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BLA 125734
Donislecel
Applicant: CellTrans, Inc.

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the Advisory Committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought the donislecel BLA to this Advisory Committee in order to gain the Committee's insights and opinions regarding the characterization and quality attributes of the proposed drug product for the proposed -treatment of "brittle type 1 diabetes. The background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the FDA for discussion by the advisory committee in the morning session of this meeting. The FDA will not issue a final determination on the issues at hand until input from the Advisory Committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the Advisory Committee meeting.

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ABBREVIATIONS

BLA	Biologics License Application
CIT	Clinical Islet Transplantation
CQA	Critical quality attribute
DTZ	Dithizone
IE	Islet Equivalent
EIN	Equivalent islet number
EIQ	Equivalent Islet Quotient same as EIN
ELISA	Enzyme-linked immunosorbent assay
FDA/PI	Fluorescein diacetate and propidium iodide
GSI	Glucose stimulation index
GSIR	Glucose-stimulated insulin release
OPTN	Organ Procurement and Transplantation Network
PP	Pancreatic polypeptide
T1D	Type 1 diabetes
UI	University of Illinois
UIH	University of Illinois Hospital and Health Sciences

1. INTRODUCTION

1.1 Product Background

CellTrans, Inc. ("the Applicant") has submitted biologics license application (BLA) 125734 seeking to market donislecel, a cellular therapy product composed of allogeneic islets of Langerhans for the treatment of "brittle type I diabetes mellitus (T1D) in adults whose symptoms are not well controlled despite intensive insulin therapy." Each lot of donislecel is manufactured from a deceased donor pancreas procured via the Organ Procurement and Transplantation Network (OPTN), and is for the treatment of one patient. Islets of Langerhans are composed of mixed populations of endocrine cells that are scattered throughout the parenchyma of the pancreas. T1D is an autoimmune disease marked by destruction of insulin-producing β cells, which leads to inadequate production of hormones in response to glucose stimulation and thus inadequate control of blood glucose levels. The proposed mechanism of action for donislecel is regulation of blood glucose levels through secretion of hormones in response to glucose stimulation.

Donislecel development at the University of Illinois Hospital and Health Sciences System (UI Health) began in 2004. The Applicant, CellTrans, acquired the rights to the donislecel development program with the purpose of supporting a BLA, but product manufacturing remains at the UI Health facility. Donislecel was granted Orphan Designation, and UI Health transferred all rights and responsibilities to CellTrans.

The manufacture of each product lot begins with acceptance of the donor pancreas at the manufacturing facility. The donor pancreas undergoes perfusion using collagenase solution, followed by a series of enzymatic and mechanical digestion steps using the Ricordi chamber [1], a sterile stainless steel chamber that facilitates mechanical tissue disruption and digestion, resulting in a (b) (4) islet preparation. The (b) (4) islet preparation containing a mix of exocrine tissues and islet cells is purified by a (b) (4) step to isolate islet fractions. The islet fractions are segregated and pooled as top, middle, and bottom fractions based on islet purity. The islets are then incubated up to 48 hours. After incubation, the islets are harvested from the cell culture, and the final suspension of islets is formulated for infusion. The final drug product is delivered into the liver via the portal vein.

1.2 Topics for Discussion

The Applicant includes purity and potency as a part of the critical quality attributes (CQAs) for donislecel. A direct correlation between a potency assay and clinical efficacy is not a regulatory requirement. Nonetheless, CQAs (potency specifically) are generally developed with the intention to demonstrate a correlation with clinical efficacy [2]. FDA's position is that the CQAs proposed by the Applicant for potency and purity do not have

a demonstrated relationship to the clinical performance of specific lots of donislecel. Without a demonstrated relationship with clinical effectiveness and/or *in vivo* potency/activity, controlling product quality through these two proposed CQAs may not be sufficient to ensure the manufacturing process consistently produces donislecel lots of acceptable safety and quality to provide the intended clinical benefit.

Each lot of donislecel is derived from one allogeneic, cadaveric donor pancreas, with each patient receiving up to three lots over the course of their treatment. Inherent variability between donor pancreata contributes to starting material variability. Thus, starting material variability of quality is one of the limitations of the manufacturing process. We ask the Committee to consider the contribution of purity, one of the CQAs, to product safety, efficacy, and manufacturing quality, and discuss whether evaluation of purity and potency is adequate to ensure that the manufacturing process will produce product lots of consistent quality. Additionally, given the limitations of the current CQAs, we ask that the Committee discuss other product characteristics of purity and/or potency not previously identified as CQAs for donislecel that might provide more meaningful measures of product quality and potency, and therefore provide better assurance of product quality from lot-to-lot.

2. REGULATORY PERSPECTIVE ON PURITY FOR CELL THERAPY PRODUCTS

A BLA may be approved on the basis of a demonstration that the biological product that is the subject of the application is “safe, pure, and potent” (42 USC 262(a)(2)(C)(i)(I)). Federal regulations provide the following definitions of purity that apply to cell therapy products:

- “Purity means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product” (21 CFR 600.3(r)).
- “Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application” (21 CFR 610.13).

These definitions allow for the presence of multiple cell types in the final product, even those that are not purported to contribute to the product’s mechanism of action. However, in general, lot release criteria are established for the cellular composition of the final formulated cellular product, including cell types that are not anticipated to have a therapeutic effect. Often, cell therapy product developers aim to identify multiple cell subsets and use certain analytical methods (e.g., immunophenotyping) to evaluate the final product composition due to the inherently heterogeneous nature of cell therapy products that are manufactured from starting material that is of complex composition, especially in cases where selection for a specific cell type is not performed in manufacturing. Additional characterization data on the cell types present in the final

product may inform decisions on specifications that limit the quantity of particular cell types to ensure product and process consistency.

Development of assays that can evaluate product purity are challenging because the cellular characteristics used for identifying these cells may not be clearly defined. For example, in the case of immunophenotyping, multiple cell types may share the same cell surface markers and, as such, in order to definitively identify the specific cell type, staining for multiple molecular attributes (e.g., cell surface markers or intracellular proteins) may be required. In cases where dyes are used to identify specific cells based on their chemical properties (such as the use of a chelating agent that relies on the presence of an ion), the dye may yield background or non-specific signal due to the presence of the target ion in other cell types, dye accumulation based on its affinity for the target ion, and cellular fluxes of ions and dyes [3].

Despite these challenges, FDA has suggested that developers of cell therapy products evaluate all the cell types present in the final product, even those that might not directly contribute to the mechanism of action. For example, for islet products, some cells could provide support during engraftment by participating in cell signaling that is important to the function of the product. In contrast, some cells may negatively impact engraftment and/or function. For these reasons, in addition to informing product and process consistency, FDA evaluates purity data to include intended and unintended cell types present in the final product [4]. Therefore, BLA applicants should include a rationale for purity and its specification.

3. REGULATORY PERSPECTIVE ON POTENCY FOR CELL THERAPY PRODUCTS

A BLA may be approved on the basis of a demonstration that the biological product that is the subject of the application is “safe, pure, and potent” (42 USC 262(a)(2)(C)(i)(I)). Federal regulations provide the following definitions of potency that apply to cell therapy products:

- “The word potency is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result” (21 CFR 600.3(s)).
- “[Potency is] the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data” (21 CFR 210.3(b)(16)(ii)).

These definitions allow for the use of laboratory tests or clinical data to demonstrate the potency of a drug product. Well-controlled clinical data can be used to demonstrate that the manufacturing process is capable of producing a potent product, and laboratory tests can be used to ensure that product potency is consistent from lot-to-lot. Laboratory tests to be used as product potency assays are most effective if they measure product

attributes that are linked to a clearly-defined mechanism of action, and/or attributes that have a demonstrated relationship with clinical efficacy.

Development of adequate potency assays, however, is challenging for cell therapy products. The *in vivo* activity of cell therapy products can be multimodal and difficult to characterize, and as a result the mechanism of action may not be clearly established. As discussed above, characterization of cell therapy products in general is complicated by the complex biological activity of cells relative to other types of drugs, as well as heterogeneity among the cells comprising the final product. Defining product quality attributes that relate to the product's clinical effectiveness, therefore, may require more extensive product characterization for cell therapy products than for other biological products. Additionally, clinical trials designed with efficacy endpoints in mind may not be adequately powered to detect association of clinical outcomes with relevant product attributes.

Notwithstanding these challenges, FDA has suggested that developers of cell therapy products may progress in two relevant ways. The first is that potency assays based on reasonable hypotheses about mechanisms of action, rather than a clearly demonstrated mechanism of action, may be adequate in some circumstances. FDA has provided guidance on potency assay development that allows a matrix approach for complex biological products, including cell therapies [2]. A matrix approach includes multiple complementary assays that measure different product attributes associated with quality, consistency, and stability [2]. The collection of assays (matrix) generally consists of a biological assay, biological and analytical assays, or analytical assays alone [2]. However, analytical potency assays may be used, if the surrogate measurement(s) of immunochemical, biochemical, and/or molecular attributes of the product can be substantiated by correlation to a relevant product-specific biological activity. Potency assays used under these conditions must be sufficiently robust in terms of reproducibility and as indicators of product quality and product stability. Furthermore, for allogeneic, cadaveric islet products, the FDA recognizes that biological assays measuring islet function are not rapid enough for use in routine lot-release testing. The FDA has encouraged developers of islet products to explore the development of rapid analytical assays that correlate to well-established biological assays, such as restoration of euglycemia in diabetic nude mice [5].

The second approach is to use clinical performance to demonstrate potency. For instance, if a product meets the primary clinical outcomes, has been extensively characterized during product development, and is produced by a well-controlled manufacturing process, these clinical data may be considered to demonstrate potency even if the mechanism of action is not completely understood. In this scenario, assays purporting to measure product attributes thought to be related to product potency must be sufficiently robust in terms of reproducibility and as indicators of product quality and stability. This approach can allow novel therapies with clearly demonstrated efficacy and well-controlled manufacturing processes to progress to licensure even if the mechanism of action and its relationship to the relevant potency assay are not completely understood. In such a case, however, when there is a need to make a change to the

established manufacturing process, demonstrating that the manufacturing change has not affected the safety, quality, and effectiveness of the product may only be possible through additional clinical studies rather than comparability studies conducted using in vitro methods.

4. DONISLECEL PRODUCT ATTRIBUTES AND CLINICAL EFFECTIVENESS

All lots of donislecel are subject to specifications designed to assure at least 30% islet purity, as assessed by dithizone (DTZ) assay. DTZ is a zinc chelating agent known to selectively stain β cells due to their high zinc content [6]. While DTZ staining is one of the commonly used methods for identifying β cells in islets, other methods, such as immunohistochemistry, can be used to identify other cell types that are present in islets [7]. In addition to measuring the product purity attribute, the Applicant relies on DTZ staining to enumerate islets (referred to as “yield” by the Applicant) as a part of the potency assay matrix. DTZ-stained islets are counted using a calibrated grid in the eyepiece of the microscope. The results of this assay are used to calculate the dose based on equivalent islet numbers (EIN), also commonly referred to islet equivalents (EIQs). Using the conversion factors established based on the average islet diameter of 150 µm, the counted number of islet cells is converted into islet equivalents and then multiplied by the dilution factor used during sampling. An islet equivalent is equal to the volume of an islet with a 150 µm diameter [8].

As noted above, a potency assay matrix is a set of complementary assays, which, taken together, measure product attributes associated with the specific ability or capacity of the product to effect a given results (e.g., therapeutic effect). Moreover, an assessment of purity can also be included in a potency assay matrix, given that purity and potency are sometimes interrelated. For example, achieving a certain level of purity for the desired cell population may be necessary to achieve the specified potency assay threshold. In this BLA, the potency assay matrix includes evaluation of insulin secretion under high glucose stimulation, as compared to low glucose stimulation (glucose stimulation index [GSI] assay), evaluation of islet viability and islet yield. A similar matrix approach has been undertaken by several groups conducting Phase 3 National Institutes of Health-sponsored Clinical Islet Transplantation (CIT) consortium trials, as illustrated in Table 1 [9].

Table 1: Purity and potency methods for islet products manufactured by CIT (Excerpt from Ricordi et al. [9]).

Test	Method
Potency: GSIR[1] (high-purity islets, pre-culture sample)	ELISA[2]
Potency: GSIR (high-purity islets, post-culture sample)	ELISA
Potency: Islet quantity	DTZ stain and microscopic examination
Potency: Viability	FDA/PI[3] stain and microscopic examination
Purity: Islet concentration	DTZ stain and microscopic examination

[1]*Glucose-stimulated insulin release*
[2]*Enzyme-linked immunosorbent assay*
[3]*Fluorescein diacetate and propidium iodide*

To control the composition of donislecel, the Applicant has implemented DTZ staining, as discussed above. This stain is specific to β cells. Islets are composed of multiple cell types, including other endocrine cells. Endocrine cells within pancreatic islets release insulin, glucagon, somatostatin, pancreatic peptide, and ghrelin, and, together, these hormones help maintain blood glucose levels within the normal range [10]. The proposed mechanism of action for donislecel is regulation of blood glucose levels through highly regulated, pulsatile secretion of multiple hormones in response to increases and decreases in blood glucose.

Given this mechanism of action, controlling the composition of the product is crucial to maintaining consistent product quality. Although use of DTZ staining (purity and EIN/IEQ), determination of viability, and evaluation of insulin secretion is consistent with the hypothesized mechanism of action of hormone-secreting activity, this approach does not evaluate the contribution of other cells present in the islets to hormone-secreting activity. For example, T1D treatment options may include standard-of-care such as insulin therapy, and less common options such as whole pancreas transplantation. Current investigational islet transplantations include autologous and allogeneic islet transplants [11], β cell-only replacement using stem cell-derived β cells or their progenitors [12], and β cells transplanted as a part of tissue-engineered

constructs [13]. The Applicant contends that in the field of autologous islet transplantation, it is very common for the final product preparation to not have undergone purification. Thus, the Applicant states, the same ratio of exocrine to endocrine tissue that is present in the pancreas is transplanted and that this has not been shown to have any negative effect on safety and efficacy. It is not clear whether the same ratio of exocrine to endocrine tissue that is present in the pancreas is maintained in donislecel because the Applicant does not evaluate the presence of other cell types.

A relationship between these *in vitro* lot release assays and the clinical effectiveness of the product has not been demonstrated. Using data from the clinical study UIH-001 and UIH-002, there were no apparent differences in the mean value of product lots given to responders or non-responders for either the potency or purity assays. Considering the available data, FDA's position is that while the CQAs identified by the Applicant and controlled in the product by *in vitro* lot release assays may have some value in assuring a consistent manufacturing process, these CQAs may not be adequate to ensure the consistent quality of the product can be provided to all patients and may not represent specific ability or capacity of the product to effect a given result (e.g., therapeutic effect).

5. PRODUCT ATTRIBUTES AND CHALLENGES FOR QUALITY ASSURANCE FOR ISLET PRODUCTS

The issue of reliable prediction of biological activity is particularly challenging for allogeneic cadaveric islet products. Evaluation of data of the Applicant's manufacturing experience revealed substantial functional (potency) and purity heterogeneity among islet lots. Furthermore, there appears to be a lack of correlation between product lot CQAs and clinical outcomes. This suggests that assays measuring quality attributes, especially those related to purity and potency, need to be robust with acceptable criteria established to ensure a consistent, quality product.

Purity:

From the perspective of assuring islet purity, the Applicant's specification of ≥30% islet purity using a stain specific to β cells permits 70% non-β cells. Owing to the technical limitations of DTZ staining, purity may be overestimated. Kitzmann et al., [14] summarized overestimation of purity by DTZ staining as follows: "Purity has been traditionally measured by visual estimation of preparations stained with DTZ, and this test gave 20%-30% erroneously higher values on average compared with more rigorous assessments of islet preparation fractions, such as electron microscopy or laser scanning cytometry……[W]hen comparing islet purity assessed by DTZ staining with results that use immunostaining to quantify total endocrine cellular composition, the DTZ-based purity assessment gave significantly higher results than those indicated by the endocrine immunostaining." The Applicant acknowledged that they cannot rule out overestimating the percent purity of the final product using the current method. The

Applicant states that if the purity percentage is overestimated, then the dosages they require could also be higher than the actual islet number; thus, the “higher” islet number required for transplant would off-set the potential overestimation. Nonetheless, the accuracy of the DTZ method remains a question, because it is not clear whether the overestimation is by a constant factor, since the above-cited study showed a range of overestimation.

Despite the factors that contribute to potentially “off-setting” the overestimation, the Applicant does not assure that the cells present in the final product are present in similar ratios as in a healthy human islet. As reviewed by Da Silva Xavier [10], a human islet consists of 30% α cells, 60% β cells, with the remainder 10% made up of δ cells, PP cells, and ε cells. The Applicant evaluates islet purity using a stain specific to β cells, but does not evaluate the presence of other (e.g., endocrine, exocrine, or stromal) cells and contends that the exocrine tissue present in the final product has not been shown to have any negative effect on product safety or efficacy. Preservation of similar ratios as in a healthy human islet may not be feasible given that the Applicant may pool fractions of different purities. The outstanding question is whether preservation of a certain ratio of different cell types in the final product is important, since scientific literature suggests that cell types other than β cells contribute to in vivo activity, as discussed below.

Animal studies evaluated the contribution of endocrine cells to graft outcomes. For instance, rats receiving mixed endocrine cell grafts (i.e., β cells with endocrine non-β cells) maintained longer-term metabolic control, as compared to rats receiving pure β cell grafts [15], while other studies showed that “exocrine tissue contamination” is deleterious to re-vascularization of islets in hamsters [16]. In mice, β cell–enriched aggregates can effectively reverse hyperglycemia and non-β cells are lost after transplantation, suggesting that non–β-cells are not essential for successful islet transplantation [17]. As reviewed by Habener and Stanojevic [18], there is scientific evidence that injured β cells might activate α cells in adult islets to promote β cell regeneration and trans-differentiation of pro-α cells into β cells is a theoretical approach to therapeutic β cell regeneration. These studies suggest that there may be a role for transplanted α cells in sustaining the transplanted β cells.

Finally, stromal cells, such as endothelial cells, may play a role in graft function [19] by providing signals that promote revascularization. For example, recent murine studies showed that angiogenic signaling provided by cilia on endothelial cells regulates islet vascularization, further building on the work demonstrating that cilia on endothelial cells regulate insulin secretion [20]. Therefore, the current literature suggests that preservation of native islet structure and relative proportions of cell types present in the islet is important to successful transplantation outcomes.

Potency:

Although the quality attributes used by the Applicant are consistent with the hormone-producing properties of β cell, these attributes may not fully capture crucial biological heterogeneity in islets. As reviewed by Papas et al. [21], several types of viability and potency assays have been developed, but many of them have limitations. For example,

the evaluation of three-dimensional islets in two dimensional planes (microscopic evaluation) using DTZ staining contributes to error both for purity and EIN determinations [21]. Moreover, the sample may not be representative of the whole preparation, while the contribution of other tissues and cell types cannot be ascertained if a method relies on evaluation of a single cell type, as in the case of DTZ staining [21]. A potency assay evaluating glucose-stimulated insulin secretion (i.e., GSI assay) has not been shown to correlate with clinical outcomes [21]. In animal studies, assays aimed at evaluating mitochondrial function (adenosine diphosphate to adenosine triphosphate ratios) have been shown to correlate with transplantation outcomes [22]. Another marker associated with mitochondrial function – glucose-stimulated oxygen consumption rate – has been shown to reflect islet quality with a potential for predictive power and reproducibility [23]. Such methods may not capture islet viability and may not differentiate between mitochondrial function of other cells in the islet preparation. Nonetheless, such assays appear to be important for quality assessment. Despite the choice of a potential potency assay, from sampling perspective, obtaining a sample from a suspension that is representative of the whole preparation is critical [21, 24].

CQAs:

While it is not established how the specific *in vitro* assays discussed above relate to the *in vivo* activity of donislecel, it is possible that the CQAs used by the Applicant and other sponsors clinical trials of investigational cadaveric islet products are not capable of detecting biological heterogeneity arising from variability related to allogeneic donor-specific differences and islet processing methods. In turn, the heterogeneity of islet preparations may also be related to the lack of correlation between the purity and potency assays and clinical outcomes.

As stated above, FDA's position is that the analytical methods used for product characterization of donislecel do not have a demonstrated relationship with clinical outcomes. Given the limitations of the current CQAs, and the state of knowledge in the field of islet transplantation, we ask the Committee to discuss other product characteristics not previously identified as CQAs for donislecel that might provide more meaningful measures of product purity, quality and potency. We ask the Committee to consider the product attributes identified by the Applicant as CQAs and discuss whether they are adequate to ensure that the manufacturing process will produce lots of consistent quality.

6. DRAFT POINTS FOR DISCUSSION BY THE ADVISORY COMMITTEE

CMC Discussion Question #1
The presumed mode of action, as proposed by the Applicant, is the "secretion of multiple hormones in response to increases and decreases in blood glucose." Each islet contains various cell types (endocrine [beta cells, alpha, gamma, delta, epsilon, PP cells], exocrine, and other cells) that may contribute to the activity of the transplanted cells. Product specifications allow for up to 70% of non-β cells (i.e., DTZ-negative cells) and a range in islet purity (mean purity: 53%; range: 30-90% for product manufactured in Phase 3 (UIH002) clinical studies). The islet yield ascertained by this method is also used as one of the measures of product potency, but this method provides no information on the presence of non-β cells in the transplanted islets.

a) What is the contribution of endocrine, exocrine, or other cell types expected to be in the final drug product to the clinical outcomes and product potency?
b) How might the relative proportions of endocrine, exocrine, or other cell types in the product play a role in clinical outcomes and product potency?
c) What are the specific types of non-β cells that the Applicant should characterize and/or, possibly, control for in the product?

CMC Discussion Question #2:
Each dose of donislecel is derived from one deceased donor pancreas. Patients are expected to receive up to three doses to attain the suggested clinical outcomes. Based on data provided from clinical trials, the critical quality attributes are highly variable, and it is not clear how the Applicant achieves lot-to-lot manufacturing consistency in terms of islet purity and potency. As outlined above, product purity is assessed by DTZ staining. Product potency evaluates glucose stimulation index (GSI), islet yield (DTZ staining), and viability.

From the scientific perspective, are the product quality attributes of purity and potency sufficient to evaluate lot-to-lot consistency in manufacturing, product quality, and product strength? If not, what additional product characteristics, not previously identified as CQAs for donislecel, would provide more meaningful measures to assess lot-to-lot product consistency?

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