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PERSPECTIVE

Current perspectives on the use of ancillary materials for the manufacture of cellular therapies

JENNIFER SOLOMON[1], LYNN CSONTOS[1], DOMINIC CLARKE[2], MARK BONYHADI[3], CLAUDIA ZYLBERBERG[4], IAN MCNIECE[5], JOANNE KURTZBERG[6], ROSEMARIE BELL[7] & ROBERT DEANS[8]

[1]STEMCELL Technologies Inc, Vancouver, BC, Canada, [2]Charter Medical Ltd, Winston-Salem, NC, USA, [3]Juno Therapeutics, Seattle, WA, USA, [4]Akron Biotech LLC, Boca Raton, FL, USA, [5]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas, MD Anderson Cancer Center, Houston, TX, USA, [6]Robertson Clinical and Translational Cell Therapy Program, Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA, [7]QIMR Berghofer Institute of Medical Research, Brisbane, Queensland, Australia, and [8]Rubius Therapeutics Inc, Cambridge, MA, USA

Abstract

Continued growth in the cell therapy industry and commercialization of cell therapies that successfully advance through clinical trials has led to increased awareness around the need for specialized and complex materials utilized in their manufacture. Ancillary materials (AMs) are components or reagents used during the manufacture of cell therapy products but are not intended to be part of the final products. Commonly, there are limitations in the availability of clinical-grade reagents used as AMs. Furthermore, AMs may affect the efficacy of the cell product and subsequent safety of the cell therapy for the patient. As such, AMs must be carefully selected and appropriately qualified during the cell therapy development process. However, the ongoing evolution of cell therapy research, limited number of clinical trials and registered cell therapy products results in the current absence of specific regulations governing the composition, compliance, and qualification of AMs often leads to confusion by suppliers and users in this field. Here we provide an overview and interpretation of the existing global framework surrounding AM use and investigate some common misunderstandings within the industry, with the aim of facilitating the appropriate selection and qualification of AMs. The key message we wish to emphasize is that in order to most effectively mitigate risk around cell therapy development and patient safety, users must work with their suppliers and regulators to qualify each AM to assess source, purity, identity, safety, and suitability in a given application.

Key Words: *ancillary materials, cellular therapy, cGMP, raw materials, regulation, stem cell research, translational medical research*

Introduction

Interest continues to grow in the development and commercialization of cellular therapies because of their potential to resolve a large number of unmet clinical indications [1,2]. Consequently, as new therapies advance through clinical trials, there is increasing scrutiny of the materials and processes used in the manufacture of the intended cell therapy product. A wide variety of starting materials may be used in the manufacturing process, some of which are integral to the final product, and in some cases, contribute to its composition or are found in the final cell product as active ingredients or as excipients. Whereby some materials used with the manufacturing process are ancillary materials, which, by definition, are components, reagents or materials used during manufacture that exert an effect on the cell product but are not intended to be part of the final cell product. It should be noted that the term ancillary material (AM) is not globally recognized by regulators and is commonly referred to as raw material in some jurisdictions, such as in Europe; however, for the sake of clarity, this paper will use the term AM throughout to describe such materials. Examples of AMs include but are not limited to: cell separation reagents, cell culture media,

Correspondence: **Jennifer Solomon**, PhD, Suite 500, 1618 Station Street, Vancouver, BC, Canada V6A 1B6. E-mail: jennifer.solomon@stemcell.com

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cryopreservation agents and disposables such as plasticware and bioprocessing bags. Many grades and compositions of AMs exist, and typically these are not approved or intended for clinical administration or use (eg, are labeled as "research use only"). Because an AM does come in contact with cells destined for clinical administration, the quality of the AM used can affect the safety, potency and purity of the cell product. As such, the long-term appropriateness of reagents and materials for use as AMs in a clinical setting must be considered under a phased and risk-based approach at each stage along the development process of a cellular therapeutic and evaluated on the basis of various criteria, including but not limited to suitability in the given application, composition, compliance, cost, availability and. ultimately, risk to patient safety.

Currently, no AM-specific regulations exist in worldwide regulatory frameworks. A growing number of guidance documents reference AM use from several national and international organizations [3–6], and, although these organizations provide a framework for strategies to control AMs, raw materials, components and starting materials, they do not precisely define the regulatory or quality requirements for AMs. More specifically, regulators provide limited guidance to cell therapy manufacturers (herein referred to as AM users) surrounding compliance requirements, generation and execution of qualification programs and the accountabilities of AM users compared with AM suppliers along development paths. Furthermore, AM suppliers do not consistently classify and name reagents intended for use as AMs, leading to further challenges to compliance. As a result, there is much confusion and mismatched expectations pertaining to the requirements for both users and suppliers. The intent of this paper is to bring further awareness to existing regulatory guidance and assist in clarifying some common misunderstandings as they relate to AMs. Moreover, this paper should serve as a resource to aid in the process of qualification and final selection of AMs for use in cellular therapy applications and ultimately to facilitate the development and commercialization of cellular therapies worldwide. Although the scope of this paper is limited, we will provide a starting point for communication between AM users, suppliers and regulators by defining commonly observed terminology, highlighting current applicable regulations and key guidance references, defining compliance and how it relates to AM use at various stages of cellular therapy development and, finally, outlining key responsibilities and accountabilities surrounding AM qualification on the basis of our combined experiences. We anticipate that this paper will be the first of a series that evaluate and help to establish standards for AM requirements globally.

Terminology

The terminology and quality or compliance claims used to describe AMs for cellular therapies can often be confusing because of inconsistent classification, naming or labeling for intended use. Very few of the more common terms are aligned across industry or region, which makes it exceedingly difficult for end-users to confidently select AMs at critical stages of the development process. In some instances, the terminology may simply be a variation in labeling or marketing techniques between different manufacturers of similar products. Such is the case with laboratory-grade and research-grade terminology frequently used to describe the same AM offered by separate suppliers. However, more frequent misunderstanding arises around current Good Manufacturing Practice (cGMP) labeling, such as products labeled as GMP, cGMP-compliant, or manufactured under cGMP, the interpretation of the requirements to label products as such and the understanding of the intended use (for example, GMP AMs labeled as research use only). Other parties have provided some guidance related to definition of terms in specific regions or as part of independent initiatives that can be leveraged [7]. However, given the criticality of the components and processes required to develop cell therapies, it is important to understand the terminology and highlight the potential differences as they relate to AMs, and materials in general, on a more global perspective. Table 1 defines the more commonly used quality and regulatory terms that describe AMs used for cell therapies that are based on existing guidances and opinion within the industry. It is recognized that there are international differences and discrepancies that are based on intended use of the AM (eg, in research versus clinical applications) and that this table does not recognize all opinions globally, despite the authors' best efforts.

It is important for AM users to investigate and fully understand the claims made by suppliers. Because of the lack of governance and consistency around AM labeling and naming, it is common that suppliers have different definitions and interpretations of standard terms for quality claims. Early and continuous communication between the users and suppliers is critical to align expectations. Furthermore, it is anticipated that dialogue between the users and their regulators will enhance user qualification requirements, and, ultimately, hold suppliers accountable for AM labeling and marketing claims.

However, to further complicate the qualification process, AM manufacturing processes and formulations are generally considered proprietary, and many

Table 1. Common quality and regulatory terminology.

Term	Meaning	Alternative or synonymous terms
Ancillary material	Materials (active or inert) that come in contact with the cell or tissue product during manufacturing but are not intended to be part of the final product [1]. Ancillary materials are commonly labeled as "not for use in clinical or diagnostic procedures" or "for *ex vivo* use only and not intended for human *in vivo* applications." Regional differences in terminology exists. Ancillary material is not used by European regulators, for example; "raw material" is synonymous with this term in Europe.	Ancillary product Ancillary reagent Processing aid Processing reagent Raw material (EU) Reagent
Animal-derived component–free (ADCF)	In the context of this paper, "animal" refers to all animals, including humans. A term used by AM manufacturers to describe products or materials that do not contain as an ingredient that is either an animal tissue or body fluid or is isolated or purified from animal tissue or body fluid. May contain recombinant proteins produced in animal cell lines or by fermentation processes. Does not necessarily limit the use of animal-derived components used in the manufacture of AM raw materials (secondary materials) or materials used further downstream (tertiary, etc), unless indicated.	Animal component–free (ACF) Animal origin–free (AOF)
Chemically defined	A term used by AM manufacturers to describe products or materials that have known chemical structures (defined by a chemical formula) and high purity, for example small molecules, salts, carbohydrates, amino acids, fatty acids and steroids (cholesterol, dexamethasone). This will be evaluated on a case-by-case basis. Does not contain proteins or other ingredients with a complex structure. Ingredients may be synthetic or biologically derived (eg, cholesterol from sheep's wool grease).	ACF Serum-free
Clinical-grade	A term used by AM manufacturers to describe products or materials that are suitable for clinical use, for example, injectable grade. Material shown to be safe and efficacious for human use through appropriate clinical trials and regulatory approvals. Usually clinical-grade products are approved as drugs by regulators, and labeling or product documentation should state sterility and safety profile. Suitable for clinical use for a specified intended use only. Clinical-grade AM for a specific and approved intended use does not mean that the AM is approved for other "off-label" processing uses without qualification and approval from the appropriate regulatory agency.	Pharmacopeial-grade Infusible-grade Active pharmaceutical ingredient (API)-grade Approved for human use
Defined	A term used by AM manufacturers to describe products or materials that o not contain undefined components such as serum, plasma, platelet lysate, tissue extracts or plant hydrolysates as ingredients. Ingredients (biologicals and chemicals) are defined in terms of origin and purity. Does not mean that ingredients are 100% pure. For example, formulation may contain bovine serum albumin (BSA), whose purity is >95% with 5% unknown composition.	Serum-free
Good Manufacturing Practice (GMP)	Refers to the current GMP (cGMP) regulations requiring manufacturers, processors and packagers to take the necessary steps to ensure that their products are traceable, safe, pure and effective. When associated with a product or material, this term denotes that the product has been prepared under some or all of the cGMP requirements to ensure proper design, monitoring and control of the manufacturing processes, facilities and the final product. cGMP regulation can be considered under a phased approach, depending on the phase and intended use of a product within a clinical application [8,9]. Intended use statements on product documentation should be reviewed by all users. Intended uses of GMP products vary and are not necessarily qualified or intended for applications outside of "research use only." Regional differences exist, where some national regulatory agencies provide GMP certificates to manufacturers of GMP AMs and others do not provide them. Term is universally recognized by regulatory authorities.	cGMP Manufactured under GMP GMP-compliant GMP-grade Compliant to 21 CFR 210, 211, 820

(*continued*)

Table 1. Continued

Term	Meaning	Alternative or synonymous terms
Home-brewed	A term used by AM end-users or manufacturers to describe products or materials prepared or manufactured in-house. Processes and components are generally not defined.	
Laboratory-grade	A term used by AM manufacturers to describe products or materials that are intended to be used within the laboratory or for research use and not intended for food, drug or medicinal/clinical use.	Research grade Research use only Laboratory use only Non-pharmaceutical–grade
Drug master file (DMF)	Confidential document that contains chemistry, manufacturing processes and controls for a product. The intention of a master file is to protect the intellectual property of a material or product. Information contained may be used to support regulatory submissions but does not guarantee that it will be accepted for use on submission to regulatory authorities. Master file type varies, based on region. For example, in the United States, the 4 most common types of DMFs are: • Type I: Manufacturing site, facilities, operating procedures, and personnel • Type II: Drug substance, drug substance intermediate, and material used in their preparation, or drug product • Type III: Packaging material • Type IV: Excipient, colorant, flavor, essence or material used in their preparation Regional differences in terminology exist. Drug master file is not used by European regulators; for example; "master file" is synonymous with this term in Europe.	Master file (EU) Regulatory support file (EU)
Protein-free	A term used by AM manufacturers to describe products or materials that do not contain proteins or polypeptides as ingredients. May contain free amino acids, dipeptides, tripeptides and peptides (up to 20 amino acids).	
Research use only (RUO)	A term used by AM manufacturers to describe the intended use of materials generally limited for use in research or pre-clinical applications only and not for use in clinical trials unless qualified appropriately and approved for use in a given application by applicable regulatory authorities. RUO products may be manufactured under various quality management systems, including ISO-certified or GMP.	Laboratory use only Research-grade Non-pharmaceutical–grade Laboratory-grade
Serum-free	A term used by AM manufacturers to describe products or materials that do not contain serum or plasma as an ingredient. May contain ingredients that are processed or derived from blood, serum or plasma such as albumin, transferrin, low-density lipids, hormones and platelet lysate. May contain other undefined ingredients that are not serum or plasma (eg, tissue extracts such as bovine pituitary extract, platelet lysate, growth factors, hormones and carrier proteins). Serum-free media (SFM) allows researchers to grow specific cell types or perform specific applications in the absence of serum.	Defined media
TSE/BSE-free	Declaration that products or materials are fully chemically synthesized or free from transmissible spongiform encephalopathy (TSE) and bovine spongiform encephalopathy (BSE) risk materials. Country of origin of material or product is important in assessing BSE/TSE risk, because some countries are categorized by the World Health Organization for Animal Health (OIE) as BSE-negligible risk or controlled for BSE risk. Testing for BSE/TSE on raw materials does not currently exist. The term is universally recognized by regulatory authorities.	
Xeno-free (XF)	A term used by AM manufacturers to describe products or materials that do not contain ingredients derived from non-human animals. May contain ingredients derived from human sources. This can include purified and processed materials as well as undefined or unprocessed materials. Recombinant materials should have human genetic DNA sequences; non-human animal DNA sequences are not allowed. Plant, bacterial or yeast DNA sequences are allowed.	

suppliers are not able or willing to disclose confidential yet necessary information despite vigilant due diligence by AM users. Recognizing this dilemma, many regulatory agencies allow suppliers to submit confidential manufacturing information directly to the agencies in the form of a master file. Master files are, however, neither necessary nor required. With the United States used as an example, a device or drug master file (DMF) for a specific AM can be submitted to the US Food and Drug Administration (FDA) by the supplier, which users can then reference in their regulatory submissions upon obtaining permission from the DMF owner (ie, the supplier). Yet, a common misunderstanding is that a master file represents regulatory approval for the use of that material. It is important to recognize that master files are not reviewed by regulators unless they are cross-referenced under an application for initiation of a clinical trial, such as an investigational new drug (IND) application in the United States or a clinical trial authorization/application (CTA) in Europe or Canada, respectively. In the United States, similar misunderstanding exists with FDA 510(k)-cleared AMs. A 510(k)-clearance covers only a very specific intended use and does not guarantee that the AM is approved or safe for use in all applications. No matter how an AM is labeled (GMP, ACF, etc) or whether a master file or previously existing clearance exists, it is the responsibility of the AM user to evaluate and qualify each AM for use in a given application. Such risk-based qualification plans are discussed in the following sections.

Regulation

Although there are regulations that describe both quality and regulatory requirements for the manufacture of cellular therapies, the regulations do not specifically describe quality requirements for AMs. They do, however, provide a framework for strategies to control these starting materials. Guidance on raw material use (and hence AM use) is available from several national and international organizations, including the US FDA [3], the International Conference on Harmonization (ICH) [4], the International Organization for Standardization (ISO) [10], and the European Medicines Agency (EMA) [5]. However, it should be noted that guidance applied to traditional medicinal products, such as small molecules, and biologic drug product, such as blood or blood products, does not translate directly to cellular therapeutics. We anticipate that regulations and minimum standards specific to AMs will come with the maturing of the regenerative medicine industry, with the prediction that AMs manufactured and maintained under quality systems compliant to the relevant cGMP regulations will be required at some point. In the interim, the following examples of regulatory documents specific to raw materials that are recognized globally will serve as starting points for evaluating, selecting and qualifying AMs:

International conference on harmonization

- ICH Q5A/D: Quality of biotechnological products
- ICH Q5E: Comparability of biotechnological/biological products subject to changes in their manufacturing process
- ICH Q6B Specifications: Test procedures and acceptance criteria for biotechnological/biological products
- ICH Q7: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients
- ICH Q8 (R2): Pharmaceutical development
- ICH Q9: Quality risk management
- ICH Q10: Pharmaceutical quality system

ISO (evaluation and selection of suppliers)

- ISO 9001: Quality Management Systems: requirements
- ISO 13485: Medical Device Quality Management System: requirements for regulatory purposes

More common than global regulations are national regulations and guidances brought forth by local regulatory authorities or partner organizations. As an example, a sampling of available region-specific guidance is presented here.

United States (FDA, USP)

In the United States, AMs used in the manufacture of cellular therapeutics are subject to different levels of regulations depending on whether the products are minimally or more than minimally manipulated. Although more-than-minimally manipulated tissue products may use a variety of complex AMs as compared with minimally manipulated tissue products, the safety and compliance requirements for the AM are the same for both types of products and are required to meet their applicable regulations. Depending on the intended use of the final product, they may also be subject to the quality systems associated with drugs [11,12], biologics [13], and devices [14]. Here is a summary of applicable regulations:

- 21 CFR 210 and 211 Current Good Manufacturing Practice (drugs)
  - 11 CFR 211.80: Current Good Manufacturing Practice for finished pharmaceuticals: components and containers/closures

  - 21 CFR 211.110: Current Good Manufacturing Practice for finished pharmaceuticals: control of in-process materials
- 21 CFR 610.15: General biologicals products standards: constituents materials
- 21 CFR 820 Quality system regulation (devices)
- 21 CFR 1271.210: Human cells, tissues supplies and cellular and tissue-based products: supplies and reagents

In addition to these regulatory documents, the United States Pharmacopeia Convention (USP) publishes a number of general chapters that define necessary qualification activities for common AMs used in the manufacture of cellular therapies [15,16]. Further USP informational chapters provide guidance on a selection of regulatory requirements and propose qualifications programs and risk-based approaches for the qualification of AMs [3,8]. Despite being US-centric, USP guidance is recognized and used globally within the cell therapy industry. The following is a summary of the USP compendial and general chapters that are directly applicable to cellular therapy products:

- <90> Fetal bovine serum quality attributes and functionality tests
- <92> Growth factors and cytokines used in cell therapy manufacturing
- <1024> Bovine serum
- <1027> Flow cytometry
- <1043> Ancillary materials for cell-, gene- and tissue-engineered products
- <1044> Cryopreservation of cells
- <1046> Cellular- and tissue-based products
- <1047> Gene therapy products

Outside of the United States, there is recognition of the need to set up quality requirements for these raw materials, but the guidance on the regulatory requirements for AM manufacture and use in some regions are less well developed. The following is a snapshot of what currently exists in other regions.

Europe (EP/EDQM/EMA)

Ancillary materials are referred to as raw materials in the EU. The following are a selection of EU-specific documents that provide general guidance for the use of AMs:

- British Standards Institution. PAS 83:2012 Guidance on codes of practice, standardized methods and regulations for cell-based therapeutics
- Directive 2004/23/EC Quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells
- Guideline on the risk-based approach according to annex I, part IV of directive 2001/83/EC applied to advanced therapy medicinal products (2013)
- Regulation (EC) No. 1394/2007, advanced therapy medicinal products regulation
- Commission Directive 2009/120/EC amending directive 2001/83/EC relating to medicinal products for human use as regards advanced therapy medicinal products
- Eudralex Volume 4: Good Manufacturing Practices
- Eudralex Volume 4: Good Manufacturing Practice Annex 2: Manufacture of biological active substances and medicinal products for human use
- Eudralex Volume 4, Good Manufacturing Practice Annex 13: investigational medicinal products
- CHMP Guideline on Human Cell-Based Medicinal Products: 2008 EMEA/CHMP/410869/2006
- EMA/CAT/GTWP/671639/2008, Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells

Australia (TGA)

Like other regions, regulatory guidance for the use of AMs within Australia is limited [17]. There is no specific mention regarding the use of AMs within relevant codes. The Therapeutic Goods Administration (TGA) governs the manufacture of cellular therapy products within Australia, and guidance exists for organizations who undertake the collection, processing, testing, storage, release and quality assurance of cellular therapy products [18]. Furthermore, Australian regulatory guidelines for biologicals exist outlining a regulatory framework for human cell- and tissue-based products under a specific group called biologicals. These guidances place cellular therapy products into one of four classes of biologicals with the use of a risk-based approach that is dependent on the methods used to prepare and process the cellular therapies during manufacture and whether the intended use is the same as their usual biological function. As in other regions, reference to USP guidance is further recommended when developing cellular therapies. Interestingly, approval by the TGA is not required to conduct phase I clinical trials in Australia. However, consideration must be given to the safety of the cellular therapy product, inclusive of the AMs used. It is required that cell products used in clinical trials beyond phase I have been evaluated

for safety, and removal of AMs must be adequately demonstrated.

Japan (PMDA)

In Japan, the Pharmaceuticals and Medical Devices Agency (PMDA) with the Ministry of Health, Labour and Welfare (MHLW) are responsible for governing the use of cellular and tissue-based products. At this time, the regulatory framework is regulated by the Pharmaceutical Affairs Law under the PMDA, and there are published guidelines on the use of cellular- and tissue-based products derived from processed cells and tissues only. These applicable guidelines are captured under the following standards:

- Standards for biological ingredients (MHLW No. 210, 2003)
- Standards for manufacturing and quality control for medical device and in vitro diagnostic reagents (MHLW No. 169, 2004)
- General principles for the handling and use of cellular/tissue-based products (MHLW No. 266, 2001)
- Guidelines on ensuring quality and safety of products derived from processed cell/tissue (MHLW notifications: autologous No. 0208003, allogenic No. 0912006, 2008)
- Guidelines on ensuring the quality and safety of products derived from processed human stem cells (MHLW No. 1314, 2000)
- Points to consider on manufacturing and quality control (MHLW notification No. 0327025, 2008)

Recently, the PMDA has established a scientific board under the Office of Cellular and Tissue-Based Products to further advance the regulatory aspects and support the PMDA to evaluate cellular and tissue-based therapies. This high-level consultative body will be responsible for making recommendations on policy for innovative products, providing regulatory guidance and ultimately will develop regulations relating to cellular- and tissue-based products, along with pharmaceuticals, medical devices and biological products.

To conclude, although some of the above guidances provide information on the eligibility of raw materials and AMs used in the processing of cellular therapy products, no actual regulation currently exists that specifically outlines the regulatory requirements for the use of AMs. There are, however, a number of consistencies between regional and international guidances. In general, various regulatory agencies consistently recommend using the highest grade of AM available that performs as required in a given application (with no stipulation on minimum-grade AM at present); following a risk-based approach to AM qualification; conducting appropriate testing to demonstrate AM removal from the final cell product; and, ultimately, that the final cell product should be safe and effective.

Compliance

In general terms, "compliance" means conforming to a rule, such as a regulation, policy, standard or law. However, in an industry in which no standard regulations exist, it becomes vital to prioritize patient safety above all else and to work with individual stakeholders to define applicable compliance requirements for applications on a case-by-case basis. Ultimately, patient safety is the responsibility of regulators who work with AM users or sponsors of an investigational or commercialized product. However, regulators generally hold the users responsible for working with their suppliers of all reagents and materials to ensure that the compliance requirements defined by their regulatory authority have been met.

Although there are similarities between cell therapy products, blood products and drug products, there are still several areas of science and regulation that do not easily translate between these industries. For example, materials used in the manufacture of a pharmaceutical drug product can generally be identified through advanced analytical test methods and thus demonstrated to be effectively removed from the final product through such testing. By nature, pharmaceutical products can be manufactured in bulk, stored and then analyzed before release. In contrast, cell products are generally prepared in small batches and require materials that are biological in nature in the manufacturing process. Few robust and sensitive analytical methods exist to detect biological AMs, making it difficult to differentiate from the biological cell product itself and hence demonstrate definitive removal of the AM from the final cell product [18]. The biological nature of many AMs also presents challenges when attempting to validate the limit of tolerance and the limit of detection along the cell therapy product development path. Further adding to the dilemma is a general lack of time available for long-term testing, as many cell products are highly labile, cannot be terminally sterilized and are intended to be administered immediately after *ex vivo* manipulation to the patient. These factors and others introduce inherent risk into cell therapy products, adding to the need for consistency around the quality claims, compliance and the need for standards governing this industry.

Regulators often require the user to demonstrate how inherent risk of materials used to manufacture cell products will be mitigated. The ICH and USP provide useful and well-recognized frameworks that can be used to develop risk assessment and qualification

plans to effectively evaluate AMs [3,19]. The level of qualification varies, depending on the amount of inherent risk associated with their composition, quality and intended use. For example, a GMP AM devoid of animal components (commonly labeled as animal-derived component–free) would require less qualification than a non-GMP reagent containing animal components. However, the conundrum is that many AMs used in the manufacture of cell-based products are inherently high–risk, and alternative low-risk AMs commonly do not exist or perform inadequately in a given application. One such example is the transition from serum-containing cell culture medium to animal-derived component–free or chemically defined medium to minimize risk of adventitious agent transmission. However, not all cell types can be easily adapted to serum-free medium and may result in a reduced yield of cells after expansion or a final cell population that lacks the therapeutic benefits of the earlier cells expanded in serum-containing medium. As such, the combined goal of regulators, suppliers and users alike is to minimize risk to patients by effectively evaluating and controlling each material or reagent used during the cell product development process.

Qualification

Similarly to all raw materials and starting materials, AMs must be qualified to varying extent on the basis of five key factors: source, purity, identity, safety and suitability, which are outlined in several regulations, guidances and draft guidances [3,5,6,11]. However, the level of detail surrounding the qualification plan depends on the intended application and the clinical phase in which the cellular therapy product will be used [9]. It is imperative to note that there is a cost associated with AM qualification activities that must be considered by both the user and the supplier. Currently, the demand for AMs for clinical applications is limited, but it is growing as the number of clinical trials increases and trials advance to later phases. Therefore, historically, when there has been little incentive for suppliers to increase compliance, suppliers are now accelerating efforts to meet this market demand. Further supporting this transitional period as the field moves to include more standardization, regulators have commonly been willing to work with users one-on-one throughout the AM qualification process to provide feedback on the proposed use of materials originally intended for research use only.

Information pertaining to AM qualification activities is typically included in regulatory submissions for clinical trials, such as in IND applications (United States) and CTA applications (EU, Canada) by a cell product manufacturer, and accountability for all qualification and risk assessment activities related to the AM falls on the user. To aid in this process, the user should establish early dialogue with regulators and the AM suppliers to create appropriate compliance requirements and product acceptance criteria, especially in light of the inconsistencies in AM labeling, and the high cost and limited availability of clinically approved or higher compliant AMs. Table 2

Table 2. User and supplier accountabilities for ancillary material use.

Qualification activity	Supplier	User
Performance in the intended application		X
Provide CoA, CoC, CoO for AM	X	
Verify country of origin to assure AM is safe with respect to source-relevant animal diseases (eg, BSE/TSE)	X	X
Conduct a risk assessment for use of AM, based on information provided by supplier, or in collaboration with the supplier, for example, failure modes and effects analysis		X
Establish and implement qualification plan for AM		X
Confirm CoA test results critical to the cell product (eg, functional assay)		X
Characterization testing of AM and set specifications (eg, identity, purity, functionality, viral contamination, animal origin, etc)	X	X
Assess effect of lot-to-lot variation of AM on the final cell product		X
Determine if biocompatibility, biodistribution, cytotoxicity or adventitious agent testing is needed (or testing results might be available from supplier, if applicable)		X
Assess presence of residual AM in the final cell product		X
Assess stability of AM	X	X
Qualify the supplier of the AM (eg, supplier audit)		X
Execute quality and supply agreement	X	X
Implement higher manufacturing standards, custom formulation or replacement of substandard components	X	X
Upgrade manufacturing process for AM under cGMP compliance (ie, in some instances, there may be requirements for shared costs and risk)	X	X
Inform the user of any changes in the manufacturing process of the AM or design/formulation of the AM (eg, under a quality agreement)	X	
Prepare and submit a master file for AM, if applicable	X	

summarizes some of the key accountabilities and responsibilities pertaining to AM qualification. It is important to note, however, that required qualification activities may be unique to each AM and global region and activities noted in the table may not be fully inclusive of these requirements.

The primary activity surrounding AMs is evaluation of their performance and identification of AMs that best perform in the intended application. Development of an optimal protocol is typically initiated in later stages of basic research with further refinement during pre-clinical studies and throughout early-stage clinical trials. In some instances, only research-grade reagents or materials may be available for the intended application at the current time, and, as such, the continuum of compliance of AMs must parallel the succession from pre-clinical studies to early-stage clinical trials to pivotal-stage trials. That is, AM compliance must increase as clinical trials advance. To ensure a successful continuum in compliance, the earlier in the development process AMs can be considered, the better. As such, as soon as the most favorable AMs are identified, a risk assessment should be conducted by the user in collaboration with the supplier. Furthermore, clearly defining where and how the AM is used in the clinical manufacturing process will help to further define the risk level of the AM. For example, an AM that is used far upstream of the final cell product, such as a cell culture medium used in the expansion of cells that will be further differentiated and processed multiple times, may impose less risk to the final cell product than a wash buffer that will be used to wash cells immediately before infusion. It is critical to keep in mind that early development and clinical work typically focus on safety, whereas later-stage clinical and commercialization efforts are geared toward consistency. Although the standard for safety is the same for both early and pivotal development efforts (ie, consistent throughout the entire qualification process), the specifications for the manufactured cell therapy product and the AMs used for its production become more stringent in terms of documentation, testing and monitoring. This further amplifies the need for evaluating risk early on.

Standard risk assessment tools, such as a failure mode and effects analysis, can be used to assist with evaluating the risks and the criticality associated with use of the AM in the proposed application. As part of the risk assessment, a robust AM qualification plan must be developed and should include a detailed list of risk mitigation activities surrounding the use of the AM. The qualification plan should be developed by the end user through the use of data generated both in research and pre-clinical activities, such as AM lot-to-lot variability and the amount of residual AM that may remain in the final cell product, and using information available from the AM supplier. For example, most AMs should come with a Certificate of Analysis (CoA) or Certificate of Conformance (CoC) to confirm that the product meets the defined quality and performance standards that demonstrate lot-to-lot consistency and outline what specific testing is conducted on the AM by the supplier. However, for many AMs, such as serum products, in which variability is inherent, it often falls on the user to establish acceptance criteria and minimum standards for each lot of material that can be evaluated with the use of internally developed assays specific to their cellular products. Further qualification activities may require a Certificate of Origin (CoO) that outlines the origin of the materials used to manufacture the AM itself. These should specify what animal-derived materials were used in the process (sometimes available for both primary and secondary ingredients) and what testing has been conducted on animal-derived materials (eg, mycoplasma and adventitious viral testing). Additional supplier documentation may include quality management system certificates, quality questionnaires and access to a master file, if applicable. On-site audits of the supplier's manufacturing facility may also be welcomed.

Dialogue between the user and supplier can help identify issues that may hamper obtaining regulatory approval. Furthermore, building a relationship between the user and supplier early on in the AM qualification process will help establish effective communication between parties so that information critical to the AM review and approval process, such as changes or enhancements to the AM manufacturing procedure, formulation and compliance, are transmitted to the user in a timely manner. It is also important to note that the availability of higher-compliance AMs will be driven by user demand, but, in some cases, both the supplier and the user may need to share both the cost and risk of increasing product compliance. These are just some of the challenges and risks associated with an industry that is still developing. Table 3 outlines specific challenges faced by users relating to the qualification and approval process for use of a specific AM. The resolution identified has been deemed acceptable by the governing regulatory agency in these instances only. Resolutions may not be representative of all applications. These case studies are provided as examples only. Each user must qualify and obtain independent approval for their own cell manufacturing process, including justification for use of all AMs. Overall, the user is responsible for the quality of the final cellular therapy product and thus must ensure that the AM used during its manufacturing is

Table 3. Case studies.

Issue	Product type	End-user concern	Resolution
Human-derived component in AM product	Cell culture medium	Concern with FDA regulations on use of human materials in the manufacture of human cell therapy products; concern about viral testing and traceability of donors.	Supplier provided product- and lot-specific CoA and CoO. Supplier provided CoO for human raw material component in medium with information on method of collection, compliance with regulatory guidelines and adventitious agent screening (because viral inactivation was not previously conducted). Supplier may provide FDA 510 (k) clearance letter for media use as a class II device for human cell tissue culture [this may not commonly be available] or reference to the medium master file previously submitted to the FDA.
Component labeled "for research use only"	Various reagents, such as cell culture medium and cell selection reagents	Concern regulatory authorities would not allow use of material labeled "for research use only" in the clinical cell manufacturing process.	User worked with AM supplier to obtain details regarding materials of construction, manufacturing, sterilization, and testing of the component. Included relevant information within end-user's IND and BLA. Following relevant regulations and guidelines applicable for medical devices, user tested the component to ensure it met applicable standards and demonstrated the component is suitable for use in the cell therapy manufacturing process. User also qualified the supplier, implemented incoming material testing and maintained continued oversight of the supplier as part of their supplier qualification and monitoring program. User executed a quality agreement within their supply agreement with the supplier to ensure end-user's quality requirements are met and appropriate controls are in place for the AMs. The approach was acceptable for use of the RUO-labeled AM in early-stage (phase I/II) clinical trials. Use of RUO-labeled product for late-stage trials and commercialization is yet to be determined.
DMSO (dimethyl sulfoxide) compatibility with packaging	Liquid medium for storage of frozen cells	Concern with placing cell product having DMSO into bag and potential leachables and extractables.	Supplier provided bag validation guide. Supplier provided leachables and extractables report and chemical resistance report with bag film material compatibility to DMSO. Supplier provided FDA 510(k) clearance (FDA) and CE mark approval letter (EMA) for bag film material use as part of a class II device for storage of hematopoietic stem cells.
Use of antibiotics in culture media	Antibiotics penicillin/ streptomycin in culture media	FDA raised issue with the presence of antibiotics in culture media and potential for allergic reactions.	Proposed including antibiotics in initial primary culture of mesenchymal stem cells with subsequent passage in media without antibiotics. In addition, validated washing of the final cell product to ensure adequate removal of the AM from the cell product before infusion.
Use of collagenase to digest tissue	Enzymes	FDA raised concern about bacterial origin and endotoxin levels.	FDA advised that if a safer alternative to collagenase is available, that should be used first. If not, only cGMP collagenase can be used in clinical applications. User needed to evaluate alternatives, and none were found. End-user needed to ensure that no residual collagenase was present in final cell product.

appropriate for use in the proposed application and satisfies the regional regulations.

Conclusions

To consolidate a global perspective on the use of AMs is challenging and constantly changing as the result of ongoing review by many stakeholders. As described throughout this paper, available information from regional and international committees, associations and regulators only provides guidance and emphasizes discretion, such as using a risk-based approach with consideration given to the direct effect on the quality and safety of the cellular therapy product. Nevertheless, it is clear that the removal of AMs within a cellular therapy process must be demonstrated through appropriate testing to indicate minimal or no AM presence in the final cell product. All literature sources consistently emphasize that all materials and reagents that come in contact with the cell product must be appropriately defined and controlled. Commonly, guidances include recommendations that the highest grade, preferably GMP or pharmacopeial-grade, AM should be used when possible, but that all materials, regardless of quality or compliance, must undergo a risk assessment and subsequent robust qualification in the context of the intended application.

What can be gleaned from previously published literature and opinions of the cell therapy community is that further consistency and standardization within the cell therapy industry, and specifically regarding AMs, is wanted and required. There are a number of global harmonization initiatives that apply to AMs but are broader-ranging than merely AM regulation and guidance, such as by the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme, jointly referred to as PIC/S, with the mission "to lead the international development, implementation and maintenance of harmonized GMP standards and quality systems of inspectorates in the field of medicinal products." This, and other ongoing initiatives by the ICH and the Regulatory Harmonization Steering Committee, will further clarify common misunderstandings surrounding compliance and quality of AMs, although the possible implementation of a common global AM standard will be challenging and is yet to be seen. There are also efforts by advocacy organizations such as the Alliance for Regenerative Medicine and the International Society for Cellular Therapy that have organized global working groups and committees to continue to solicit feedback from the cell therapy community and work with governing bodies to facilitate the advancement of cellular therapies, which includes standardization efforts and consolidated approaches to cell therapy manufacturing around the world. This white paper is one such initiative.

There are still many challenges related to AMs in the development of cell therapeutics, but we are starting to see trends to reconcile these challenges. Challenges faced by AM users, such as extensive and expensive qualification activities, are being mitigated by suppliers who are steadily increasing compliance and, thus, reducing the inherent risk by transitioning from materials containing animal-derived components to animal component–free and chemically defined materials, manufacturing reagents under GMP, generating master files and registering AMs with local regulatory agencies. Additional challenges have been addressed through the continued flexibility and understanding of regulators during the clinical trial application process regarding the use of wide-ranging AMs that are available and cost-effective. Continued review of regulatory submissions on a case-by-case basis that are based on both data and common sense, open dialogue on qualification activities for AMs between users and regulators, and subsequent communication with suppliers all remain critical for further advancement of AM development and increasing compliance. Many of us have observed encouraging trends along these lines with open communication between regulators, users and suppliers occurring early in the development process. This has almost certainly had a positive impact on the incidence of applications being approved. Additional challenges are being addressed on an ongoing basis. These include, for example, the development of analytical test methods and potency assays for AMs that are biological in nature to assist with key qualification activities assessing purity, identity, safety and even suitability.

Because the cellular therapy industry is rapidly growing and starting to mature, specific guidances, regulations, and standardization surrounding AMs and their use at various aspects of the development process are sure to arise. Regulation will provide clarity surrounding the requirements and responsibilities of AM users and suppliers, which may either foster or impede commercialization efforts. Although clarity around the use of AMs is required, we must ensure that efficient and realistic regulations are set that allow cost-effective yet safe, innovative cell product development. We hope we have provided a basis for continued discussion between users, suppliers and regulators in the cell therapy field and ultimately to help guide strategies for AM use to facilitate the development and commercialization of effective cell therapies globally.

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