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Comparison of guidelines for biological ancillary materials used for the manufacture of gene and cellular therapy products in Asia

Toshimitsu Tanaka[1,**], Keiji Yoshimura[2], Ryan Chang[3,*], Bryan Choi[4,*], Ying Gai[5,*], Pawan Kumar Gupta[6,*], Udaykumar Kolkundkar[6,*], Shing-Mou Lee[7,*], Sunray Lee[8,*], Wenbin Liao[9,*], Xiang Zhao[10,*], Koji Takakura[11]

[1] *Chemistry, Manufacturing and Control Regulatory Affairs, Regulatory Affairs, Astellas Pharma Inc, Tokyo, Japan*
[2] *Japan Tissue Engineering Co, Ltd, Aichi, Japan*
[3] *Acepodia Inc, Taipei, Taiwan*
[4] *Strategic Center for Regenerative Medicine, Inha University College of Medicine, Incheon, South Korea*
[5] *China Medicinal Biotech Association, Beijing, China*
[6] *Stempeutics Research Pvt, Ltd, Bangalore, India*
[7] *EMO Biomedicine, Taipei, Taiwan*
[8] *CEFO Co, Ltd, Seoul, South Korea*
[9] *Baylx, Inc, Beijing, China*
[10] *Shenzhen Easeng Medical Science Co., LTD, Shenzhen, China*
[11] *Astellas Pharma Inc, Tsukuba, Japan*

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ABSTRACT

Background aims: Although biologiocal ancillay materials (AMs) have specific risk associated with their derivations, it plays key role to manufature cell and gene therapy (CGT) products. It is important to understand the regulation relevant to AMs for developers.
Methods: The authors investigated the guidelines and pharmacopeia (hereinafter referred to as "guidelines") for biological AMs used for the manufacture of CGT products in Asia (China, India, Japan, Korea and Taiwan). In addition, the authors benchmarked the relevant guidelines in the United States (US) and European Union (EU).
Results and discussions: The guidelines could be classified into two types based on whether specific AMs are scoped: (i) general guidelines for risk assessment of AMs and (ii) guidelines for specific AMs. The authors compared the risk categories for each type of AM provided in the general guidelines between the US and China and the specific requirements for bovine serum and trypsin in the guidelines of China, Japan, Taiwan, US and EU. The authors further compiled in-depth descriptions of the respective regulations in China, India, Japan, Korea and Taiwan. There is limited availability of some guidelines for specific AMs. Moreover, there are no common requirements established across the surveyed countries and regions. Therefore, the authors suggest a risk assessment approach for AMs with consideration of their biological origin and traceability, production steps applied and ability to control or remove AMs from the final medicinal product over the CGT manufacturing process.

Introduction

The Forum for Innovative Regenerative Medicine (FIRM) is an industrial association for regenerative medicine in Japan. Since 2018, FIRM has organized the Asia Partnership Conference of Regenerative Medicine (APACRM), an annual conference of regulators, industries and scientists on various regulatory subject areas within the life cycle of product development and industrialization of regenerative medicine in Asian countries and regions.

The purpose of APACRM is to promote regulatory harmonization among Asian countries and regions to develop an ideal environment for the clinical application of regenerative medicine. Its specific goal is to establish a realistic platform for an optimized regulatory framework in Asia. This international platform should serve as the basis for development of a common scientific understanding of the nature of

** Correspondence: Toshimitsu Tanaka, Chemistry, Manufacturing and Control Regulatory Affairs, Regulatory Affairs, Astellas Pharma Inc, 2-5-1, Nihonbashi-Honcho, Tokyo, Japan.
* These authors contributed equally to this work.
E-mail address: toshimitsu.tanaka@astellas.com (T. Tanaka).

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regenerative medicine by establishing a dialogue between industries and health authorities to identify common challenges. It is also expected to help expedite distribution of these advanced therapeutic products in Asian markets. FIRM has taken the role of secretariat of APACRM and has closely collaborated with industrial groups, including the China Medicinal Biotech Association in China, Association of Biotechnology Led Enterprises in India, Council for Advanced Regenerative Medicine in Korea, Singapore Association of Pharmaceuticals Industries in Singapore and Biotechnology and Pharmaceutical Industries Promotion Office in Taiwan. The activities of FIRM in APACRM have been supported not only by the Ministry of Health, Labor and Welfare and the Pharmaceuticals and Medical Devices Agency in Japan but also by other Asian health authorities, including the Central Drugs Standard Control Organization in India, Ministry of Food and Drug Safety in Korea, Health Sciences Authority in Singapore and Center for Drug Evaluation in Taiwan.

Working group 2 (WG2), under the auspices of APACRM, was established to discuss and investigate issues regarding the quality of cell and gene therapy (CGT) products. In 2020, WG2 reported issues to consider when assessing the eligibility of mesenchymal stromal cells as a starting material and the comparability of mesenchymal stromal cell-based products before and after changes in manufacturing process [1]. Based on these investigations, a panel discussion with Asian health authorities took place at the 2021 APACRM conference, and a subsequent article was published in *Cytotherapy* in February 2022 [2].

In 2022, WG2 further conducted comparative research on regulations in Asian countries and regions as well as the United States (US) and European Union (EU) with regard to biological ancillary materials (AMs), which come into contact with starting cellular materials, intermediates or final products during manufacturing but are not intended to be present in the final CGT product [3]. The present article summarizes the studies comparing regulations in each Asian country or region and further suggests points of consideration that could arise from these regulatory differences.

Comparison of Guidelines and Pharmacopeia in the US, EU and Asian Countries and Regions

Overview

The authors investigated guidelines for biological AMs, which are defined as materials used in the manufacturing process but not intended to be present in the final product [3], in the US, EU and Asia (China, India, Japan, Korea and Taiwan). Table 1 shows the direct AM guidelines in each country surveyed. However, indirect guidelines such as CGT quality guidelines that mention AMs in one of their chapters are not listed. In addition, the International Organization for Standardization standard [4–6] and World Health Organization technical report series [7] may be useful as recommended information but are not listed here because the authors focused on regulatory requirements in countries or regions. The authors classified guidelines into two types based on whether specific AMs are scoped: general guidelines that explain how to perform a risk assessment for AMs [3,8–10] and guidelines describing specific AMs [11–24], including bovine serum, trypsin and recombinant proteins.

General guidelines

The US, EU, China and Taiwan have general guidelines for risk assessments associated with animal- or human-derived AMs.

Guidelines regarding specific AMs

In Japan, there are three standards for human, ruminant and animal materials relevant to AMs in the Standards for Biological Materials [11]. In the US, there are specific guidelines for bovine serum (USP90 [12] and USP1024 [13]), monoclonal antibodies (Guidance for Industry Monoclonal Antibodies Used as Reagents in Drug Manufacturing [22]), recombinant porcine trypsin (USP89 [23]) and recombinant human IL-4 (USP92 [24]). Similarly, in the EU, there are specific guidelines for bovine serum (EP2262 [12] and EMA/CHMP/BWP/457920/2012 [15]), control of transmissible spongiform encephalopathy (TSE)-relevant animals (EP5.2.8 [16] and EP1483 [17]) and trypsin (EMA/CHMP/BWP/814397/2011 [20] and EP0694 [21]). China and Taiwan have specific guidelines for bovine serum (Pharmacopeia of the People's Republic of China, Volume III, 3604 [18] and Taiwan Pharmacopeia v.9 5094 [19]). India and Korea do not have independent guidelines specific to AMs.

Risk assessment

The use of AMs has a significant impact on the quality, safety and efficacy of CGTs and needs to be carefully assessed by developers and manufacturers with the ultimate goal of protecting patients. No single measure or combination of measures can guarantee the quality, functionality and safety of AMs for their intended use. Therefore, an overall risk assessment must consider the biological origin and traceability of AMs, production steps applied and ability of the CGT manufacturing process to control or remove AMs from the final medicinal product (EP5.2.12) [8]. USP1043 [3] also mentions that, as a part of the risk assessment process, a rational and scientific qualification program should be designed for each AM, and the qualification programs for AMs should reflect the extensive scrutiny to which these were subjected during development and manufacture. Risk assessment should also consider the amount of AMs used, the stage at which AMs are used in the manufacturing process and the residual amount of AMs remaining in the final CGT product.

The risk evaluation of AMs is itself a critical component of the comprehensive risk assessment of CGT products. USP1043 [3] and Pharmacopeia of the People's Republic of China, Volume III [9] present the potential risk categories of AMs as a guide to aid CGT developers in designing a risk assessment. Table 2 summarizes the risk categories for each AM type. The risk categorization is very similar between the two countries.

Licensed biologics and drugs are considered to have the lowest risk. However, adopting them as AMs could fall outside the scope of intended use; therefore, their suitability as AMs requires careful qualification in the CGT product manufacturing process (USP1043) [3]. Well-characterized AMs produced under an established quality system (e.g., current Good Manufacturing Practice [GMP]) are categorized as having the second lowest risk. Although these AMs may be provided with the label of "clinical-grade" or "GMP-grade," CGT product manufacturers are ultimately responsible for ensuring that these AMs meet the necessary functional, quality and documentation requirements demanded by the relevant regulatory authorities (USP1043). Recombinant proteins, including antibodies not intended for CGT production, have mainly a moderate risk. These AMs are relatively straightforward to characterize and assess for adventitious agents associated with the cell lines and materials used for production. AMs derived from animal/human tissues and fluids, the components of which are complicated and not sufficiently known, are considered to have the highest risk. Bovine serum, antibodies, enzymes and animal/human cells used as feeder layers fall into this category. For this category of AMs, extensive qualification is needed prior to being used in the manufacture of CGTs or additional processing steps may be required, including (i) upgrading the AM manufacturing process; (ii) treating AMs to inactivate or remove adventitious agents, disease-causing substances or specific contaminants; and (iii) performing additional testing of each AM lot for quality control purposes. An alternative strategy is to validate the complete removal or inactivation of these AMs and the potential adventitious agents associated with the CGT manufacturing process.

Table 1
Guidelines specifically describing AMs.

Scope of material		Country/region	Guidelines	
General		US	USP1043 [3]	Ancillary materials for cell, gene and tissue-engineered products
		EU	EP5.2.12 [8]	Raw materials of biological origin for the production of cell-based and gene therapy medicinal products
		China	Pharmacopeia of the People's Republic of China, Volume III [9]	Quality control of ancillary materials and excipients during the production of biological products
		Taiwan	Taiwan Pharmacopeia v.9 5040 [10]	Biological materials required for CGT products
Human origin	Human	Japan	Standards for Biological Materials [11]	Standard for Human Materials[a]
Animal origin	Animal	Japan	Standards for Biological Materials [11]	Standard for Animal Materials[a]
	Ruminant animal	Japan	Standards for Biological Materials [11]	Standard for Ruminant Materials[a]
	FBS	US	USP90 [12]	FBS-quality attributes and functionality tests
	Bovine serum	US	USP1024 [13]	Bovine serum
	Bovine serum	EU	EP2262 [14]	Bovine serum
	Bovine serum	EU	EMA/CHMP/BWP/457920/2012 rev 1 [15]	Use of bovine serum in the manufacture of human biological medicinal products
	TSE-relevant animal	EU	EP5.2.8 [16] (identical to EMA/410/01 rev 3)	Minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products
	TSE-relevant animal	EU	EP1483 [17]	Products with risk of transmitting agents of animal spongiform encephalopathies[b]
	Newborn bovine serum	China	Pharmacopeia of the People's Republic of China, Volume III, 3604 [18]	Newborn bovine serum
	Bovine serum	Taiwan	Taiwan Pharmacopeia v.9 5094 [19]	Bovine serum
	Porcine trypsin	EU	EMA/CHMP/BWP/814397/2011 [20]	Use of porcine trypsin in the manufacture of human biological medicinal products
	Animal-derived trypsin	EU	EP0694 [21]	Trypsin
Materials produced using substances of human or animal origin (recombinant protein)	General	Japan	Standards for Biological Materials [11]	Standard for Human Materials[c]
	General	Japan	Standards for Biological Materials [11]	Standard for Animal Materials[c]
	Monoclonal antibodies used as reagents in drug manufacturing	US	Guidance for Industry [22]	Monoclonal Antibodies Used as Reagents in Drug Manufacturing
	Recombinant porcine trypsin produced in yeast	US	USP89 [23]	Enzymes used as ancillary materials in pharmaceutical manufacturing
	Recombinant human IL-4 expressed in *Escherichia coli*	US	USP92 [24]	Growth factors and cytokines used in cell therapy manufacturing

[a] Standards for Biological Materials are supported by the implementation guideline [25].
[b] Refers practically to EP5.2.8 [16].
[c] Applies to recombinant proteins using human or animal cell lines.

Bovine serum and trypsin

Bovine serum and porcine trypsin have been widely used as cell culture medium components in CGT manufacturing. Similar to other animal-derived materials, their quality can pose risks to the quality, efficacy and safety of CGTs. In particular, animal-derived materials carry a risk of contamination with adventitious agents. Additionally, there is a risk of TSEs such as bovine spongiform encephalopathy (BSE) since bovines are ruminant animals (TSE-relevant animals).

The requirements for bovine serum and porcine trypsin as AMs in the US, EU and Asia are summarized in Table 3. With regard to bovine serum in the US and EU, the requirements for sourcing, production,

Table 2
Risk categories for each type of AM in the US and China.

Risk category	USP1043		Pharmacopeia of the People's Republic of China, Volume III	
1 (lowest)	Highly qualified material, such as licensed biologic, drug or medical device	Recombinant insulin for injection Human serum albumin for injection Injectable monoclonal antibodies	Biological drugs or sterile preparations that have obtained marketing authorization	Human albumin injection
2 (low)	Well-characterized material produced under an established quality system, but AM is not a licensed or approved medical product	Recombinant growth factors, cytokines produced (non-mammalian, recombinant sources) Human AB serum Proteolytic enzymes	Chemical raw materials or animal origin-free, pharmaceutical-grade protein hydrolases produced in accordance with China's "current GMP"	Pharmaceutical-grade protein hydrolases from non-animal sources
3 (moderate)	Intended for research use, locally produced under laboratory conditions or not intended for use in CGT product manufacturing	Recombinant growth factors, cytokines Monoclonal antibodies Proteolytic enzymes	Produced, not intended for use as pharmaceutical substance	Protein hydrolases from non-animal sources Monoclonal antibodies
4 (high)	Produced for industrial or research use and may contain harmful impurities and/or may contain animal- or human-derived components harboring adventitious agents	Animal- and human-derived materials Purified enzymes Animal or human cells used as feeder layers	Toxic chemical entities with known biological mechanism and most animal-derived tissues and body fluids with complex components	Bovine sera Animal-derived enzymes Ascites-derived antibodies or proteins

Table 3
Comparison of requirements for bovine serum and porcine trypsin in the US, EU and Asia.

Country/region	China	Japan		Taiwan	US		EU			EU	
Material	BS	Animal	Ruminant	BS	BS		BS			PT	
Guideline	CP[a]	St. 4, 3[b]	St. 4, 1[c]	TP[d]	USP90	USP1024	EMA GL[e]	EP2262	EP5.2.8	EMA GL[f]	EP0694
Starting material											
Healthy animal		✓		✓		✓	✓	✓		✓	✓
Collection			✓[g]	✓		✓		✓[h]	✓		
Virus inactivation/removal											
Applying the process		✓		✓		✓	✓	✓		✓	
Validation		✓[i]				✓	✓	✓		✓	
Condition		✓		✓		✓	✓	✓		✓	
Quality control											
Safety tests	✓			✓	✓	✓	✓	✓		✓	✓
General tests	✓				✓	✓	✓	✓			✓
Storage					✓	✓		✓			✓
Labeling					✓	✓		✓			✓
Traceability											
Record		✓	✓	✓		✓	✓	✓		✓	
COA		✓[j]		✓		✓	✓			✓	
COO			✓[j]		✓[k]	✓	✓			✓	
TSE risk											
Geographical sourcing			✓			✓			✓		
Animal parts			✓						✓		
Age						✓[l]			✓[m]		
Risk assessment			✓	✓		✓			✓		

BS, bovine serum; BSE, bovine spongiform encephalopathy; COA, certificate of analysis; COO, certificate of origin; PT, porcine trypsin; Q&A, question and answer; TSE, trasnsmissible spongiform encephalopathy.

[a] Pharmacopeia of the People's Republic of China, Volume III, 3604 [18].

[b] Standard for Animal Materials [11].

[c] Standard for Ruminant Materials [11].

[d] Taiwan Pharmacopeia v.9 5094 [19].

[e] EMA/CHMP/BWP/457920/2012 rev 1 [15].

[f] EMA/CHMP/BWP/814397/2011 [20]

[g] Information for "procedures for collection of animal cell/tissue material" must be recorded for ruminant-derived materials.

[h] It is described that "further guarantee of the safety and quality of serum may be ensured by the use of a controlled donor herd."

[i] It is recommended to refer to ICH-Q5A for inactivation/removal process assessment in the implementation guideline (PFSB/ELD No. 1002-1, PFSB//ELD/OMDE No. 1002-5, October 2, 2014) [25] and Q&A (June 30, 2015).

[j] "A copy of certificate" is described as an example of the records in "8. Miscellaneous" in the implementation guideline (PFSB/ELD No. 1002-1, PFSB//ELD/OMDE No. 1002-5, October 2, 2014) [25].

[k] Indicating "country of origin on product labeling" is described as an additional requirement.

[l] It is described that "BSE infectivity may increase with animal age. Although bovine serum is considered a low-risk material for TSE transmission, some end users consider it prudent to source serum from dams below a set maximum age" [13].

[m] It is described that "as the TSE infectivity accumulates in bovine animals over the incubation period of several years, it is prudent to source from young animals," etc. [16].

characterization, traceability, certification, testing for adventitious agents, toxicity to cell growth, viral inactivation/removal and/or BSE risk assessment are described in the US Pharmacopeia (USP90 [12] and USP1024 [13]), European Pharmacopeia (EP2262 [14] and EP5.2.8 [16]) and EU guidelines (EMA/CHMP/BWP/457920/2012 [15]). With regard to porcine trypsin in the EU, the requirements for sourcing, production, testing for adventitious agents, viral inactivation/removal, quality control and risk assessment of trypsin purified from porcine pancreatic glands are described in the EU guidelines (EMA/CHMP/BWP/814397/2011 [20]), and the required characterization of trypsin extracted from the mammalian pancreas is described in the European Pharmacopeia (EP0694 [21]). There is no stipulation regarding porcine trypsin in the US.

In Asia, the requirements for sourcing, production and/or characterization of bovine serum are described in the Pharmacopeia of the People's Republic of China, Volume III, 3604 [18] and Taiwan Pharmacopeia v.9 5094 [19]. However, there is no stipulation regarding bovine serum or porcine trypsin use in other Asian countries. Japan follows the Standard for Animal Materials in the Standards for Biological Materials [11], which is subject to all animal-derived materials, including bovine serum and porcine trypsin. In addition, the Standard for Ruminant Materials in the Standards for Biological Materials describes the requirements for sourcing and traceability of raw materials derived from TSE-relevant animals, including bovine serum.

The general points to be considered are (i) traceability, (ii) virus inactivation/removal steps in the production process and (iii) quality testing and certificate of analysis (COA) for bovine serum and porcine trypsin. Traceability from starting materials to final raw material products should be qualified with appropriate records, a certificate of origin and animal health status. Detailed descriptions of the selection of animals and/or collection of starting materials are listed in the US, European and Taiwan pharmacopeias.

In principle, it is necessary to apply validated inactivation/removal steps during the production processes of these materials in the US, EU, Taiwan and Japan. Examples of methods such as filtration, gamma irradiation (e.g., >25 kGy [13,19] or >30 kGy [14] for bovine serum and >30 kGy [20] for porcine trypsin), ultraviolet treatment and/or heat inactivation (e.g., >56°C for 30 min [13,19] for bovine serum) are shown in the referred guidelines.

For quality testing and COA of bovine serum and porcine trypsin, the US, EU, China and Taiwan provide examples of test methods (Table 4) for sterility, mycoplasma and viral contaminants [12–15,18,19]. Details regarding specific virus testing requirements in the US, EU and Taiwan are shown in Table 5. Examples of characterization methods, including identification and functionality of materials, are also addressed in [12–15,18]. Bovine serum exerts its effects on cell cultures through complex biological activities; thus, its characterization is required for quality assurance.

Finally, it should be noted that the fundamental and common requirement for bovine serum is to minimize BSE risk. Despite the fact that bovine serum has a low TSE risk, its animal origin should, in principle, be sourced from countries or regions with no or low BSE

Table 4
Examples of test methods for bovine serum and porcine trypsin described in guidelines or pharmacopeias in the US, EU and Asia.

Country/region		China	Taiwan	US		EU		EU	
Material		BS	BS	BS		BS		PT	
Guideline		CP[a]	TP[b]	USP90	USP1024	EMA GL[c]	EP2262	EMA GL[d]	EP0694
Tests for adventitious agents	Sterility	✓	✓		✓	✓	✓	✓	✓[i]
	Mycoplasma	✓	✓		✓	✓	✓	✓	
	General tests for virus	✓	✓		✓[e]	✓[e]	✓[e]	✓	
	Specific tests for virus		✓		✓	✓	✓	✓[h]	
Characterization	Identification[f]			✓	✓		✓		
	Hemoglobin	✓		✓	✓		✓		
	Chemical profile				✓		✓		
	Endotoxin	✓		✓	✓		✓		
	pH	✓		✓					✓
	Osmolality	✓		✓	✓		✓		
	Total protein	✓		✓	✓		✓		
	Cell growth properties[g]	✓		✓	✓	✓			
	In vitro cytotoxicity				✓				
	Other								✓[j]

BS, bovine serum; PT, porcine trypsin, TAMC, Total aerobic microbial count; TYMC, total combined yeast/moulds count.
[a] *Pharmacopeia of the People's Republic of China, Volume III*, 3604 [18].
[b] Taiwan Pharmacopeia v.9 5094 Annex II [19].
[c] EMA/CHMP/BWP/457920/2012 rev 1 [15].
[d] EMA/CHMP/BWP/814397/2011 [20].
[e] Should be performed by inoculation of serum on at least two distinct detector cell lines, one of which is of bovine origin.
[f] Includes confirmation by electrophoretic pattern and immunochemical methods such as radial immunodiffusion.
[g] Tests include FBS functionality tests, growth promotion curve, clonal assay and *in vitro* cytotoxicity.
[h] Specific consideration should be given to widely distributed viruses that are difficult to inactivate (e.g., *porcine circovirus and porcine parvovirus*) and have zoonotic potential (e.g., *hepatitis E virus*).
[i] Tests for microbial contamination, including TAMC, TYMC and absence of *Escherichia coli* and *Salmonella*, are listed.
[j] Tests for the appearance of trypsin solution, specific absorbance, chymotrypsin, loss on drying and trypsin activity are described.

risk (USP1024 [13]) or with negligible BSE risk (classified as "Category A" by the Office International des Epizooties) (EP5.2.8 [16] and Standard for Ruminant Materials [11]), and blood should be collected without cross-contamination from other high infectivity tissues (USP1024 [13] and EP5.2.8 [16]) using appropriate procedures (USP1024 [13], EP2262 [14] and Taiwan Pharmacopeia v.9 5094 [19]). Since there is currently no available validated method for routine antemortem screening of asymptomatic animals and inactivation/removal of TSE agents (USP1024 [13]), strategies for risk assessment and risk reduction are crucial for materials derived from TSE-relevant animals.

Table 5
List of viruses tested in bovine serum by country and region.

Country/region	Taiwan	US	EU
Material Guideline	BS TP[a]	BS USP1024	BS EP2262
Bovine viral diarrhea virus	✓	✓	✓
Bovine parvovirus	✓	✓	✓
Bovine adenovirus	✓	✓	✓
Bluetongue virus	✓	✓	✓
Bovine respiratory syncytial virus	✓	✓	✓
Rabies virus	✓	✓	✓
Reovirus	✓	✓	✓
Other viruses (if necessary)	✓	✓	✓[b]
Akabane		✓	
Bovine herpesvirus 1	✓	✓	
Parainfluenza 3 virus	✓	✓	
Bovine leukemia	✓	✓	
Bovine rotavirus	✓	✓	
Bovine circovirus	✓	✓	
Bovine polyomavirus	✓	✓	
Coronavirus	✓	✓	
Torovirus	✓	✓	
Bovine enterovirus	✓	✓	
Bovine astrovirus	✓	✓	
Foot-and -mouth disease virus	✓	✓	
Rinderpest	✓	✓	

BS, bovine serum.
[a] Taiwan Pharmacopeia v.9 5094 Annex II [19].
[b] It is described that "depending on the country of origin, specific tests for other viruses may be needed."

Introduction to AM Regulations in Asian Countries and Regions

China

General principles and guidelines for raw materials, AMs and excipients for biological products are provided in the chapter "Quality controls of raw materials and ancillary materials for biological products" from the Pharmacopeia of the People's Republic of China, Volume III (2020) [9]. AMs are categorized into four tiers (category of risk, 1–4) according to level of risk with regard to source, toxicity to biological product and risk of contamination. Requirements for bovine serum are provided in the chapter "Newborn bovine serum" [18], in which quality attributes, including total protein, hemoglobin, pH value, osmolality, sterility, mycoplasma, viruses and endotoxins and their respective criteria as well as recommended testing methods, are described. Each lot of serum should be tested for its ability to support the *in vitro* growth of Sp2/0-Ag14 cells and another specific cell line (HFL1, Mv1Lu, Vero or Chinese hamster ovary) for qualification. Qualification requirements for AMs based on Chinese Pharmacopeia risk level are listed in Table 6.

Another provision, "Considerations on quality control and nonclinical studies for chimeric antigen receptor T-cell therapeutics (2018)" [26], also provides useful information for the selection and risk control of AMs in cellular products, although this guideline document is focused on chimeric antigen receptor T-cell manufacturing. Generally, it is recommended to consider and classify the risk of each AM at an early stage of product development. The developer is recommended to use low-risk (versus high-risk), sterile (versus nonsterile), GMP (versus non-GMP), clinical-grade (versus non-clinical-grade) and animal-free (versus animal origin) materials whenever possible and appropriate. Quality attributes, test methods and release criteria must be established and continuously improved during the development of each AM according to its risk category. For research-grade AMs, purity, potency and biological effects on cell viability and

Table 6
Risk-based qualification requirements for AMs of biological products in China.

Risk category	1	2	3	4
Marketing license (e.g., BLA, production permit)	✓	✓		
Vendor GMP certificate	✓	✓		
Vendor release qualification report	✓	✓	✓	✓
Qualification certificate (from national agency)	✓ (if available)			
Testing report (compliant with national pharmacopoeia guideline)		Sampling (lot)	✓	✓
Testing report on critical quality attributes	✓	✓		
Adventitious agents				✓ (if animal origin)
Additional processing or purification			✓ (if necessary)	✓ (if necessary)
Source certificate				✓ (if animal origin)
Compliance with safety requirements of China and country of origin (e.g., TSE)				✓ (if animal origin)
Vendor audit	✓	✓	✓	✓

BLA, Biologics License Application; GMP, Good Manufacturing Practice; TSE, Transmissible Spongiform Encephalopathy.

functions also need to be considered as part of quality control, in addition to safety tests on mycoplasma, sterility, viruses and endotoxins.

India

AMs used in CGT product development are critical for a reproducible and robust manufacturing process. Furthermore, AMs used in the manufacturing process must meet stringent quality standards and satisfy the necessary regulatory criteria. For successful transition to clinical trials and commercialization, the best strategy is the use of proper AM sourcing in the early stage of cell therapy development.

Generally, in India, AMs used in manufacturing cell therapy products are not regulated. However, regulatory guidelines (National Guidelines of Stem Cell Research issued by the Indian Council of Medical Research and Department of Biotechnology in 2017 [27] and National Guidelines for Gene Therapy Product Development and Clinical Trials issued by the Indian Council of Medical Research, Department of Biotechnology and Central Drugs Standard Control Organization in 2019 [28]) recommend that CGT developers use clinical/pharmacopeia-grade AMs manufactured under GMP conditions whenever possible because of their potential influence on the characteristics and safety of the final CGT product. Unfortunately, therapeutic-grade versions do not exist for all types of AMs used in CGT manufacturing. In these cases, the best option would be to choose an AM manufactured under the appropriate GMP.

AMs used in the manufacture of CGT products are evaluated from selected vendor manufacturing through controlled GMP quality systems according to the principles of the pharmaceutical quality system. AMs of animal origin are selected according to their source and country of origin. Fetal bovine serum (FBS), trypsin and Matrigel (Corning, Corning, NY, USA) are common raw materials of animal origin and are compliant with the requirements for ingredients of animal origin used for the production of biologics described in the US and EU guidelines in general. FBS is assessed based on the "risk management and mitigation" approach with regard to TSE/BSE and country of origin. Furthermore, animal-derived materials/reagents such as FBS, bovine serum albumin and trypsin should be tested for adventitious agents (e.g., TSE agents). Table 7 shows that CGT developers must focus on key AM characteristics when choosing AMs for CGT manufacturing.

Japan

The Standards for Biological Materials [11] were established as standards for materials derived from humans, animals or other living organisms (excluding plants) for use in pharmaceutical products, including CGT products, in 2003 and amended thereafter. The aim is to ensure the quality and safety of pharmaceutical products by establishing standards for raw materials. The Standards for Biological Materials consist of two general rules and six standards (human cell/tissue materials, human urine materials, human materials, ruminant materials, animal cell/tissue materials and animal materials) and essentially cover all ingredients in biological materials. Three of the six standards are AM-relevant standards. The Standard for Human Materials focuses on human-derived materials and materials produced using substances of human origin (e.g., recombinant proteins produced from HEK293 cells). The Standard for Animal Materials focuses on animal-derived materials and materials produced using substances of animal origin (e.g., recombinant proteins produced from Chinese hamster ovary cells). With regard to materials produced using substances of animal or human origin that are within the scope of these standards, although there is no clear description in the standards regarding how far upstream of materials should be regulated, it is commonly interpreted that it is regulated until secondary materials. The Standard for Animal Materials and Standard for Human Materials regulate broader AMs and thus do not focus on one specific material. These standards require (i) safety of starting materials or their derivatives, (ii) inactivation or removal of adventitious viruses in the manufacturing process and (iii) records of facilities where the materials are prepared, data associated with material preparation, test results, lot numbers and other information required to ensure the quality and safety of CGT products.

An implementation notice [25] was issued in 2014 in connection with these standards. This notice provides explanations of the texts used in the standards and examples of materials that meet the

Table 7
Key characteristics for selecting AMs.

Attribute	Consideration
Identity and free of microbial or viral contamination	Details of composition or formulation If material is proprietary, documentation of the active components without concentration COA, pathogen testing for animal-derived materials Required viral testing and donor screening documentation for human-derived materials
Purity and impurity	Documentation of purity levels Documentation of identification of impurities Assays to detect residual impurities
Consistency	Lot-to-lot consistency on COA GMP-manufactured materials
Storage and stability	Supplier's recommended storage conditions for temperature, light and humidity, demonstrating that raw materials maintain consistent performance Shelf life and stability of AM

regulations. In terms of materials derived from animals, verified heating conditions for virus inactivation are indicated for specific materials. Specific materials that have been inactivated under each condition are not required for the assessment of virus infection risk. In addition, the notice provides examples of animal materials that are semi-synthesized or have undergone a robust purification process and are excluded from the regulations from the perspective of bacterial, fungal and viral inactivation. As the risk of adventitious agent contamination and risk of TSE should be considered separately, materials derived from ruminant animals must comply with the Standard for Animal Materials and Standard for Ruminant Materials. The Standard for Ruminant Materials is used to control TSE risk. This standard describes (i) parts of the body that must not be used, (ii) risk of TSE being of negligible country/region origin and (iii) records to ensure quality and safety, such as country/region of origin, data associated with material preparation, conditions for rearing and slaughtering, processing and procedures for preventing TSE and lot numbers of materials. Further points of consideration for countries and regions of origin and the list where the risk of BSE can be ignored are explained in the implementation notice. The notice refers to the types of ruminant animals and also indicates the high temperature and alkali conditions that eliminate TSE risk and examples of materials produced under these conditions. These materials are beyond the scope of the Standard for Ruminant Materials. In addition to the Standards for Biological Materials, there are other guidelines regarding CGT products [29–33] that indirectly refer to biological materials, but they only describe compliance with the Standards for Biological Materials. A series of studies on these guidelines to ensure the quality and safety of cell-based products have also been published in a number of journals [34–38]. There are no pharmacopeias or guidelines stipulating the requirements for specific AMs.

Korea

AMs used for manufacturing CGT products should be tightly controlled, and the required information should be provided at the time of Investigational New Drug Application. The Provision on Product Approval and Review of Biopharmaceuticals [39] defines general requirements that should be submitted with regard to raw materials and AMs. Article 14 of the provision states that information should be submitted with regard to the origin, source, identity, purity, potency and quality control of AMs, as should safety data showing no adventitious agents in the case of animal-derived materials. It also requires information regarding residual amounts of AMs, how they were removed and how they were verified. Detailed information with respect to AMs can be found in the Guideline on Requirements for Quality Dossiers of CGT Products [40] and the Guideline for Cell Bank Evaluation of Cell Therapy Products [41]. They can referr to the Guideline on the Characterization of Cell Substrates Used to Produce Biologicals [42]. According to the guidelines, it is recommended to use AMs of clinical grade; otherwise, information proving the origin, safety and performance of AMs, such as COA and certificate of origin, should be provided, and additional assays, such as residue tests, are required to ensure quality and safety. The guidelines particularly emphasize that the use of beta-lactam antibiotics, such as penicillin, or hormonal components, such as steroids, should be avoided; otherwise, these substances need to be inactivated by neutralization or removed by repeated washing followed by a residue test for verification of complete removal. The guidelines also note that the source, manufacturing methods, quality control results/methods of AMs (particularly with regard to human serum albumin), FBS and recombinant trypsin should be reviewed carefully to detect the potential risk of contamination, and if necessary, any methods to devitalize or remove contamination of all possible adventitious agents, including TSE from ruminants, adenovirus and porcine parvovirus, from AMs of animal origin should be adopted.

Taiwan

CGT products use multiple biological AMs that have been developed using diverse processing and control methods. AMs include human and animal components and materials originating from humans and animals, such as extracted or recombinant proteins. The users of these materials should bear the ultimate responsibility to ensure the quality, safety and viability of the final products for their intended applications.

Chapter 5040 of the latest Taiwan Pharmacopeia [10] provides an appropriate risk control and validation framework to help manufacturers validate the quality and consistency of biological AMs used over the life cycle of CGT product manufacturing. The chapter also recommends that manufacturers provide additional comparable evidence in the event of changes in the raw materials used during the process. There are no single or combined methods that can guarantee the quality, functionality and safety of AMs used to produce the final product. Therefore, the risk assessment needs to take into consideration the sources, traceability and control or removal in each processing step over the course of manufacturing. Every risk factor as well as its residual impacts needs to be evaluated within the context of the patient's clinical benefit and exposure risk.

AMs covered in 5040 include serum (e.g., human and 5094 bovine) [19] and serum substitutes (e.g., human platelet lysate) as well as proteins produced by recombinant DNA technology (e.g., collagenase), proteins extracted from biological materials (e.g., porcine trypsin, transferrin and albumin) and vectors. The chapter 5094 provides suggestions for the purchasing, production and characterization of bovine serum to ensure its safety for application. Manufacturers should consider and follow the control procedures described in this chapter to confirm that the components of bovine serum are safe for use in research or in the production of therapeutics. Bovine serum sources include fetal, newborn calf, calf, donor and adult bovine serum, and the relevant guidelines and regulations for these sources are listed in Annex I of 5094. All types of bovine sera and blood should be collected from government-inspected and government-registered premises (slaughterhouses, abattoirs and donor farms). Blood should be collected by trained operators following written procedures approved by the serum manufacturer. Either single-use disposable collection devices or reusable collection equipment should be used along with validated cleaning procedures. Sera manufacturers should verify the inactivation of sera. Specific testing and control methods for adventitious pathogens in serum products are outlined in paragraph 7 of 5094. Specific viruses, including bovine viral diarrhea virus, bovine parvovirus, bovine adenovirus, bluetongue virus, bovine respiratory syncytial virus, reovirus and rabies virus, should be tested using fluorescent antibodies after at least 21 days of cultivation. Other viruses, described in Annex II of 5094 including bovine herpesvirus 1, parainfluenza 3 virus, bovine leukemia, bovine rotavirus, bovine circovirus, bovine polyomavirus, coronavirus, torovirus, bovine enterovirus, bovine astrovirus, foot-and-mouth disease virus and rinderpest, and may also need to be considered for testing under the manufacturers' risk assessment plan. Viral tests described in 5094 include inoculation of the serum on at least two distinct detector cell lines for at least 21 days of cultivation and monitoring by cell stain/microscopic observation as well as hemadsorption test and fluorescent antibody technique for specific viruses.

Furthermore, according to Chapters 3071 [43] and 5063 [44], sterility and mycoplasma testing are also required for the serum used in manufacturing. Serum manufacturers should maintain traceability to slaughterhouses and donor animal farms where sera are collected from donor animals. As an integral part of traceability and risk management, sera manufacturers should provide the specific COA for each lot, import and export documents and manufacturing reports of TSEs.

Discussion

There are specific guidelines for a few AMs, such as bovine serum and porcine trypsin, in several countries and regions included in the authors' survey. When we use AMs complying with these guidelines for the manufacturing of CGT products, it would be a strong justification in the risk assessment for patient protection. However, the available guidelines are limited to only a few AMs in some countries and regions, and there are no common regulations for specific AMs across Asian countries and regions. Because concerns regarding risks of AMs may vary by country or region (e.g., TSE risk for bovine serum, contamination risk of prevalent virus in a specific area for human-derived materials), each country or region may have different speed to issue or revise guidelines depending on accumulated experiences. Therefore, a risk assessment approach that considers the biological origin and traceability of AMs, production steps and quality controls over CGT manufacturing processes becomes even more important. The authors retrieved some published articles [45,46] containing useful references for manufacturers. Moreover, the authors recommend moving to defined serum-free media and recombinant trypsin to reduce the risks associated with bovine serum and porcine trypsin and to simplify the risk assessment while confirming the impact on CGT quality, efficacy and safety.

As the field continues to evolve as a result of new knowledge and experience, the authors recognize that it will still take time to establish common standards. The authors recommend that various stakeholders in each country and region meet in order to discuss how to harmonize the risk assessment approach, testing, certification and process controls for AMs.

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Declaration of Competing Interest

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Authors Contributions

Conception and design of the study: TT. Acquisition of data: TT, KY, BC, UK, RC, YG, PKG, SL, SML, WL, XZ and KT. Analysis and interpretation of data: TT, KY, BC, UK, RC, YG, PKG, SL, SML, WL, XZ and KT. Drafting or revising the manuscript: TT, KY, BC, UK, RC, YG, PKG, SL, SML, WL, XZ and KT. All authors have approved the final article.

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