Regulatory Oversight of Cell and Gene Therapy Products in Canada

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Abstract Health Canada regulates gene therapy products and many cell therapy products as biological drugs under the Canadian *Food and Drugs Act* and its attendant regulations. Cellular products that meet certain criteria, including minimal manipulation and homologous use, may be subjected to a standards-based approach under the *Safety of Human Cells, Tissues and Organs for Transplantation Regulations*. The manufacture and clinical testing of cell and gene therapy products (CGTPs) presents many challenges beyond those for protein biologics. Cells cannot be subjected to pathogen removal or inactivation procedures and must frequently be administered shortly after final formulation. Viral vector design and manufacturing control are critically important to overall product quality and linked to safety and efficacy in patients through concerns such as replication competence, vector integration, and vector shedding. In addition, for many CGTPs, the value of nonclinical studies is largely limited to providing proof of concept, and the first meaningful data relating to appropriate dosing, safety parameters, and validity of surrogate or true determinants of efficacy must come from carefully designed clinical trials in patients. Addressing these numerous challenges requires application of various risk mitigation

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strategies and meeting regulatory expectations specifically adapted to the product types. Regulatory cooperation and harmonisation at an international level are essential for progress in the development and commercialisation of these products. However, particularly in the area of cell therapy, new regulatory paradigms may be needed to harness the benefits of clinical progress in situations where the resources and motivation to pursue a typical drug product approval pathway may be lacking.

Keywords Health Canada • Regulation of cell therapy • Regulation of gene therapy • Safety of Human Cells, Tissues and Organs for Transplantation Regulations • CTO Regulations • More than minimally manipulated

1 Introduction

Cell and gene therapy products (CGTPs) offer the prospect of improved treatments, and potential cures, for currently intractable diseases and conditions and have therefore attracted much public interest and hopeful expectation. At the same time, these products and aspects of their use involve new and exploratory techniques and potential risks to patients. An appropriate regulatory framework must provide clear pathways to investigational scientists in industry and academia with informational requirements that address the risks associated with products and procedures without serving as an impediment to innovation and product development.

Canada has a dynamic medical research community and a high-quality healthcare system that is advantageous for drug development despite accounting for a relatively small proportion of world drug sales. Since the first Canadian gene therapy clinical trial in 1994, there have been close to 100 clinical protocols approved. The marketing approval granted to Glybera (alipogene tiparvovec) in the European Union (EU), for the treatment of monogenic lipoprotein lipase deficiency (LPLD), has a Canadian connection in that two of three interventional clinical trials (that provided 19 of the 27 patients) were conducted in Canada by Dr. Daniel Gaudet and collaborators drawing on a LPLD "founder population" in Eastern Québec [1–3]. The connection extends much earlier and more broadly through the work of Dr. Michael Hayden's research group at the University of British Columbia [4].

The Canadian cellular therapy research community has also been active for many years and should be significantly advantaged by the recent addition of CellCAN, a new Network of Centres of Excellence (NCE) that will bring together the efforts of many stakeholders in stem cell research and promote cooperation, partnership development and innovation in regenerative medicine and cell therapy. The NCE operates a suite of national funding programmes on behalf of the three federal granting agencies, the National Science and Engineering Research Council (NSERC), the Canadian Institutes of Health Research (CIHR) and the Social Sciences and Humanities Research Council (SSHRC), in partnership with Industry Canada and Health Canada. Seven main organisations are at the heart of the new network which will standardise practices and promote innovative treatments for various diseases such as diabetes, cardiovascular disease and cancer.

Canada became the first country to grant marketing approval to a stem cell therapy product with the issue of a Notice of Compliance with conditions (NOC/c) for Prochymal (remestemcel-L) in May, 2012. Prochymal is a population of adult mesenchymal stem cells for intravenous infusion in the management of acute graft-versus-host disease and has since been approved in other regulatory jurisdictions.

In the following sections, this chapter will address the regulatory framework and applicable pathways for CGTPs in Canada and will provide an overview of some of the regulatory expectations for these classes of products. The applicability of Canadian, international, pharmacopoeial and various non-Canadian guidance documents will be discussed.

2 Regulatory Framework

Canadian regulatory frameworks are comprised of various elements: (1) statutes, or Acts, provide scope, high-level principles and the legal authority to make regulations; (2) regulations interpret an Act and provide general details on what must be done; (3) guidelines interpret and provide details of how to meet the regulations (being faster and simpler to introduce (not legally binding), they allow flexibility and adaptation to change); and (4) policies clarify and/or modify the intent of regulations (they usually relax or simplify, providing a "quick fix" pending re-drafting). All elements play a role in the regulation of CGTPs.

At present, there is no formal Canadian regulatory definition of either gene therapy or cell therapy; and these products are not specifically listed on *Schedule D* to the *Food and Drugs Act* (*F&D Act*) [5] which identifies biological drugs (or biologics) and which brings to bear a specific set of regulations under *Part C*, *Division 4* of the *Food and Drug Regulations* (*F&D Regulations*) [6]. Nevertheless, some of these products are captured by one or more class listings on *Schedule D* and so, logically, and in step with other regulatory jurisdictions, gene therapy products (GTPs) and many cell therapy products (CTPs) are regulated as biologics. Confirming the status of these products as biologics will be addressed by changes to *Schedule D*, or by other means, as part of ongoing regulatory modernisation.

Despite the lack of a formal definition, the transfer and expression of an exogenous gene typically associated with compensating for a missing or non-functioning endogenous gene has enduringly been identified as gene therapy. In addition, various other approaches to intervention are considered to be gene therapy by Health Canada, including (1) if nucleic acid (DNA or RNA) is transduced by viral vector or other means (directly in vivo, or into cells ex vivo followed by administration) and subsequently expressed (transcribed or translated) into messenger RNA, protein or "regulatory" RNA (e.g. small interfering RNA (siRNA)); (2) if cells intended for treatment are modified using various approaches to the introduction of site-directed mutations (for gene repair or modification of gene expression without actual gene transfer); and (3) the use of oncolytic viruses to treat cancer. In contrast, direct treatment with synthetic, regulatory RNAs, or with proteins that bind DNA (in a typical

drug approach), or with cells loaded ex vivo with such RNAs or with proteins would not be considered gene therapy.

The concept of therapeutic or prophylactic effect is important to the identification as a GTP. Introducing genetic changes to cells that are not related to the mode of action, for example, to make them better vectors or carriers of loaded antigens, is not considered to be gene therapy. However, any potential misclassification is not critically important to the review process since there is no distinct pathway or set of rules for GTPs; and the same part of Health Canada would be tasked with evaluation of the product using the same regulatory framework as for other biologics.

The regulatory approach to CTPs involves two major categorisations each supported, primarily, by a different set of regulations. There is stringent regulatory oversight for CTPs considered to be drugs, governed by longstanding and widely applicable parts of the *F&D Regulations* [7]; and a standards-based regulatory approach to allogeneic transplantation governed by the more recently developed *Safety of Human Cells, Tissues and Organs for Transplantation Regulations* (*CTO Regulations*) [8].

In addition, certain provisions of the *Assisted Human Reproduction Act of Canada* (*AHR Act*) will apply to embryonic stem cells. The creation of an embryo for any purpose other than for reproduction is prohibited in Canada; however, unused embryos can be donated for research purposes with appropriate consent. The *Act* extends to gene therapy in that it prohibits "knowingly altering the genome of a cell of a human being or in vitro embryo such that the alteration is capable of being transmitted to descendants" [9].

CGTPs and medical devices whose components are integrated into a singular product are regulated as combination products. Where the principal mechanism of action for the claimed effect or purpose is achieved by pharmacological, immunological or metabolic means, the *F&D Regulations* apply; in certain other circumstances, the Medical Device Regulations may apply [10]. The Health Products and Foods Branch (HPFB) Therapeutic Products Classification Committee may be engaged to reach a final decision regarding classification; however, regardless of the outcome, appropriate expertise from across the Branch is used to assess combination products.

Under the *Canadian Environmental Protection Act, 1999* (*CEPA, 1999*), and attendant *New Substances Notification Regulations* (*Organisms*), an environmental assessment is required for new substances and microorganisms not already on the "Domestic Substances List" [11]. The relevant definition of a microorganism includes viruses (but not plasmids) and so is applicable to many GTPs. Individuals, organisations or companies that file submissions are identified as sponsors. Sponsors planning to file a New Drug Submission (NDS) or Clinical Trial Application (CTA) for a viral or bacterial vector should notify Environment Canada. The assessment is actually completed within Health Canada by staff in the Healthy Environments and Consumer Safety Directorate (HECS). This process is largely about maintaining awareness and looking for any significant lack of consideration of potential problems by the sponsor. Thus far, there has been no prevention of clinical trial activities. Information can be obtained by sending an enquiry to substances@ec.gc.ca.

Prior to the conduct of a clinical trial, the Research Ethics Board (REB) and Biosafety Committee at each institution will examine the clinical protocol to ensure it meets the institutional requirements.[1] The Biosafety Committee will also be aware of municipal requirements regarding waste management and spills.

3 Regulatory Pathways

As outlined in Sect. 2, GTPs, CTPs (unless certain specific criteria apply—discussed below) and cellular products whose regulatory status is unclear but whose development requires the conduct of clinical trials are regulated as biologics. Responsibility rests with the Biologics and Genetic Therapies Directorate (BGTD) which forms part of the HPFB.

The clinical development and marketing application process is generally similar to that in the United States (USA) and other major, ICH[2]-observant, regulatory jurisdictions. As for all investigational studies in humans, Health Canada requires that clinical trial protocols for CGTPs obtain Research Ethics Board approval at each clinical site. However, there is no involvement of a standing, dedicated, government-associated committee like the US National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC).

For biologics, the regulatory requirements are defined within Divisions 1A (Establishment Licensing), 2 (Good Manufacturing Practices [GMP]), 4 (Biologics), 5 (Clinical Trial Applications) and 8 (New Drugs) of the Canadian *F&D Regulations* [7]. Through the application of Division 4, these products are subject to On-Site Evaluation (OSE) of manufacturing sites and the testing of consistency lots as part of the premarketing evaluation process and to the Lot-by-Lot Release Programme, the extents of which are discretionary by Health Canada following a risk-based assessment [12]. The Lot-by-Lot Release Programme can incorporate suitably modified approaches to reflect the small, or even single-treatment, lot sizes and the reality of some retrospective testing for products requiring use immediately after manufacture. Guidances and policies relating to biologics also apply, as do the target time frames for drug review. A product that meets the requirements and conditions for marketing approval is issued a Notice of Compliance (NOC) with the regulations, which constitutes an authorisation for sale. Sites where biologics are manufactured also require an Establishment License, which is reviewed and approved separately by the HPFB Inspectorate.

[1] Information and guidance regarding the bio-containment of gene therapy vectors is available from the Office of Laboratory Safety (OLS), Centre for Emergency Preparedness and Response (CEPR) and Public Health Agency of Canada (PHAC). Requirements will depend on the type of virus used as a vector, any association with human or animal disease, and the amount of the virus genome that remains.

[2] International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use.

CGTPs are often evaluated in relatively small patient populations which can present challenges for the generation and analysis of statistically meaningful data. A NOC/c may be granted in certain situations where sufficient product safety has been established, where preliminary evidence is supportive of clinical efficacy, and where a particular patient population, and the process of collecting additional clinical data, would benefit from early market access. The conditions must be met within a defined period after the NOC/c is issued. An "Orphan Drug" programme, which could help address many of the regulatory issues surrounding the clinical development of products for small patient populations, has been lacking in Canada, but the introduction of new "Orphan Drug Regulations" is in progress.

Some cellular therapies that meet certain criteria or that have an established safety profile and therapeutic use (such as bone marrow transplantation) are subjected to a less stringent, regulatory approach under the *CTO Regulations* [8]. These regulations came into force in December 2007 with the purpose of minimising the potential health risks to Canadian recipients of human cells, tissues and organs (CTOs), e.g. transmissible diseases. The focus is on activities performed by establishments such as cell and tissue banks, transplant establishments for living donors and organ donation organisations. The regulations are standards based and directly reference sections of standards developed by the Canadian Standards Association (CSA) [13] that are related to the safety of human CTOs. There is no premarket review and a NOC is not issued; however, establishment registration with Health Canada is required, along with suitable attestations. The regulations also empower the inspection of registered establishments. Comprehensive information is published elsewhere on the evolution of these regulations [14] and with specific respect to blood stem cell products [15].

The *CTO Regulations* are applied only to CTPs that are allogeneic, minimally manipulated and intended for homologous use. Regarding cells, "minimally manipulated" means that the processing does not alter the biological characteristics that are relevant to their claimed utility; and "homologous use" means that the cell performs the same basic function after transplantation (both as defined in the *CTO Regulations*) [8]. The regulations prohibit the transplantation of CTOs unless they have been processed by a registered establishment and determined safe for transplantation (except under the provision for "exceptional distribution", which, for example, could cover a situation where both donor and recipient were positive for hepatitis B virus). In this context, "safe" means processed in accordance with the *CTO Regulations*, and "processing" means any of the following activities: donor screening, donor testing, donor suitability assessment, retrieval (except organs and islet cells), testing and measurements performed on the CTO after retrieval, preparation for use in transplantation (except organs), preservation, quarantine, banking and packaging and labelling.

In general, cells of human origin that do not have an established therapeutic use should undergo investigative studies authorised under Division 5 (Clinical Trials) of the *F&D Regulations* (if conducted in Canada).

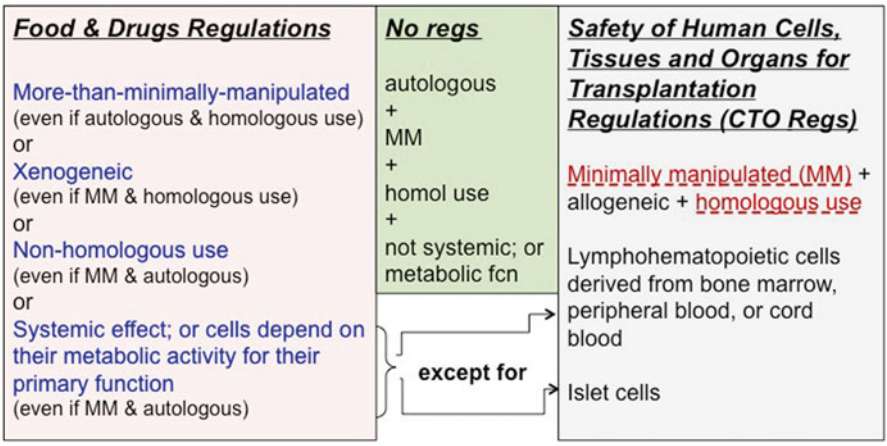

Fig. 1 Regulations governing cell therapies. Two distinct sets of regulations are available and applied based on specific criteria. Cells for human treatment meet the definition of a drug, but autologous cells that are minimally manipulated are not covered by regulations if (1) they perform the same basic function, (2) there is not a systemic effect, and (3) the primary function is not dependent on metabolic activity. "Minimally manipulated" (MM) means cell processing does not alter the biological characteristics that are relevant to their claimed utility. "Homologous use" means the cells perform the same basic function after transplantation. Two exceptions to the regulatory approach regarding CTPs with "systemic effect" or "metabolic activity" are islet cells and certain lymphohematopoietic cells. This image was originally published in [***The Regulation of Cell Therapy Products in Canada***] in [*Biologicals*, 2015, DOI: 10.1016/j.biologicals.2015.05.013]. ©[Elsevier 2015]

Essentially, there are a few "trump card" descriptors that dictate the application of the *F&D Regulations* to CTPs if any of the following apply: (1) xenogeneic, (2) more than minimally manipulated, (3) for nonhomologous use or (4) have a systemic effect or depend on their metabolic activity for their primary function (see Fig. 1). Despite this last criterion, for various practical reasons, the *CTO Regulations* are applied to lymphohematopoietic cells derived from bone marrow, peripheral blood or cord blood and to islet cells. Otherwise, with respect to cells, application of the *CTO Regulations* is restricted to cellular products that are minimally manipulated, intended for allogeneic and homologous use and not combined with non-cell or non-tissue products.

No clinical trials involving xenotransplantation have been approved by Health Canada to date, and issues unique to xenogeneic cells will not be discussed further in this chapter.

Health Canada currently has no applicable regulations for cellular products that are autologous, minimally manipulated, intended for homologous use and do not have a systemic effect or depend on their metabolic activity for their primary function.

By comparison to allogeneic cells with the same characteristics, these autologous cells are considered to represent a lower risk and are currently not a regulatory focus; however, to improve clarity, formal regulatory exemptions under the *F&D Regulations* that would cover some examples in this product class may become possible.

4 Regulatory Harmonisation and Guidance

Health Canada is a contributor to the ICH and adopts all ICH guidelines. Many of these guidelines are applicable to the therapeutic use of CGTPs, and even though some guidelines contain a product scope that excludes these products, many of the principles may still be relevant. There are also three ICH Considerations documents that address gene therapy with regard to germline integration, oncolytic viruses and vector shedding [16–18].

In addition to direct participation on ICH Expert Working Groups and Discussion Groups, Health Canada participates under the umbrella of the ICH-affiliated, International Pharmaceutical Regulators' Forum (IPRF), on the Cell Therapy Working Group and the Gene Therapy Working Group. Health Canada also participates in the Advanced Therapy Medicinal Product (ATMP) "Cluster Meetings" held regularly between the US Food and Drug Administration (FDA) and European Medicines Agency (EMA).

In the absence of specific Canadian or ICH guidance, Health Canada encourages the use of relevant regulatory guidance developed by the FDA and EMA. Three US Pharmacopeia (USP) documents are also highly relevant to the manufacturing and processing of these products: <1043> Ancillary Materials for Cell, Gene and Tissue-Engineered Products; <1046> Cellular and Tissue-Based Products; and <1047> Gene Therapy Products [19–21].

In addition, the CIHR has developed guidance for human pluripotent stem cell research and created a National Stem Cell Oversight Committee to oversee grant applications involving these cells. Those guidelines were recently incorporated within the "Tri-Council Statement: Ethical Conduct for Research Involving Humans" [22]. The Tri-Council is the research funding arm of Health Canada and comprises the CIHR, the National Science and Engineering Research Council (NSERC) and the Social Science and Humanities Research Council (SSHRC). All clinical trials undertaken in Canada should be in accordance with the guidelines outlined in the Tri-Council Policy Statement.

Health Canada is also taking steps to try to address the need for guidance for CTPs following a Stakeholder workshop co-sponsored by Health Canada and the Canadian Stem Cell Network held in December 2010. The needs of the research community that were expressed, and the input provided at this workshop, along with other regulatory considerations, form the basis for a document titled "Guidance for Sponsors: Preparation of Clinical Trial Applications for use of Cell Therapy Products in Humans" which should become final in 2015.

5 Overview of Specific Considerations and Expectations for CGTPs

5.1 Product Manufacturing

Cells used with or without gene transfer in the manufacture of CTPs and cell-based GTPs encompass a wide variety of types derived from patients undergoing treatment (autologous cells) or from donated cells or established cell lines (allogeneic cells). These include cells of somatic or embryonic origin, derived from various tissue sources, at different stages of differentiation and subjected to various degrees of manipulation. The gene therapy vectors used to transduce cells ex vivo or for direct administration are also diverse, including nucleic acid (both DNA and RNA) formulated in buffer or complexed with agents to aid transduction, and viral vectors of many origins. Like other biologics, the manufacturing of CGTPs features the inherent risks associated with biological starting materials, the potential introduction of adventitious agents during manufacturing, the inherent variability of products derived from processes that use living systems, and the difficulty in precisely controlling the manufacturing process. Viral vector design and manufacturing control are critically important to overall product quality and linked to safety and efficacy in patients through concerns such as replication competence, vector integration and vector shedding. It is often stated that for biologics, processing defines the characteristics of the product; and this is particularly valid for CGTPs.

Similar to other biological drugs, the risks associated with CGTPs can be mitigated to a large extent through tightly controlling starting materials and the manufacturing process and suitably evaluating intermediates and the final product. In the following sections, we have divided CGTPs into three categories: CTPs, cell-based GTPs and virus-based gene therapy vectors, in order to discuss the risk mitigation strategies and regulatory expectations for manufacturing these products.

5.1.1 Manufacturing Process for CTPs and Cell-Based GTPs

Often, the manufacturing process for CTPs and cell-based GTPs begins with the isolation of cells from blood or various tissues with or without dissociation with digestive enzymes. The cells can also be subject to various degrees of manipulation including, but not limited to, ex vivo expansion in culture, genetic modification or reprogramming, activation to induce expression of genes or cell surface receptors, differentiation, photochemical treatment and/or irradiation or combined with biologic or nonbiologic matrices or supporting structures. Further, the ex vivo expansion of cells may involve (1) a continuous process with no intermediates, (2) a cell banking system involving one or more cryopreserved intermediates such as master or working cell banks and/or (3) the use of culture media supplemented with reagents and growth factors from a wide variety of sources (e.g. animal serum, pooled human serum, autologous serum, human platelet lysate or recombinant growth factors).

In addition, CTP and cell-based GTP manufacturing is often critically time dependent due to cell passage number limitations, the inability for hold times and the limited shelf life of hours to days of non-cryopreserved living cells held at room or refrigerated temperatures. Cryogenic storage may be necessary to extend product shelf life but can have deleterious effects on cell viability and function.

Variability in the biologic starting materials and the manufacturing process could potentially affect the safety and identity/composition of the cellular product, as well as its biologic activity in vitro and in vivo. A well-controlled and validated manufacturing process will help minimise the potential variability attributable to the manufacturing process itself and help maintain product quality, safety, identity and potency. An important regulatory expectation is a detailed description of the process with flow charts and diagrams that identify the critical control steps.

Another aspect of CTP and cell-based GTP manufacturing, important for avoiding mix-ups, is the segregation, labelling and tracking of different batches, which may be numerous and small in size. This is especially relevant for autologous cells, directed allogeneic cells (i.e. intended for a specific patient) or small-scale allogeneic cells, situations that also require tightly controlled cleaning and changeover procedures. Closed systems and automation can help with product segregation and also provide greater manufacturing consistency.

5.1.2 Starting Materials for Human-Derived CTPs and Cell-Based GTPs

The infectious disease risks associated with starting materials containing live cells cannot be mitigated via sterilisation or other pathogen inactivation or removal processes. Consequently, a combination of donor screening and infectious disease testing serve as critical control steps in the manufacturing process. For allogeneic CTPs and cell-based GTPs regulated under the *F&D Regulations* [7], the donor suitability assessment, donor screening and infectious disease testing requirements in the CSA Standards [13] that are referenced in the *CTO Regulations* [8] are mostly considered appropriate by Health Canada. These Standards consist of the National Standard for Cells, Tissues and Organs for Transplantation: General Requirements and four subset standards with specific requirements for lymphohematopoietic cells, tissues, ocular tissues and perfusable organs [13] and are further clarified in a specific guidance document [23]. However, since the *CTO Regulations* are intended for cells and tissues that are not subject to the *F&D Regulations*, alternative practices may be considered acceptable if they are supported by adequate evidence and rationales.

A controversial issue regarding the controls for starting materials relates to the testing of autologous donors for donor-derived viral pathogens, since they do not pose a risk to themselves. However, Health Canada recommends testing autologous donors for the infectious disease agents that pose a significant risk to patients in order to address concerns regarding the cross-contamination of products manufactured in the same facility for other patients and/or potential viral propagation during culture.

Another challenge relates to the use of established cell lines derived from donors that were not screened or tested in accordance with current requirements. The appropriate risk mitigation measures in these cases must include, among others, a risk assessment, re-screening and/or retesting donors where possible and testing the cell lines with appropriately validated tests.

5.1.3 Ancillary Reagents, Excipients and Materials

CGTPs are highly complex, and it is difficult to confirm critical quality attributes via final product testing. Thus, product safety, potency, quality and consistency are assured by implementing controls for various critical components. These include the ancillary reagents, excipients and certain other materials used during manufacturing. These materials, typically purchased from commercial sources, are usually not of GMP or pharmacopoeial grade, as would be preferred for use in manufacturing CGTPs, and exhibit various risk profiles. Of particular concern are materials derived from human or animal sources, which are associated with the risk of contamination with viral and bacterial pathogens as well as transmissible spongiform encephalopathies (TSEs). Consequently, critical components must be appropriately qualified prior to use in manufacturing via vendor qualification and audits, review of Certificates of Analysis and adequate in-house quality control testing as described, for example, in the USP Guidelines on ancillary reagents [19].

5.1.4 Process Validation

Process validation is required to help reduce lot-to-lot variability associated with the factors mentioned above and minimise the potential for release of CGTPs that do not meet specifications. This is especially important where release must take place before the availability of certain test results.

Some aspects of validation and process controls should be implemented during early clinical trials, with emphasis placed on aseptic process validation and the use of safety tests for sterility, mycoplasma and endotoxin. A preliminary assessment of other parameters should also be performed at this stage. Process validation should be subject to continuous improvement at later stages of product development and should demonstrate the consistency of the manufacturing process. This should include demonstrating consistency of products derived from different donors and, where applicable, the consistency of multiple lots derived from the same donor.

There are unique challenges associated with process validation for CGTPs derived from autologous donors as it may not be appropriate to retrieve cells or tissues from patients for validation purposes. In these cases, the process validation may be carried out using allogeneic donations. Potential differences between patient- and donor-derived cells or tissues are then assessed when the former becomes available.

5.1.5 Product Characterisation and Specifications

The characterisation of CGTPs typically supports the establishment of specifications for key parameters that could be used to monitor the consistency of the manufacturing process, as it relates to product safety, quality and potency. This is particularly important in cases where extensive testing cannot be performed, or where the products are released for administration to patients prior to the availability of results of final product testing. Examples include autologous products with limited samples available for quality control testing, or products with limited shelf lives.

The characterisation of CTPs and cell-based GTPs is particularly difficult because of their cellular complexity; i.e. along with the desired cell populations, they typically contain various cellular impurities such as nonviable cells, non-functional live cells and cell types that are not at the intended maturity. Other challenges, in some cases, include (1) the presence of immunogenic and tumorigenic cells that cannot be removed; (2) further maturation, migration and/or differentiation in vivo, of the desired cell types in the final drug product; (3) the contribution of more than one cell type in a heterogeneous array of cells to the effectiveness of some CTPs; and (4) the lack of reference standards.

The establishment of specifications for autologous and directed allogeneic products also warrants special consideration as this is often the only product available to these patients. Donor-to-donor variability makes it difficult to consistently meet stringent specifications, thus resulting in fairly broad specifications that reduce the number of products rejected during clinical trials. However, an appropriate level of stringency needs to be applied to these products once adequate supporting data has been accumulated.

Given the challenges identified above, emphasis is placed on safety-related parameters when setting specifications during the early phases of clinical trials, followed by the refinement and tightening of specifications for other parameters as product development progresses. The key parameters used to establish specifications for CGTPs include, but are not limited to, viability, identity, yield, purity and potency, as well as safety tests for sterility, endotoxin, mycoplasma and other adventitious agents. In some cases, the complexity of CGTPs and/or the technical limitations of the analytical methods employed may require the use of multiple tests for a single parameter. Some of the particular challenges associated with the characterisation of CGTPs are discussed below.

5.1.5.1 Identity and Purity

Assays for cell identity and purity (absence of cellular contaminants) are usually based on the expression of cell surface markers, which can be affected by the tissue source, the culture conditions or cell cycle progression. Establishing the criteria for cell identity or purity can be challenging if not all the cell types responsible for the product's biological activity and functions are known. As well, the comparison of cells manufactured in different facilities based on cell surface marker expression could be problematic if the same manufacturing process is not employed.

5.1.5.2 Viability

The choice of analytical methods used for the determination of cell viability can be an issue. Cell death may be due to necrosis or to apoptosis. Necrotic cells lose their membrane integrity and are able to take up vital dyes that are either colorimetric (e.g. trypan blue) or fluorescent (e.g. propidium iodide). In contrast, apoptosis is triggered by biochemical events that lead to characteristic cell changes. While cells in the late stages of apoptosis exhibit loss of membrane integrity, those in the early stages do not and are unable to take up these dyes [24]. Consequently, the use of dyes that are only taken up by leaky cells could lead to an underestimation of nonviable cells.

5.1.5.3 Potency

Potency testing is especially important for complex products. Assays are ideally based on the mechanism of action and should be demonstrated to contribute to the prediction of clinical efficacy for each lot (but it is understood that other analyses contribute to a summation of evidence that product will perform appropriately). CTPs could exert their effects via different mechanisms, including cell engraftment and paracrine signalling. The latter may involve the secretion of factors with anti-apoptotic, anti-inflammatory, immunomodulatory and/or angiogenic effects. In most cases, the mechanism of action of a CTP is not well understood. Consequently, potency assays and their specifications and/or acceptance criteria are difficult to develop, and multiple assays may be required to characterise these products. It is also important to provide quantitative test results for product release (this could involve a quantitative physical assay that correlates with, and is used in conjunction with, a qualitative biological assay).

Given the challenges with product characterisation that are noted above, truly relevant potency assays are generally not established for products used in early-phase clinical trials. Nevertheless, it is valuable early in development to have the ability to reject subpotent material. Thus, the evaluation of in vitro and in vivo candidate assays should begin in early-phase clinical trials and be refined as product development progresses. A suitable potency assay should be selected and implemented prior to applying for market access, in order to generate sufficient data to include in the marketing application.

5.1.5.4 Safety

CTPs and cell-based GTPs cannot be sterilised or subjected to pathogen removal/inactivation procedures because they contain live cells. Further, some CTPs and cell-based GTPs must be released for administration to patients within hours to days of final formulation. Under these circumstances, pharmacopoeial methods (e.g. USP <71> for sterility testing) [25] cannot be used as prospective lot release tests due to the time required to obtain the test results. The use of rapid,

non-pharmacopoeial methods for sterility and mycoplasma testing that are as sensitive as the pharmacopoeial methods could serve as useful lot release tests in some cases.

CGTPs must also be tested for viral agents to rule out the introduction of viral contaminants during processing. Two ICH Quality Guidelines (Q5A R1 and Q5D) provide recommendations for adventitious agent testing for various human and animal viruses by PCR assays and other in vitro and in vivo tests [26, 27]. Although intended for cell lines and banks used for the preparation of biotechnological/biological products, the guidance could be adapted for CTPs and cell-based GTPs in some situations. A risk–benefit analysis should be employed when determining the appropriate level of testing.

5.1.6 Analytical Method Validation

Biological assays involving complex CTPs are highly variable, and there are currently no reference standards for these products. Assay variability can be controlled to a large extent by using analytical methods that have been validated to establish method sensitivity, specificity, accuracy, precision and robustness. Given the challenges with the development and validation of some of the analytical methods employed, clinical trial sponsors are not required to submit full validation data to Health Canada during early-phase clinical trials. Nonetheless, all methods must be appropriately qualified for their intended use, and the methods for safety-related parameters must be appropriately validated to ensure the safety of clinical trial subjects.

5.1.7 Release Criteria

As discussed above, some CTPs and cell-based GTPs must be released for administration to patients within hours to days of final product formulation and before some final product test results (e.g. sterility, mycoplasma) are available. In these cases, the missing tests could also be performed as in-process controls, and as close to the final product as possible, to ensure preliminary results are available prior to product release.

5.1.8 Batch Analysis

Typically, sponsors are required to submit data for at least three consecutive batches of the final drug product prior to clinical use. For CTPs and cell-based GTPs, one batch of starting material could be used to produce one to several batches of drug product. To account for the variability in the starting materials, the batch analysis should also include final product manufactured from at least three consecutive batches of starting materials.

5.1.9 Stability Studies

A major challenge with CTPs and cell-based GTPs relates to the relatively short shelf life of fresh or thawed cells and the impact of storage and transportation conditions or delivery systems on cell integrity and function. Stability studies should be designed to assess the impact of all these factors on stability-indicating parameters such as cell count, viability and potency. These studies should cover the proposed product shelf life, and testing should be performed after final product formulation and following storage and/or transportation. In-use stability studies should also be performed to assess product when it is subjected to the conditions employed to prepare the cells for administration, including any hold period prior to administration.

5.1.10 Additional Considerations/Expectations for Virus-Based Gene Therapy Vectors

Bacterial plasmids and other nucleic acid-based gene therapy vectors present fewer challenges compared to other biological therapeutics than do virus-based and cell-based vectors and are not specifically addressed in this chapter. While manufacturing challenges associated with cell-mediated gene transfer have been covered in the preceding sections, some challenges particular to viral vectors are addressed below.

5.1.10.1 Replication Competence

In most situations, virus-derived GTPs are designed to be replication incompetent. In these cases, testing to confirm this attribute is an important quality and safety consideration. Aspects of vector design and manufacturing control may virtually eliminate the possibility of recovery of replication competence during product manufacturing, but with large-scale production involving high virus titres, rare events can occur. Natural infection of patients by wild-type versions of the virus upon which the GTP is based may present the opportunity for recombination or complementation events potentially capable of restoring replication competence and should be avoided, controlled and/or risk rationalised. In these instances, replication-competent virus is a form of adventitious virus.

Replication incompetence can be important for control of targeting of the vector and reducing pathogenicity. However, conditionally replicating vectors may have distinct advantages for some indications and uses (e.g. oncolytic viruses).

5.1.10.2 Vector Integration

Minimising the potential for unintended viral integration, and associated risk of insertional mutagenesis, is an important issue. Again, this is largely controlled through choice of vector and vector design, and intentional vector-mediated

integration is generally accomplished in the more-controlled conditions of ex vivo gene transfer. Replication competence presents particular concerns for integrating vectors such as retroviruses, but again, cell transduction is conducted on cells ex vivo, with cryopreservation and storage prior to patient administration, thus allowing time and opportunity for extensive analysis.

5.1.10.3 Virus/Vector Dose and Expressed Product Dose

Measurement and control of vector dose is important since this could have an impact on several factors including expressed product dose and immunogenicity. A high titre of vector could induce a sudden immune response with adverse consequences for the patient. Immunogenicity can also cause reduced efficacy via rapid removal of vector and negatively affect the potential value of repeat administration. Regulation of expression of the transduced gene is important for targeting the effect and will depend on aspects of the gene construct (e.g. tissue-specific promoter), the cellular target and the cellular host range of the vector. While obtaining sufficient expression may be a typical concern, the consequences of overexpression may also be undesirable (such as for a highly potent cytokine).

5.1.10.4 Testing for Adventitious Viruses

Testing for adventitious virus in a virus product can present significant challenges. Special adaptations may be needed for some analytical methods. For example, a conditional replication-competent oncolytic virus can interfere with assays for detection of other viruses. One approach is to use vector-specific neutralising antibodies for in vitro, cell-based assays for adventitious viruses; however, if these are not available or suitably efficient, one must rely on in vivo assays.

5.1.10.5 Potency

Biological potency assays are especially important for complex biologics like GTPs and should be well described, justified and eventually validated. There is some flexibility regarding stage of product development: early on, ability to quantify the expression of a gene therapy vector product may suffice, but later in development, the assay should measure an appropriate biological activity.

5.2 Nonclinical Evaluation

The principles in the ICH Safety guidelines (e.g. ICH S6 (R1)) [28] and the scientific content in FDA and EMA gene and cell therapy guidelines [29, 30] are applicable to clinical trial and market authorisation applications for CGTPs in Canada.

Prior to administration of an investigational product in a clinical trial in Canada, the sponsor must provide adequate nonclinical data and information in relevant animal model(s). Relevant animal species in which the CTP or GTP is immune-tolerated and biologically active should be used in the toxicology studies, if available. Studies in healthy animals can be a useful means to collect toxicology information; however, due to their distinctive features, animal models of disease/injury may be more preferable to assess product activity and safety. Due to the species-specific nature of the GTP or CTP (e.g. some vector-expressed human transgenes; human-derived cells), testing these products often requires the use of immune-compromised animals. In some cases, however, it may be necessary to investigate the safety profile of a product in an immune competent environment. As such, testing of an analogous animal product, or testing in transgenic animals, may provide suitable alternatives [28]. In these situations, the design of the nonclinical testing programme is considered on a case-by-case basis and should incorporate the fundamental principles of pharmacological and toxicological testing that underlie traditional nonclinical studies. The type, duration and scope of animal studies required vary with both the duration and nature of the proposed clinical studies as well as the inherent risk/safety profile of the product itself.

With that said, certain aspects of pharmacology and toxicology, such as absorption, metabolism, and excretion, may not be applicable to many CGTPs. The unique aspects of product characterisation and the mechanism(s) of action of CGTPs set them apart from chemical pharmaceuticals and from other biologics (such as therapeutic proteins); the traditional, standardised battery of nonclinical toxicity studies required for drug development and testing may not be appropriate for assessing their safety.

Rodents have been invaluable for the study of GTPs. While mice may provide proof of principle and allow testing of a variety of therapeutic products, murine models do have a variety of limitations, including a genetic background and organ systems that differ greatly from humans. As a consequence, some rodent studies may not directly translate to the human setting. Large animals (e.g. cats, dogs, sheep, pigs, goats and horses) may provide an acceptable substitute when adequate justification is provided. Large animals can allow for longitudinal studies and may be more applicable to the human situation in many cases. In addition, large animal models typically have more heterogeneous genetic backgrounds compared to inbred rodent models, resulting in studies that may more closely resemble clinical outcomes. Overall, Health Canada is in agreement with FDA guidelines which propose that nonclinical testing paradigms may include the use of (1) large and small animal models, (2) multiple small animal models or (3) only large animal models, depending upon the nature of both the product and the intended indication [29].

Viruses are the most commonly used vectors for gene therapy. The risk of spreading of a viral vector via secreta and excreta from the treated patient is a safety concern for healthcare professionals, family members and others. A nonclinical shedding study may be valuable to monitor the secretion and excretion profile of the vector which can then be used to make estimates on shedding in patients, such as, the likelihood of occurrence, the extent and the kinetics. One of the challenges of

investigating viral vectors in nonclinical studies is the relevance of the animal species, as a large number of viral GTPs used in clinical studies are derived from parental strains that do not readily infect and rarely replicate in nonhuman species. Therefore, the shedding profile might not directly correlate with that in humans. Prior to use in the nonclinical studies, the susceptibility of study animals to infection from the viral vector under investigation has to be considered.

5.3 Clinical Evaluation

5.3.1 Conduct of Clinical Trials

Many CGTP proof-of-concept clinical trials are being conducted in cancer, inherited disorders, immune system disorders, infectious diseases and cardiovascular disorders. However, advancing CGTPs from the nonclinical studies and early-phase clinical trials into late-phase clinical trials and marketing authorisation has proven to be challenging.

Issues that are unique to CGTPs may make it difficult to categorise clinical trials involving these products into the traditional developmental phases used to investigate pharmaceuticals. Phase I studies in healthy individuals, for example, would not be considered ethical for the majority of CGTPs. Extrapolation of nonclinical data may not be feasible for defining the appropriate dose or dose range which must then be determined on a case-by-case basis and should incorporate current knowledge regarding the biodistribution, engraftment, tumour forming potential and immunogenicity. In such cases, conservatively designed early clinical trials may still proceed if it can be clearly argued that the potential benefits of the therapy outweigh the potential risks within a specific patient population. When possible, dose estimation should be based on previous clinical experience with similar cell types. In principle, clinical trials should be designed to detect clinically meaningful endpoints that assess the therapeutic effect and duration of a CTP or GTP as well as short- and long-term adverse events. Valid surrogate endpoints are acceptable for CGTPs, particularly for those products developed for rare diseases.

Traditional pharmacokinetic studies to assess biodistribution in humans may be challenging and may require the development of appropriate cell or vector tracking technologies. The presence of CTPs or GTPs in non-target sites should be further investigated and the risks fully evaluated whenever feasible. Health Canada may insist on pharmacokinetic assessment for products associated with higher risks of tumorigenicity or ectopic tissue formation prior to the initiation of trials in a large number of patients.

5.3.2 Specific Challenges Regarding Safety and Efficacy

There are efficacy and safety concerns clearly associated with gene therapy. Increased efficiencies of vector and transgene delivery and expression may affect dosing regimens, therapeutic indices and safety profiles. The duration of gene

expression and the impact of immunological responses directed against the delivery vector or transgene are also important considerations for gene therapeutics. The transgene introduced into target cells may show only transient expression and so may not provide long-term effectiveness, perhaps implying that patients might need multiple rounds of GTP administration. However, the loss of transgene expression may be caused by the development of immune responses against the vector or the transgene. This is known to occur frequently when using adeno-associated virus (AAV) vectors, as humans are frequently exposed to wild-type AAV in the context of pathogens during childhood. In such cases, neutralising antibodies to AAV can be generated after the first injection of vector, inducing immunogenicity that would normally reduce the usefulness of repeated vector administration [31].

The type of the induced immunogenicity depends on the route of administration of the vector, the target tissue, the vector serotype and dose, the disease targeted and the expression level of the transgene. There are a number of scientific means to try to counter this immunogenicity. Co-delivery of pharmacologic and vector-encoded immunosuppressive agents may prolong vector expression. Alternatively, vectors could be developed to produce higher transgene expression levels at much lower vector doses. There might also be some value in repeating treatment using different vector serotypes.

Viral vectors present a variety of potential problems to the patient: toxicity, inflammatory response, immunogenicity and gene control and targeting issues. In addition, there is the concern that the viral vector, once inside the patient, may recover its ability to cause disease. Viral shedding should be considered as a possible source of transmission to other individuals. For most clinical applications, a viral vector should be safe and well tolerated, should not elicit a strong immune response, and should also be replication incompetent in humans. Note, however, that for oncolytic viral vectors, conditional replication competence and an eventual immune response may be desirable.

Additional concerns related to GTPs include the risk of delayed adverse events. Factors likely to increase such risks include:

- Persistence of the viral vector
- Integration of genetic material into the host genome
- Prolonged expression of the transgene
- Altered expression of the host's genes

Persistence of the viral vector could permit continued expression of the transgene. Although it may be necessary for the product to provide a continuing clinical benefit, the persistence of the viral vector could have adverse effects upon normal cell function and place patients at risk for development of adverse events, some of which may be delayed by months or years. Integration of a viral vector into the host cell genomic DNA raises the risk of malignant transformation. Prolonged expression of the transgene may also be associated with long-term risks such as uncontrolled cell growth and malignant transformation. Altered expression of the host genes could also result in unpredictable and undesirable events, such as autoimmunogenicity or cancer.

Issues that are more specific to CTPs include graft failure, tumour formation, immune responses, ectopic tissue formation, inflammatory events, viral activation and the distribution and engraftment of the cells throughout the body. Concerns specific to product administration should also be addressed, including:

- Lung emboli formation
- Respiratory and cardiac adverse effects
- Both local and systemic toxicities

5.3.3 Monitoring and Risk Management

CGTPs require longer than normal monitoring and follow-up periods compared to other biologics. Even in early clinical trials, patient monitoring may be required for 1 year or more.

The precise length of time for monitoring is dependent on considerations such as the product characteristics, the anticipated time for the occurrence of delayed adverse reactions, the clinical indication and the expected life expectancy of the treated patients. Long-term monitoring should be focused on survival and serious adverse events (e.g. oncologic, hematologic, immunologic, etc.). Detailed plans should also be put in place proactively to maintain long-term monitoring in cases of early stoppage. EMA and FDA guidances suggest 5 years and 15 years, respectively, for the follow-up of gene therapy. With the marketing authorisation application, a Risk Management Plan (RMP) should be submitted by the sponsor and can be based on the EMA's RMP with a Canadian context [32].

Measures to identify and mitigate potential long-term risks of study subjects should be discussed and carefully planned from the outset. In the absence of any detectable serious adverse events, it may be possible to initiate later stage trials with larger patient populations prior to completion of long-term monitoring. Such trials would require specific stopping rules that are directly linked to outcomes from ongoing early investigations.

6 Future Directions and Possibilities

An appropriate level of regulatory oversight has the potential to protect patients by minimising the risk of adverse events while also enabling scientific advancement by maintaining sufficient flexibility to support innovation. Health Canada has established, and continues to adapt, a regulatory framework that strives to meet these goals and is committed to working with sponsors from academia and industry, other regulatory authorities and other interested stakeholders to facilitate entry to the market of promising CGTPs.

There is already a process for conditional approval of drug products (leading to a NOC/c), and there will soon be in place a programme for Orphan Drugs. Although

these regulatory tools may be particularly useful for products targeting small patient populations, new regulatory paradigms may be needed in some situations. The cost of bringing a new drug product through the marketing approval process typically runs into hundreds of millions of dollars; and, to protect such an investment, industry requires a strong proprietary position and a suitable, projected, financial return. In the absence of biopharmaceutical industry sponsorship, some products/therapies with significant potential may face an unsure future with investigational status at a limited number of treatment sites. Additionally, a centralised manufacturing approach presents many challenges for the distribution of CTPs and cell-based GTPs that utilise autologous cells; and some such products may require the need for additional steps at the treatment site prior to administration that would currently constitute product manufacturing. An alternative regulatory option that requires proof of safety and efficacy demonstrated through clinical trials but then allows wider use at registered/licensed establishments committed to established procedures, and meeting appropriate standards, might be useful in many situations [33].

Finally, Health Canada is a strong proponent and active participant in efforts geared towards international regulatory harmonisation and convergence. The sharing of scientific expertise and regulatory experiences is always positive and will be especially valuable in this still developing field of endeavour, encompassing such a wide variety of products.

If you want to go fast—go alone ….
If you want to go far—go together (African Proverb)

Note: Some text on regulatory information in this chapter has been adapted from a conference report by Ridgway appearing in the Journal *Biologicals*, volume 43/5, in press.

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