy documents on CAR-T cell therapy products

Date	policy	Issuing Dep.	Content
May 2009	Administrative Measures for Clinical Application of Medical Technology	Ministry of Health	Immune cell therapy technology is included in the third category of medical technology, which needs to be strictly controlled and managed by the health administrative department. Its safety and effectiveness still need to be further verified by standardized clinical trial research, and it needs to be technically audited by the third category of medical technology audit institution of the Ministry of Health
June 2009	Management Specifications for Treatment Technology of Autoimmune Cells (T Cells, NK Cells) (Trial)	Ministry of Health	For the field of immunocellular therapy, detailed technical specifications have been formulated, including the scope of immunocellular therapy technology, requirements of clinical application institutions, personnel requirements, quality control standards, hardware requirements,

Date	policy	Issuing Dep.	Content
			etc. This management specification is only applicable to medical technologies that have completed safety and effectiveness certification in clinical practice and meet ethical requirements, only involving T cells and NK cells as treatment means.
July 2015	Notice of the National Health and Family Planning Commission on Canceling the Examination and Approval of the Clinical Application of Category III Medical Technology	Former National Health and Family Planning Commission	Cancel the approval for clinical application of the third category of medical technology, and list immunocyte therapy technology as "clinical research"
October 2017	Provisions for the Administration of Drug Registration (Revised)	Former State Food and Drug Administration	It is stipulated that cell therapy products can be declared according to the corresponding category requirements of biological products for treatment
October 2017	Requirements for Registration Classification and Application Materials of Biological Products (Trial)	Former State Food and Drug Administration	Clarified the classification of various cell therapy products and standardized supervision
December 2017	Guidelines for Acceptance and Examination of Registration of Biological Products for Treatment (Trial)	Former State Food and Drug Administration	1. Data acceptance department; 2. Basic requirements for application materials; 3. Key points of application form review; 4. Key points of application materials review
December 2017	Guiding Principles for Research and Evaluation of Cell Therapy Products (Trial)	Former State Food and Drug Administration	To guide the research, development and evaluation of cell therapy products according to drug management specifications for reference before clinical trial declaration and clinical research
December 2017	Considerations on Pharmaceutical Research and Application for Clinical Trial of Cell Therapy Products	Center for Drug Evaluation, NMPA	Provide reference for research and development of cell therapy products and preparation of application materials
June 2018	Consideration Points of CAR-T Cell Therapy Product Quality Control Testing Research and Non clinical Research	China Institute for Food and Drug Control	The regulations on the application scope and quality control testing of CAR-T cell therapy products were clarified
November 2019	GMP Appendix - Cell Therapy Products (Draft for Comments)	NMPA	The GMP requirements for cell therapy products are specified in terms of personnel, plant facilities, materials, production management, quality management and product traceability system
February 2021	Guiding Principles for Clinical Trial Technology of Immune Cell Therapy Products (Trial)	NMPA	Provide guidance for the overall planning, trial scheme design, trial implementation and data analysis of clinical trials of immunocyte therapy products
May 2022	Guiding Principles for Pharmaceutical Research and Evaluation Technology of Immune Cell Therapy Products (Trial)	Center for Drug Evaluation, NMPA	The general principles of pharmaceutical research of immunocyte therapy products at different stages, such as clinical trial application, market application, process change, were clarified, and detailed pharmaceutical technical requirements were proposed for production materials, production process, quality research and quality control.

2.2 Problems in the Industrialization Development of CAR-T Cell Therapy Products in China

As of March 2022, The Center for Drug Evaluation (CDE), NMPA has accepted 135 applications for cell therapy products. Most of them are experimental new

drug (IND) applications. Of the 135 applications, 70 were CAR-T. At present, CAR-T cell therapy products in China are mostly in the clinical research stage, and only a few have reached the later stage of commercialization. Due to the particularity of CAR-T cell therapy products and various difficulties in production, CAR-T cells still have some problems to be solved in the process of industrialization and clinical application, mainly including the following three points: First, CAR-T cell therapy products still have many safety risks, mainly including a series of adverse reactions including cytokine release syndrome (CRS), neurotoxicity, on target/off tumor recognition and anaphylaxis, which can lead to death in severe cases; Second, there is no more targeted regulatory rules for the commercial production of CAR-T cell therapy products in China. How to ensure the safety, effectiveness and quality controllability of products and continuously produce products that meet the quality requirements when products are scaled up from the laboratory to a reliable and economical commercial production scale; Third, the personalized treatment of CAR-T cell therapy products has kept their production costs high. How to reduce production costs, reduce the economic burden of patients, and improve the accessibility of products. This paper mainly studies and discusses the second aspect. For the production of CAR-T cell therapy products, there are some common industrial problems in the implementation of GMP, including production process, pollution and cross contamination, confusion, storage, transportation and use, quality inspection, etc. (5) Therefore, how to use the control method of "Quality by Design(QbD)" is particularly important to promote the industrialization of CAR-T cell therapy products.

3. Application of QbD in Kymarih product quality control

The concept of QbD first appeared in Q8 issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), It is defined as "a set of systematic research and development methods based on sufficient scientific knowledge and quality risk management, starting from predetermined goals, emphasizing the understanding of products and processes and process control". (6) In the concept of QbD, drug quality is not determined by inspection, but by design, production and management. QbD can be applied to the development and production of different types of drugs, and its core principles and methods are universally applicable. Therefore, this paper will take Novartis Kymarih (common name: tisagenlecleucel, product code: CTL-019), the first CAR-T product in the world, as an example to deeply analyze the application of QbD method in the quality control of its commercial production products, with a view to providing suggestions for the quality control of CAR-T cell therapy products in China.

3.1 Product description and production process

Kymriah is a gene therapy product containing autologous transgenic T cells. The product is composed of autologous T cells genetically modified with anti-CD19 chimeric antigen receptor (CAR) encoded by lentivirus vector, targeting CD19 positive tumor cells and normal B cells. CAR is composed of mouse single chain antibody fragment (scFv) against CD19, CD8 hinge and transmembrane region, and intracellular signal transduction region with 4-1BB (CD137) and CD3 zeta. (7) CAR structure is shown in Figure 1.

The production process of Kymarih products mainly includes two major production links: (7) first, the production of lentivirus vectors, including upstream production processes: unfreezing the working cell bank (WCB), expanding the production cell bank, plasmid transfection, induction and harvesting; Downstream purification process: filtration, chromatography and nuclease treatment to obtain carrier material, which is then subjected to aseptic filtration, concentration and filling to obtain carrier products. The second is the production of CAR-T cell end products. The process is as follows:

a) Cell collection: collect blood samples from patients in the company's certified institutions;

b) T cell separation and sorting: peripheral blood mononuclear cells (PBMC) were isolated and T cells were sorted by immunomagnetic beads;

c) T cell activation: T cells were activated by magnetic beads coated with anti-CD3/CD28 monoclonal antibody;

d) CAR gene transduction: transfect the lentivirus vector containing anti-CD19 CAR gene into T cells;

e) CAR-T cell expansion: expand the CAR expressing T cells in the cell culture to a sufficient number;

f) Cell washing and harvesting: wash cells to remove impurities, and add glucose, sodium chloride, human serum albumin and other infusion media to prepare a preparation;

g) Cryopreservation of CAR-T cell products: add DMSO into the preparation and place it in the gas-phase liquid nitrogen refrigerator for freezing.

h) Product transportation: Put the product in gaseous liquid nitrogen and transport it to medical institutions by qualified transport personnel.

Kymarih production is a continuous process, from cell separation, activation, CAR gene transduction, cell expansion to harvesting suspension, it is continuous, timely and cannot be interrupted, and takes a certain quality and quantity of CAR-T positive T cells as the ultimate goal. In addition, the whole production process from cell collection to product generation and transfusion back to patients is controlled by a computer-based identification chain system to ensure product identity and product traceability. (7)

3.2 Implementation of QbD in Kymarih products

The implementation of QbD includes the following five elements, and the implementation route is shown in Figure 2:

a). Define the quality target product profile (QTPP): QTPP is the design basis for product research and development. The factors to be considered in determining the QTPP include: expected clinical use, route of administration, dosage form, administration system, dosage specification, container sealing system, etc., as well as the product quality requirements, such as sterility, purity, stability, etc. (6)

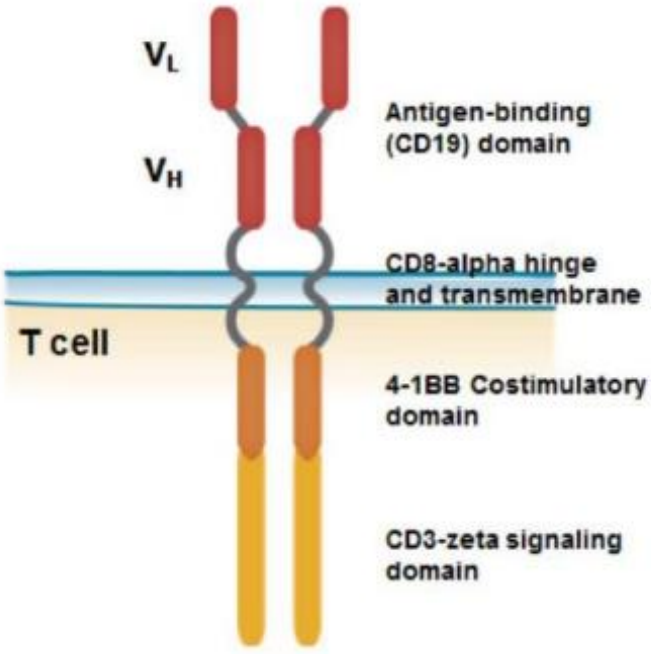

Figure 1. CAR structure of Kymarih

b). Determination of critical quality attributes (CQA): obtained by assessing the impact of each quality attribute on product quality, safety and effectiveness through risk analysis. (6)

c). Clarify critical process parameter (CPP) and critical material attribute (CMA): based on production process research, use risk analysis to evaluate the impact of material attributes and process parameters on product quality. (6)

d). Establish the process design space (DS): confirm the acceptable change range of each CPP and CMA through the experimental design in the process characterization and process validation, within which the change of process parameters shall enable the product to meet the predetermined quality requirements. (6)

e). Set quality control strategy: control various parameters according to the design space or real-time release inspection scheme, including the specification of raw materials, excipients and preparations, as well as the control of the production process, to ensure the continuous production of products that meet the quality requirements. (6)

The implementation process of QbD can be divided into three stages: quality design, quality control and quality improvement, which run through the whole life cycle management of products. Therefore, QbD can not only improve the ability and speed of product development, but also contribute to the continuous improvement of product quality. The implementation of QbD needs to be based on a thorough understanding of product characteristics and production processes, and the integrated application of process analysis techniques and quality risk management methods to the whole process of product quality control.

3.2.1 Define the QTPP

The QTPP describes the characteristics of the required final product, which can be defined from the aspects of expected clinical use, administration route, dosage form, administration system, dosage specification, container closure system, and product quality requirements, which is the starting point of QbD. According to Kymarih's product information, the following QTPP are listed, as shown in Table 2.

3.2.2 Determine the CQA

In the process of developing a production process, it is very important to determine the CQA of the product to ensure the product quality. These CQAs are physical, chemical, biological or microbial attributes within the appropriate limits or distribution ranges to ensure the desired product quality. Therefore, CQA is a quality attribute directly related to the safety and effectiveness of products. Table 3 shows the CQA judgment matrix of Kymarih end products. First, list all the quality attributes of the product, judge whether each quality attribute is CQA by judging the severity of the hazards (safety and effectiveness) caused to patients when it does not meet the requirements, and score according to the weight of CQA in the product quality attributes (scoring criteria: three grades, from low to high, the scores are 1, 3, 5 respectively)

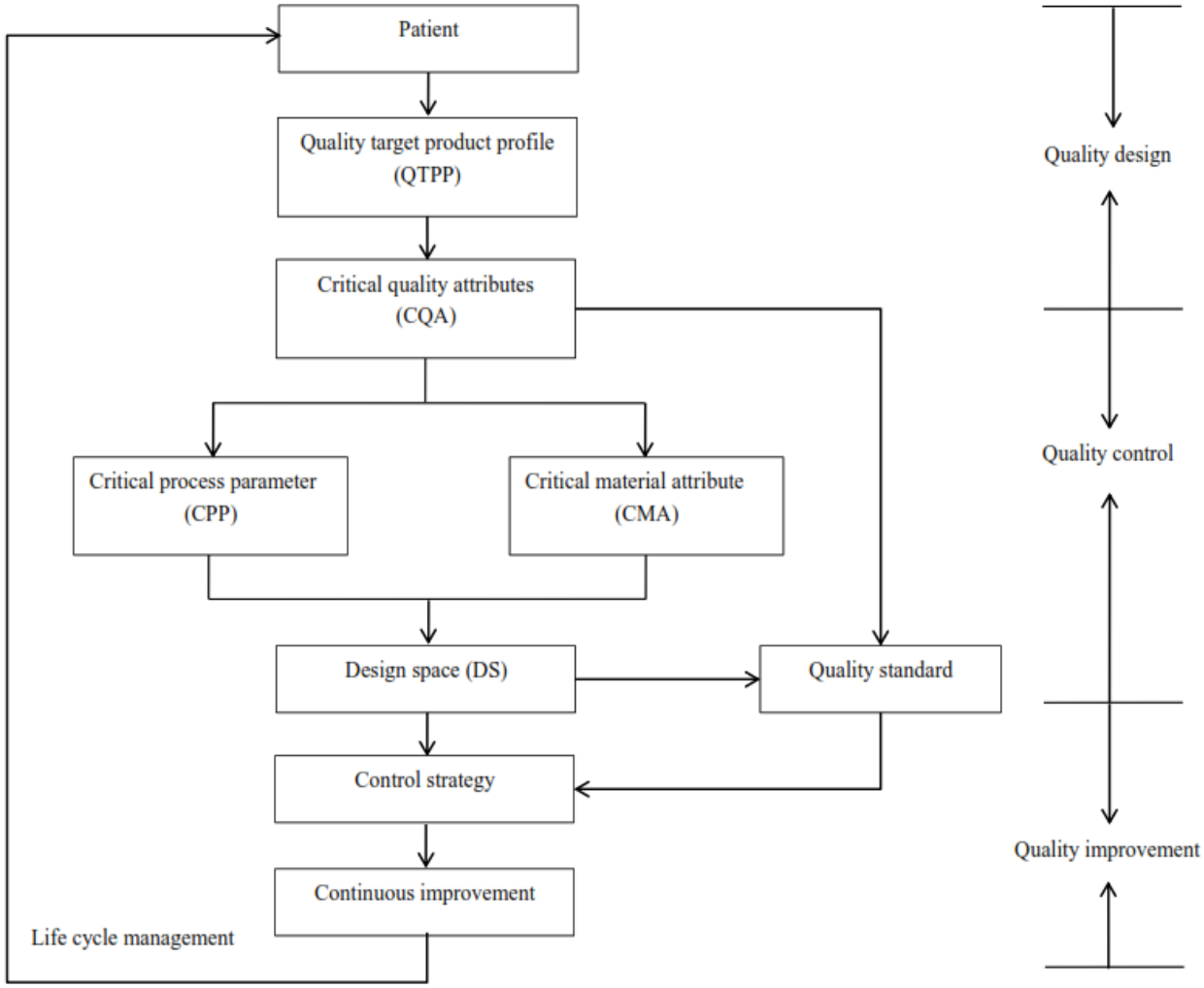

Figure 2. QbD Implementation Route

Table 2. QTPP List of CAR-T Cell Therapy Product Kymarih

Item	Index
Indication	Refractory or relapsed B-cell acute lymphoblastic leukemia under 25 years old, and adult refractory or relapsed large B-cell lymphoma
Main action mechanism	Through the release of perforin and granzyme, and the secretion of cytokines, direct cytolytic killing effect is initiated on tumor cells
Main factors affecting the mechanism of action	Expression of CAR and binding of CD19 antibody to antigen
Preparation	Injection
Specifications	50mL and 250mL
Mode of administration	Intravenous injection
Product quality	Meet USP and European Pharmacopoeia
Product validity	Store at minus 120 ℃ for 9 months, and store at room temperature 20-25 ℃ for 30 minutes after thawing
Inner packaging materials of products	Ethylene vinyl acetate (EVA) infusion bag
Appraisal	CAR gene successfully transduced and expressed on T cells
Effectiveness	Can correctly identify and effectively kill target cells
Purity/impurities	Cell impurities and process impurities shall be controlled within specified limits
Microbial safety	No microbial contamination such as bacteria, fungi and mycoplasma

Table 3. CQA Judgment Matrix

Product quality attributes	Quality standard description	Attribute Target	Judgment analysis	Critical or non-critical	Weights of different CQA in product quality attributes (from low to high score 1,3,5)
Appearance	Colorless to light yellow	No quality defects observed visually to ensure product safety	The appearance reflects the product quality, process level and product stability to a certain extent, which may affect the safety of drugs. If the red blood cell residue exceeds the limit, it can be seen from the product appearance color.	Critical	3
Identify	CAR q-PCR test is positive	Determine whether it is the product	Identification is the key to safety and effectiveness.	Critical	3
Number and percentage of living cells	Compliance	Ensure product effectiveness and use for dose calculation	Determine the dose for the product, which directly affects the therapeutic effect of the product	Critical	3
Total number of cells	NA	Used to calculate the number and percentage of living cells	No direct impact on product safety and effectiveness	Non critical	NA
CAR positive rate	Compliance	Ensure product effectiveness and use for dose calculation	Determine the dose for the product, which directly affects the therapeutic effect of the product	Critical	3
Biological effectiveness	Compliance	Ensure the effectiveness of products	The biological effect directly affects the therapeutic effect of the product	Critical	5
Purity/impurities (endotoxin, magnetic bead residue)	Compliance	Ensure product safety	If the process residues such as endotoxin or magnetic beads do not meet the requirements, the patient's safety will be affected.	Critical	5
Microorganism (sterile, mycoplasma)	The test result is negative	Ensure product safety	If sterile or mycoplasma and other microorganisms do not meet the requirements, the patient's safety will be affected	Critical	5
Replication lentivirus	The test result is negative	Ensure product safety	If replication lentivirus exists in the product, it will affect the safety of patients, but this indicator has been fully detected in the virus carrier stage	Critical	1

Note: NA means not applicable

3.2.3 Determine CPP and CMA

3.2.3.1 CPP

CQA directly determines product quality, while CPP indirectly affects product quality by influencing CQA. Table 4 shows the CPP judgment matrix of Kymarih end products. First, all process parameters (PP) are listed according to the production process and operation, and then the impact of each process parameter on CQA is scored respectively. Finally, the total score is calculated according to the following formula:

Total score=(CQA1 * PP1+CQA2 * PP2) * Existing control level

Note: CQA1 and CQA2 are the weight scores of different critical quality attributes, and PP1 and PP2 are the scores of the influence degree of a certain process parameter on the above two critical quality attributes. If a certain process parameter has influence on multiple critical quality attributes, they shall be added in turn.

Judgment criteria: Since three-level scoring criteria are adopted (1, 3, 5 from low to high), if the weight of CQA on product quality attributes is medium (value 3), the existing control level is medium (value 3), and the impact of process parameters on CQA is medium (value 3), then the total score is 3 * 3 * 3=27, so the total score ≥ 27 is determined as the judgment criteria for CPP.

Since the data in this paper are from the product registration and declaration materials published on the official websites of FDA and EMA, and the information such as process parameters is hidden from the data, the CPP judgment matrix in this paper mainly provides a judgment method and does not do quantitative calculation. The judgment of CPP requires the use of experience, experiment, modeling and various analytical techniques to clarify the relationship between related PP and CQA and the degree of impact on CQA, and then conduct quantitative assessment to determine CPP.

After determining the CPP, it is also necessary to conduct quality risk assessment on these parameters. Common quality risk management tools include Failure Mode and Effect Analysis (FMEA), Hazard Analysis Critical Control Point (HACCP), Hazard and Operability Analysis (HAZOP), etc. Measures shall be taken to control the assessed high-risk projects, and risk assessment shall be conducted again for the controlled results to ensure that the residual risks are within the acceptable range.

3.2.3.2 CMA

CMA, like CPP, indirectly affects product quality by influencing CQA, and its judgment method is the same as CPP. First, list all material attributes (MA), then analyze the impact of material attributes on CQA one by one, and determine CMA based on scoring and calculation. The scoring standard, calculation principle and judgment standard of CMA are the same as those of CPP. The calculation formula is:

Total score=(CQA1 * MA1+CQA2 * MA2) * Existing control level

Note: CQA1 and CQA2 are the weight scores of different critical quality attributes, and MA1 and MA2 are the scores of the influence degree of a certain material attribute on the above two critical quality attributes. If a certain material attribute has influence on multiple critical quality attributes, they will be added in turn.

In Kymarih's production process, the materials used can be divided into two categories: materials used to produce lentivirus vectors and materials used to produce cell end products. The materials used to produce lentivirus vectors include lentivirus, plasmid, production cells, etc; Materials used for the production of cell end products include culture medium, PBMC separation reagent, T cell separation reagent, activator, cytokines, serum, human serum albumin, human platelet extract, frozen solution DMSO, etc.

3.2.4 Establish process design space

The implementation of QbD is not to eliminate the deviation in the production process, but to establish a production process that can adjust the deviation within a certain range to ensure the stability of product quality. (8) This range can be determined by establishing a process design space. The process design space describes the impact of CPP and CMA on CQA and the variable range to maintain product quality. The variability of CPP is closely related to the production process of cell therapy products. Because of the variability of cell raw materials, CPP may need to be adjusted accordingly. Changes in the design space are not considered process changes because their impact on CQA has been studied and determined to be acceptable. FDA believes that it is not necessary to apply for changes to the operating parameters in the design space on the basis of reliable scientific data and sound quality system. Therefore, this feature of QbD greatly reduces the burden of regulators.

Design space is developed from a deep understanding of processes, experiments, and system modeling. Although it is difficult to establish a high level of confidence in the process design space of complex CAR-T cell therapy products, there are two complementary tools to guide the development of the process design space, including design of experiment (DOE) and system modeling.

4. Establish the quality control strategy of CAR-T cell therapy products

According to the concept of QbD and the above case analysis of Kymriah products, this paper believes that the quality control strategy for CAR-T cell therapy products should include at least three dimensions: the whole process quality control based on the production process, the whole factor quality control based on the production factors, and the hierarchical control based on the risk level.

Table 4. CPP Judgment Matrix

Process steps	**Process parameters**	**Existing control level (score from low to high: 1,3,5)**		**CQA（Score：1,3,5）**							**Total score (Impact of PP on CQA）**
			appearance（3）	**identify（3）**	**Number and percentage of living cells（3）**	**CAR positive rate（3）**	**Biological effectiveness（5）**	**Purity /impurities（5）**	**Microorganism（5）**	**Replication lentivirus（1）**	
Cell sorting	Amount of sorting reagent	——	——	——	——	——	——	——	——	——	——
	Centrifugal velocity	——	——	——	——	——	——	——	——	——	——
	Centrifugal time	——	——	——	——	——	——	——	——	——	——
Cell activation	Amount of activator	——	——	——	——	——	——	——	——	——	——
	Activation time	——	——	——	——	——	——	——	——	——	——
CAR gene transduced by cells	Dosage of viral vector	——	——	——	——	——	——	——	——	——	——
	Centrifugal time	——	——	——	——	——	——	——	——	——	——
	Centrifugal velocity	——	——	——	——	——	——	——	——	——	——
Cell expansion	Culture temperature	——	——	——	——	——	——	——	——	——	——
	Culture humidity	——	——	——	——	——	——	——	——	——	——
	Carbon dioxide concentration	——	——	——	——	——	——	——	——	——	——
	pH	——	——	——	——	——	——	——	——	——	——
	dissolved oxygen	——	——	——	——	——	——	——	——	——	——
	Mixing speed	——	——	——	——	——	——	——	——	——	——
Cell washing and harvesting	Amount of magnetic bead	——	——	——	——	——	——	——	——	——	——
Cell cryopreservation	DMSO dosage	——	——	——	——	——	——	——	——	——	——
	Cooling procedure	——	——	——	——	——	——	——	——	——	——

4.1 Whole process quality control based on production process

In the concept of QbD, the quality control object is not an independent production unit, but the entire production system. It pays attention to the quality control of each unit, and also pays attention to the connection between each unit in combination with the system, emphasizing the quality transfer in the process. Therefore, the quality control strategy of CAR-T cell therapy products should focus on the whole production process and carry out the quality control of the whole process.

CAR-T cells, as a special drug, have complex preparation process and numerous quality control links, so it is necessary to carry out quality control in the whole process based on its production process. Unlike the production process of traditional drugs, which starts with feeding, passes the product inspection and ends with release, the production process of CAR-T cell therapy products refers to the whole process of a series of in vitro operations from obtaining target cells from the donor to importing finished cells into the recipient. (9) Therefore, based on the production process of CAR-T cell therapy products, its quality control should include the quality control of production materials, production procedure, product release inspection, storage and transportation, sample collection and product return.

4.1.1 Quality control of production materials

Materials for the production of CAR-T cells refer to all substances or materials used to produce the cell therapy products. It includes donor cells, vectors for gene modification, and raw materials and excipients. Raw materials refer to all biological and chemical raw materials used in the production process. They are not the target components of CAR-T cell products, such as culture media, PBMC separation reagents, T cell sorting reagents, activators, cytokines (such as IL-2, IL-7 and IL-15), serum or serum substitutes. Auxiliary materials refer to the auxiliary materials used in the product formula, which are the components of cell products, such as human blood albumin, human platelet extract, cryopreserved solution (such as DMSO), etc.. Production materials are directly related to the quality of products. Therefore, a good and standardized quality management system for production materials should be established, including use risk assessment, audit and quality inspection of production material suppliers.

4.1.2 Quality control of production procedure

Due to the small sample size and short validity period of CAR-T cell therapy products, product release inspection will be limited by sample size and time. Therefore, risk assessment and trade-off between the minimum sample size, the shortest time and the maximum control of product quality are required. Therefore, it is necessary to make up for the limitations of product release inspection by strengthening procedure control and procedure inspection, and develop flexible and feasible product release strategies based on the principle of risk management.

4.1.3 Product release inspection

Before the release of CAR-T cell therapy products, appropriate tests must be carried out to ensure that the products meet the clear release criteria. The basic principle of the release standard is to provide sufficient inspection to ensure that all aspects of the product properties meet the requirements. The release inspection items of CAR-T cell therapy products shall at least include: appearance, identification, number of live cells, percentage of live cells, CAR positive rate, cell vitality, purity, sterility, endotoxin, mycoplasma, process residues and replication virus.

4.1.4 Quality control of storage and transportation

The quality control in the storage and transportation phase refers not only to cell products, but also to cell samples collected from patients. The collected cell samples should be transported to the centralized GMP production base in a reasonable packaging form and cold chain. CAR-T cell therapy products need to be frozen at minus 120 ℃, which requires higher low temperature. Generally, they are stored in liquid nitrogen storage tanks. (10) In order to ensure cell activity, the temperature and duration of transportation must be strictly controlled. Therefore, it is necessary to verify the transportation system to ensure that the control of various indicators of the transportation system meets the requirements; Risk assessment shall be carried out for the transportation process, risk points shall be identified and measures shall be taken for control to ensure the safety, effectiveness and quality controllability of products during transportation.

4.1.5 Quality control of sample collection and cell reinfusion

Although the collection of patient cell samples and product reinfusion are not operated in the centralized GMP production base, they are also part of the production process of CAR-T cell therapy products. They are two important links, one at the beginning and one at the end, and should also be included in the quality control system.

Since the collection of cell samples and cell reinfusion are carried out in medical institutions, the quality control of these two processes depends on the cooperation of medical institutions. Kymriah, a CAR-T product of Novartis, has adopted the mode of cooperation with designated hospitals in the process of commercial operation. Only hospitals that have undergone strict screening and training can be included in the clinical application network of its CAR-T products. (11) China can also use this model for reference. Enterprises should establish standard operating procedures for the evaluation and qualification of medical institutions, and strictly screen medical institutions; At the same time, the enterprise should also establish document standards for training medical institution personnel, and conduct training and examination for relevant personnel as required to effectively ensure the quality control of sample collection and cell reinfusion.

4.2 Whole factor quality control based on the production factors

As a drug, the production of CAR-T cell therapy products must strictly comply with GMP specifications, and the management and control of the five major elements in drug production (human refers to the personnel who produce the product, machine refers to the equipment used to produce the product, material refers to the materials used to produce the product, method refers to the method used to produce the product, and the environment in which the product is produced) should be done well, As the material aspect has been analyzed in "Quality control of production materials", it will not be repeated here.

4.2.1 Personnel

In view of the fact that the active ingredient of CAR-T cell therapy product is living cells, which has its special product characteristics, the personnel involved in production and quality management should have professional knowledge in microbiology, cell biology, immunology, biochemistry, etc., and be able to deeply understand the special requirements of this product for production and quality management. The training of personnel mainly includes two aspects. First, the training in theoretical learning, through which the personnel can have a deep understanding of GMP specification requirements, product characteristics, production processes, operation processes, etc., and firmly abide by relevant requirements; The second is strict training in technical practice, and the actual operation level of personnel shall be assessed and qualified, especially the aseptic operation training. Only qualified personnel can engage in aseptic operation in cell production.

4.2.2 Plant, Facilities and Equipment

In terms of plant and facilities, scientific and reasonable layout shall be carried out according to the technological process. The flow direction of personnel, materials and wastes shall be reasonably designed to prevent pollution and cross contamination. It is suggested to subdivide the production units into modules, and conduct risk assessment on the impact of each production module and different modules. According to the results of risk assessment, corresponding control measures shall be taken according to the risk level, and reasonable layout design shall be carried out according to the degree of influence between modules. For production modules with high risk level and high degree of interaction, independent plants, facilities and equipment shall be used respectively. For example, the production of cell therapy products, virus vectors and plasmid vectors shall be carried out in their own independent production areas, and independent air conditioning purification systems shall be equipped. (12)

In terms of equipment, closed equipment and pipelines should be used as far as possible for the production and operation of cell therapy products, disposable consumables should be used as far as possible for sterile consumables directly contacting cell therapy products, and automatic equipment should be encouraged to achieve safe, efficient and controllable production, and minimize the risk of pollution and cross contamination. In view of the small production volume of CAR-T cell therapy products, the miniaturized equipment can more flexibly meet the production requirements. In addition, for the initial plant, equipment installation qualification, operation qualification and performance qualification are essential, and detailed maintenance plan shall be formulated to maintain the equipment continuously.

4.2.3 Method

All methods used in production and quality control shall be subject to methodological validation, especially for quantitative testing methods. The specificity, accuracy, sensitivity and precision of the method shall be emphasized. The applicability of the methods included in the pharmacopoeia should be validated, and the rapid and micro new detection methods developed should be comprehensively and fully validated. In addition, in the early use of the new testing method, the new testing method should be carried out in parallel with the pharmacopoeia method. After obtaining sufficient comparison data, the release strategy should be designed in combination with the risk assessment of the whole production process to replace the pharmacopoeia method.

4.2.4 Environment

As cell therapy products are highly sterile and sensitive to temperature and other environmental conditions, the environment, equipment, personnel and samples should be closely monitored to ensure that the products are not contaminated and cell vitality is not damaged. The contents of environmental monitoring include the monitoring of floating bacteria, settling bacteria, dust particles, differential pressure, temperature and humidity and other parameters of the plant, the monitoring of microorganisms on the surface of equipment, the monitoring of multi-point microorganisms such as operators' gloves, chest, shoulder and head, and the monitoring of microorganisms in samples, so as to monitor environmental microorganisms in many ways.

On the other hand, the production process of CAR-T cell therapy products is complex and involves many operations, which may lead to pollution in all production links. Therefore, culture medium simulation test shall be conducted for the production process of virus vectors and cell therapy products to ensure that the aseptic operation in the whole process meets the requirements.

4.3 Hierarchical control based on the Risk Level

The concept of QbD requires that the understanding of products and processes should be combined with quality risk management, so that fluctuations in material properties and process parameters can be adjusted in an appropriate way, which is conducive to production process control, so as to ensure the continuous stability of product quality. In fact, the concept of quality risk management should run through the whole implementation process of QbD. Therefore, enterprises should widely carry out risk assessment in quality management and formulate corresponding quality control strategies based on the risk assessment results.

4.3.1 Risk assessment

The premise of risk control is risk assessment. The implementation method of QbD helps us analyze CQA according to product characteristics and production process, and then analyze CPP and CMA, which is the embodiment of risk based hierarchical control. Distinguish between critical process parameters and general process parameters, critical material attributes and general material attributes, so as to formulate quality control strategies at different levels.

4.3.2 Risk control

The risk level can be divided into high, medium and low levels. For projects with high risks, necessary risk control measures should be taken. The implementation of risk control measures should be determined according to the results of risk assessment. However, there are some key problems in the production process of CAR-T cell therapy products, which are not only closely related to critical quality attributes, but also often become high-risk points, and should be focused on, including viral carriers, microbial safety, effectiveness, etc.

4.3.2.1 Virus carrier safety

There are two main risk points in the production process of viral vectors. One is that the host gene may be inactivated due to the integration of inserted genes into the host cell genome, and the other is that replication capable viruses may be generated due to gene recombination. These two potential risks will have an important impact on the safety of the product, so we must pay attention to the biological safety of the virus vector and do a good job in controlling the relevant risks. The whole process control system shall be established for the production of virus vectors, including the quality control of raw materials, production processes and key points. In terms of production, we can establish a cell bank by screening stable virus production cell lines to ensure product quality consistency as much as possible; In terms of detection, the product quality is strictly controlled through virus carrier copy number detection, replication virus detection, etc.

4.3.2.2 Microbial safety

In the production process of CAR-T cells, the main risk points related to microorganisms include bacterial pollution, endotoxin pollution, mycoplasma pollution, exogenous viral factor pollution, etc. These risk points are directly related to the safety of the product and should be paid attention to. The control of the microbial safety of CAR-T cells mainly involves the strict detection of production materials, the strict control of the production process according to GMP requirements, and the microbial detection of products. An enterprise shall establish a biosafety management system and records, have facilities and equipment to ensure biosafety, prevent and control biosafety risks in the production process of products, and prevent the introduction or transmission of pathogens. (13)

4.3.2.3 Effectiveness test

The efficacy test is used to detect whether CAR-T cell therapy products have the target therapeutic ability, which is closely related to the efficacy of the products. (14) Factors that may affect the efficacy of CAR-T cell therapy products include transfection efficiency, CAR structure, gene vector, culture conditions, cell types and their proportions. The general test items include the number of T cells transduced, the biological efficacy of antigen specific T cells, and the amount of CAR expression. Due to the complex nature of CAR-T products, it is often difficult to reflect the effectiveness of the products by a single effectiveness test. Therefore, it is recommended to develop a variety of testing methods in the product development stage, so that with the accumulation of experience, the most indicative testing methods can be selected more flexibly.

5. Conclusion

This paper focuses on the quality control problems that CAR-T cell therapy products must face when they are commercially produced, and systematically analyzes them with the concept and method of QbD. Taking Kymriah, the world's first CAR-T cell therapy product, as an example, based on its product characteristics and production process, this paper discusses the application method and implementation process of QbD, and on this basis, combined with relevant laws, regulations, policies and guiding principles, proposes a quality control strategy for establishing CAR-T cell therapy products from three dimensions.

Although there are still many challenges in the commercial production of CAR-T cell therapy products in China, and the quality control strategy also needs to be improved in many aspects, the application of QbD can create flexible and applicable quality control methods based on the characteristics of CAR-T cell therapy products and production processes to adapt to the particularity of CAR-T cell therapy products and the complexity of the entire production process.

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Conflict of Interest

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Wei Chen has made substantial contributions to the conception and design of this work. Wenmiao Tian has taken part in revising work critically for important intellectual content. Yi Liang has revised work and approved the final version to be published.

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