Chapter 17
MSCs: The US Regulatory Perspective

Adrian P. Gee

Abstract In the USA, the Food and Drug Administration (FDA – the Agency) regulates cellular therapies, primarily through the Center for Biologics Evaluation and Research (CBER) Office of Cellular, Tissue and Gene Therapies. The rapid expansion of these therapies has prompted the Agency both to determine the applicability of existing regulations and to develop specific new laws. The strategy that has evolved is based upon perceived risks to the donor and recipient of the cell product and to the product itself by *ex vivo* manipulation during the manufacturing process. Mesenchymal stromal cell (MSC) products are considered to be more-than-minimally manipulated, due to the requirement for expansion of the cells in culture. As such, the product must be manufactured under current Good Manufacturing Practices (cGMP) and clinical trials carried out under an Investigational New Drug (IND) application. The development of this regulatory strategy and the factors involved in cGMP manufacturing and applying for an IND are reviewed in this chapter.

Introduction

The resurgence of interest in cellular therapies has excited the attention of national regulatory authorities. Their concerns primarily relate to the potential development of commercial products and services associated with the new therapies, the rapid expansion of novel technologies, and the risk of blurring the boundary between research activities and billable clinical therapies.

A.P. Gee (✉)
Departments of Medicine and Pediatrics, Center for Cell and Gene Therapy,
Baylor College of Medicine, Houston, TX 77030, USA
e-mail: agee@bcm.edu

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Regulation of Somatic Cell Therapies in the USA

In the USA, regulatory responsibility falls to the Food and Drug Administration (FDA – the Agency) and, more specifically, to the Office of Cellular, Tissue and Gene Therapies in the Center for Biologics Evaluation and Research (CBER). Prompted by the use of cells in a variety of therapeutic applications, the Agency has worked to develop a regulatory strategy to encompass these diverse and developing therapies and products while ensuring the safety of patients and donors.

When seeking to regulate a new area, the FDA will usually review existing regulations to determine if they could be applied and whether they require supplementation. The Agency identified applicable regulations within the United States' Public Health Service (PHS) Act of 1912 and the Federal Food, Drug and Cosmetic Act of 1938. In 1993, they summarized which existing regulations could be applied to somatic cell and gene therapies in the Federal Register [1]. This document served to define somatic cell therapy products and to categorize them as biological products subject to the provisions of the PHS Act but noted that they also fell within the definition of drugs. As such, cellular therapy products would be subject to regulation under Investigational New Drug (IND) laws and would be manufactured under current Good Manufacturing Practice (cGMP) regulations. They would also be subject to establishment and product licensure.

The Agency recognized, however, that the existing regulations were insufficient to address current activities in a comprehensive manner. The solution has been to develop a unifying strategy for regulation based upon the potential risks [2, 3]. These include risks to the donor of the cells, risks posed by *ex vivo* handling, and risks posed to the intended recipient(s) by administration of the cellular product.

Manipulation

The risk-based regulatory strategy placed particular emphasis on the hazards posed by *ex vivo* handling of the cells. This was considered to be related to the degree to which the cells were manipulated. Manipulation was subdivided into two categories, "minimal manipulation" which posed a lower risk than the second category "more-than-minimal manipulation." Attempts were made to define how various *ex vivo* procedures should be classified, and after some initial confusion, a definition was developed, which was published in 1997 by the FDA [4]. Minimal manipulation was processing that did not alter the original relevant characteristics of the cells. More-than-minimal manipulation would include processing such as expansion, encapsulation, activation, or genetic modification. Cell selection, by contrast, was eventually considered not to be more-than-minimal manipulation [5]. Subsequently, more-than-minimal manipulation was broadened to include cells that were used in a nonhomologous manner, that is, were not being used in the recipient to perform the same basic function as they did in the donor. Examples would be marrow-derived cells that were being administered to treat cardiac or neurologic

diseases. By defining these two categories of manipulation, the FDA determined the regulations to be followed during product manufacturing. More-than-minimally manipulated cells would fall under cGMP, and clinical trials using these cells would require an IND.

Further information on the regulation of cell and gene therapies was provided in March 1998 by publication of the Guidance for Human Somatic Cell Therapy and Gene Therapy [6]. This provided guidelines for characterization and release testing of cells for cell-based and gene therapies, including information on preclinical studies and gene vectors. This guidance is particularly valuable for investigators developing therapies using genetically modified MSCs, since it describes the preparation and testing of cell and virus banks used to manufacture the vector and testing on the final transduced cell product. When using gene-modified cells, the clinical protocol will require testing of the recipients for the presence of replication-competent virus.

For some time, it was not clear which manufacturing regulations applied to minimally manipulated cells. This was clarified in 2005 with publication of the current Good Tissue Practices (cGTP) regulations [5]. These closed the loop by providing a regulatory framework for these types of cellular products (Fig. 17.1). cGTP regulations were published as Subpart D of a new part (Part 1271) of Title 21 of the Code of Federal Regulations. This established the regulations regarding human cells, tissues, and cellular and tissue-based products (HCTPs). Specifically excluded from HCTPs are vascularized organs for transplant, whole blood and blood components, secreted or extracted human products, and minimally manipulated bone marrow for homologous use and not used in combination with another article, for example, scaffold or matrix. Part 1271 described the general provisions of the regulations (Subpart A), including the requirement to register your establishment annually with the FDA and to list the activities performed and products manufactured (as described in Subpart B) and to determine the eligibility of donors to provide cells (described in Subpart C). Subpart D describes in detail the cGTP regulations to be followed when handling minimally manipulated cell products. In essence, these are a "light" version of the cGMP regulations, containing many similar elements. Subpart E addresses enforcement of Part 1271.

MSCs require *ex vivo* expansion before clinical use. This places their manufacture into the more-than-minimal manipulation category and subject to the cGMP regulations and clinical use under the IND mechanism. This position has been legally challenged (unsuccessfully) by a commercial entity involved in MSC-based therapy [7].

Investigational New Drug Applications

The IND application provides the FDA with a summary of the preclinical data generated (including animal studies where performed); the details of manufacturing, testing, and criteria for release; and labeling of the cellular product (contained in the

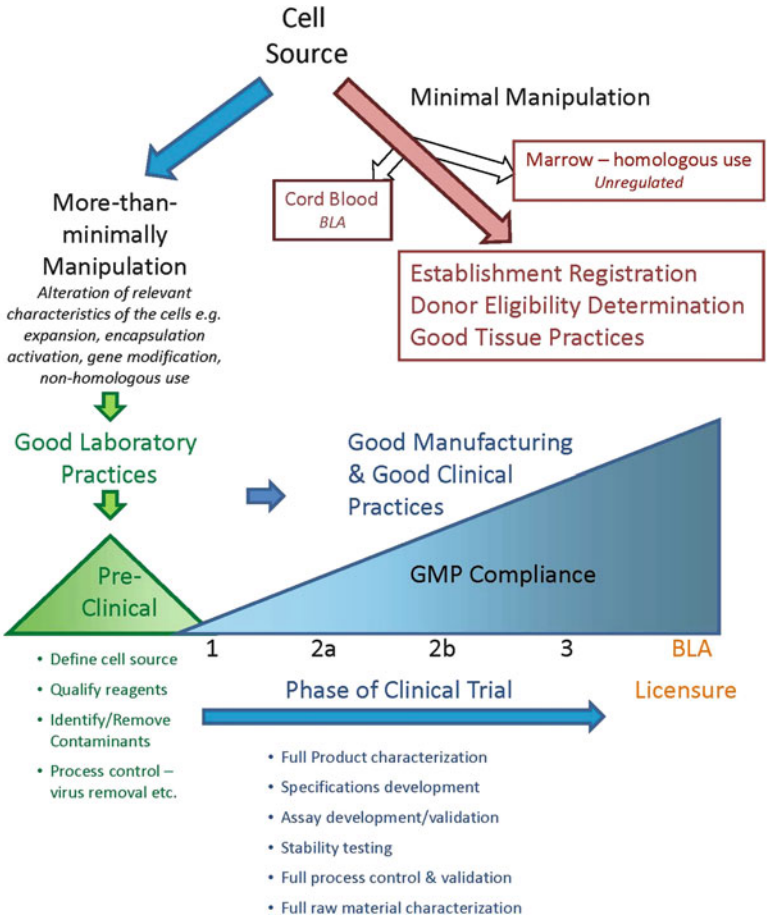

Fig. 17.1 Summary of FDA regulatory pathways for cellular therapy products. This figure summarizes the pathways for regulation of cellular therapy products based upon risk. The major differentiation is based upon the degree of manipulation of the cells *ex vivo*. Minimally manipulated cells are subject to manufacturing under current Good Tissue Practices, whereas manufacturing of more-than-minimally manipulated cells falls under current Good Manufacturing Practices (*cGMP*). As products move to later phases of clinical trials, the cGMP regulations become applicable with increasing stringency. *BLA* biologic license application

Chemistry, Manufacturing and Control (CMC) section of the application), the clinical trial design, and the evaluation criteria, including stopping rules. The most comprehensive assistance for preparation of an IND is found on the FDA webpage at www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/default.htm and in the guidance "Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products" [8]. When preparing to submit an IND application, the investigator

is strongly advised to initiate a "pre- or pre-pre-IND meeting" with the FDA. A pre-pre-IND meeting is usually a general discussion of the purpose and structure of the proposed study to gauge the initial response of the FDA to the intention to submit an IND. The pre-IND meeting provides the opportunity to address areas of confusion and to clarify questions that may have arisen during the preparation of the application. The investigator should make a written request for the meeting and provide the Agency with a list of specific topics that are to be addressed. Within 60 days, the FDA will arrange a conference call that will be attended by selected representatives of the Agency with expertise in the areas to be covered. The call will be of specified duration and provides the investigators with an excellent opportunity to resolve problems and amend the application accordingly. The value of these types of initial interactions cannot be overstated. Carefully structured pre-IND meetings can greatly expedite the review and approval of the final IND application. The types and scope of meetings that can be held with the Agency are described in the 2009 Guidance document "Formal Meetings Between the FDA and Sponsors or Applicants" [9].

It is important that the preclinical experimental and toxicity data submitted in the IND are generated using a product manufactured under the same conditions as those proposed for the clinical trial. Where possible, the product proposed for the trial should be available as a single lot, or the manufacturing process should have been sufficiently validated to show lot equivalence where more than one lot will be used. These types of issues are frequently on pre-IND meeting agendas.

The formal IND application will proceed more smoothly if the CMC section is carefully written. A template for this section is available from the FDA "Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)" [10]. Although intended for reviewers of IND applications, it provides a stepwise approach to constructing the CMC section to contain all of the required information in a format that is familiar to the Agency. The main elements include description of the origin of the cells and the reagents and excipients that will be used during collection and manufacturing. This is usually presented in tabular form. Wherever possible, media, reagents, and additives should be of clinical grade. Where this is not possible, the purest available alternatives should be proposed, and certificates of analysis (CsofA) from the manufacturers should be submitted to indicate the level of testing that is performed. The Agency may require additional testing prior to the use of such materials for product manufacturing.

The procedure for manufacturing is provided in detail, including the timeline for production and details of any in-process storage and the final formulation that will be used for administration. The manufacturing process must have been qualified to provide assurance that different batches of cells can consistently meet specifications. A detailed listing is required of the tests that will be performed on the product to demonstrate identity, purity, residual contaminants, endotoxin, and freedom from microbiological agents. Potency testing is listed but is not formally required until initiation of phase 3 clinical trials. Cell dose, viability, and stability testing results

should be provided. For the latter, it is advisable to include the anticipated stability under cryopreservation and the stability of the product once thawed. A draft CofA should be provided. This lists the tests to be performed (and their sensitivity or limit of detection), the identity of the testing laboratory, and the specification for release. Release will be based on results that will be available prior to administration of the product. Additional testing may be performed (and required by the FDA) for which the results will be received post administration. A procedure should also be submitted for dealing with out-of-specification test results that are received after administration.

A system for tracking and tracing the product between collection and administration should be described. It is advisable to provide a copy of the proposed label for the final product, ensuring that it contains the required FDA terminology. A description of the product container is required together with the proposed route for administration. In cases where catheters will be used for delivery, a validation of the delivery system should be provided to demonstrate that the product is not altered or adversely affected by the means of administration.

Standard operating procedures should be referenced for procedures not described fully in the body of the CMC. It is usually not necessary to submit copies with the IND application, although the investigator may subsequently be asked to provide selected examples. It is important to coordinate the CMC section contents with information in the remainder of IND application, which is frequently multiauthored by researchers, clinicians, statisticians, regulatory staff, and manufacturing technologists.

Once the application has been filed, the FDA has 30 days in which to reply. If there are no issues, the application will be approved. More frequently, it will be put "on hold" pending answers to questions raised by the Agency. These are generally provided by the investigators in a written reply which carefully and specifically addresses the issues raised. An approval may include "non-hold" issues that allow the trial to be initiated but point out that additional information will be required subsequently, for example, by the start of the phase 3 studies [9]. All communications with the FDA during the application process should be documented to ensure that there is a written record of interactions. Follow-up written confirmation of important points raised during telephone calls should be copied to the Agency to avoid misunderstandings.

GMP Manufacturing

New investigators often misinterpret cGMP manufacturing requirements. A common misconception is that a clean room facility is required [11]. Such facilities are now commonplace in larger academic institutions but are not a prerequisite. For phase 1/2 studies, the FDA is primarily concerned that the product is safe and manufactured by a reproducible procedure. The cGMP infrastructure is designed to provide this [12, 13], predominantly in the form of documentation. The regula-

tions require that there be adequate space, personnel, equipment, etc., and these must be described. There must be documentation of staff training and competency, control of environmental conditions (when specified), written manufacturing procedures, a quality program, methods for handling reagents and materials, procedures for release of the product, etc. At first glance, the regulations may appear intimidating but, with familiarity, become routine in even a small manufacturing facility [11]. The Agency has recognized that not all components of cGMP are appropriate at the start of clinical trials. Full cGMP compliance is "phased in" as part of what has been called the cGMP continuum, such that by the initiation of phase 3 studies, all of the major regulations must be followed (Fig. 17.1). To assist investigators performing phase 1 studies, the FDA published in 2008 a guidance "cGMP for Phase 1 Investigational Drugs," which outlines the Agency's expectations for compliance. The "c" in cGMP indicates "current" and updates to the regulations can be found on a special FDA web page at www.fda.gov/AboutFDA/CentersOffices/cder/ucm095412.htm.

Mesenchymal Stromal Cell Products

The Agency tends to look for specific items when reviewing the CMC section of an IND application. The GTP regulations are based on risk, including that posed to the cells during manufacturing of the product; this same philosophy can be applied to more-than-minimally manipulated products manufactured under cGMP regulations. Potential risks and methods for their elimination or avoidance should be addressed in the CMC section.

For MSC products, the investigator should propose eligibility determination of the donor within 7 days of collection of the product. The collection method should be described in detail, stating the source of the material, the collection method, and precautions taken to protect both the donor and the cells. Wherever possible, functionally closed systems should be used for cell handling. These include the use of disposable bags, culture systems, and tubing sets that can be sterile connected. In some cases, especially when starting with small numbers of cells, this is not possible and "open" culture systems are initially used. Under such circumstances, the investigator should describe precautions taken to prevent contamination and cross-contamination of the products during handling. Where multiple products are handled in a facility, a procedure should be described for changeover between handling of cells from different donors. Reagents used during cell culture should be described in detail, and CsofA submitted in the IND application. Where the materials are not of clinical grade, justification for their use should be provided, and the CofA included for the proposed source. Antimicrobial agents should be avoided if possible, and where their use is justified, evidence should be provided to indicate the maximum residual amount that could be present in the product at the time of administration. It is also advisable to demonstrate that final sterility testing of the product is not adversely affected by the presence of residual antibiotics or other additives

that may interfere with the sterility assay. This is accomplished by performing a bacteriostasis/fungistasis assay in which the product or excipient is examined for its ability to suppress or stimulate bacterial and fungal growth.

A major question that arises when products are manufactured by *ex vivo* expansion is the use of serum in the culture medium. In an ideal situation, the culture medium would consist of salt solutions containing non-proteinaceous supplements, but successful cell growth under such conditions is difficult to achieve. Ideally, the MSC culture medium should be free of animal sera [14]. In reality, attempts to come up with such formulations have met with varying success [15, 16]. Substitution with human AB [15] or autologous serum is an option. Pooled serum requires the appropriate screening for infectious agents and usually needs to be sourced carefully to minimize batch to batch variation. Autologous serum may be difficult to obtain in sufficient quantities and will often show subject-specific variability. The FDA has accepted protocols using media containing animal, pooled human, autologous sera and platelet lysate. The responsibility for justification of the serum/protein type lies with the investigator, in showing that the chosen source is essential to manufacture products with the required characteristics, and that alternatives which potentially are of lower risk are not capable of producing the same results. It should be appreciated that the type of serum and culture conditions chosen may have an important effect on the composition, phenotype, and function of the resulting MSC cell product [17, 18]. As clinical trials progress toward licensure, there may need to be substitution of previously acceptable supplements.

Attention should also be paid to the use of cytokines. The use of each should be justified. It is not acceptable in a proposed manufacturing procedure to add a "cocktail" of growth factors without demonstrating that each component is required. This evidence can be provided in the preclinical section of the IND application and/or published justifications provided.

In MSC therapeutic regenerative medicine applications there should be justification that the cells in the final product either retain the ability to differentiate along multiple pathways or have been primed toward a particular lineage. This is most frequently achieved by the use of multi- or unipotential colony-forming assays. Although of limited value for demonstrating therapeutic potential, these assays remain an important indicator of cell function and will normally be expected to be on the list of release tests. In addition, retention of multi-potentiality and replicative capacity may diminish with time in culture [19] and, thereby, limit the degree of expansion possible if, for example, aiming to generate a large bank of MSC. In this context, the use of colony-forming assays coupled with gene expression studies may be invaluable. Colony information provided on the final product will only be available after clinical administration, and in-process testing may offer useful supplementary information. If large numbers of MSCs are to be generated for an allogeneic bank, in addition to the question of how many times can the cells be passaged, are the issues of when a "cell bank" is considered to have been generated (requiring more complex and extensive testing) and the effects of cryopreservation and thawing on the cells.

When any cell with ability to differentiate along multiple pathways is proposed for therapeutic application, a major concern is that of aberrant differentiation and mutagenesis. Cells administered with the intent of differentiation into myocytes could potentially grow into bone, or senescent cells could reactivate and mutate into tumor. Initial reports described the development of malignant cells in MSC cultures [20, 21]. In at least one such report, the findings were eventually attributed to cross-contamination of the cultures with malignant cells [22]. This reinforces the importance of developing manufacturing procedures that eliminate the potential for cross-contamination and also for thorough screening of cell donors. A recent review of the risks associated with MSC therapy concluded that "the conditions for safe expansion of MSC without generating tumorigenic cells are now well documented" [23]. This concern may be additionally addressed in appropriate preclinical animal models using cells of the type proposed in the clinical trial. This does not, however, provide indisputable evidence for cell fate, due to the well-known vagaries of these models. As described above, the in vitro colony assays may provide some additional evidence, and investigators have examined the genotype and morphology of cultured cells during the manufacturing process to detect changes. A major problem is that genotypic changes occur with varying frequency during cell culture and their potential clinical significance is not always completely understood. The value of these assays and the relevance of their results to the clinical study plan are excellent points for discussion with the FDA at the "pre-" or even "pre-pre-" IND meeting. The earlier these issues are discussed with the Agency the better, as the answers will affect preclinical studies, manufacturing, and trial design.

The weak immunogenicity of MSCs has led to their use in immunomodulation [24] and for allogeneic regenerative medicine studies [25]. Immunosuppressive activity of MSCs on a mixed leukocyte reaction may be evaluated as a release criterion in these applications. HLA matching has, therefore, not been a major stumbling block when using MSCs clinically. It has been reported, however, that during differentiation in vivo, allogeneic MSCs may provoke an immune response in the recipient [26]. Similar responses can also occur to MSC culture constituents [27]. Many of these differences are due to the multiple methods for generating MSCs, and it is clear that a variety of cell types have initially been used under this name. In attempt to address this issue, the International Society for Cellular Therapy developed minimal criteria for defining multipotent MSCs, based on immunophenotype, plastic adherence, and trilineage (osteoblasts, adipocytes, and chondroblasts) differentiation capacity [28]. The abbreviation MSC itself has been redefined over time as representing mesenchymal stem cells, mesenchymal stromal cells, and, finally, multipotent mesenchymal stromal cells, further indicating the complexity and variety of the cell types under study.

Under such circumstances, each cell product essentially stands alone when it comes to regulatory interpretation. Where there is clear and indisputable identity between a cell type proposed for study and one that is already in clinical trials, it is helpful to ask the principal investigator (PI) of the clinical trial for permission to cross-reference his or her IND. This provides the Agency with additional information

on the cell type under study and its use in clinical studies. One potential drawback is that any product-related adverse events on the existing or new trial may result in both studies being placed on hold.

In the absence of such cellular identity, the investigator must provide the regulatory authority with a stand-alone submission. This may cross-reference other studies with similar cells types but provides independent data on the characteristics, manufacturing, and proposed clinical use of his or her specific MSC product.

Conclusions

As our understanding of the identity, properties, and clinical applications for MSC populations grows, the regulatory requirements and procedures for manufacturing, release, and administration are likely to change. This chapter can, therefore, only provide a general overview. This is especially true for a cell type with plasticity and with seemingly multiple applications. Investigators wishing to start a new clinical study should always revisit the regulations and talk with the Agency to determine the current regulatory strategy for their specific MSC product.

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