

BACKGROUND INFORMATION FOR ALLOGENEIC ISLET MANUFACTURE

Purpose: Provide background information and context for FDA's regulatory concerns about different stages of the manufacturing process for allogeneic islets, which encompass pancreas procurement, processing and product characterization.

Specific FDA questions regarding manufacture of islets are provided at the end of this background information.

Pancreas Procurement

▪ **Methods of harvesting and handling**

Historical data from the International Islet Transplant Registry (ITR) and published sources indicate that certain practices in pancreas procurement, handling and organ allotment, are detrimental for preparation of suitable islet preparations. FDA is concerned that these practices may result in the use of a "substandard" pancreata resulting in "substandard" and inconsistent islet preparations. To ensure high quality islet preparations can be consistently made, FDA believes only the highest quality pancreata should be used.

Pancreas Processing

▪ **Control and consistency/reproducibility of manufacture**

Pancreas age, size, duration and conditions used for dissociation will impact islet yield, size distribution and viability. If the manufacturing process is not controlled, it will be difficult to consistently produce an equivalent product from lot to lot and consequently make it difficult to identify the critical parameters necessary to ensure the desired clinical effect.

Demonstration of control in islet processing

Data available from the ITR clearly indicates that producing high quality islet preparations consistently is a complex process which requires considerable expertise. In such situations, FDA frequently requests that sponsors of new INDs or new clinical manufacturing sites provide data demonstrating that the therapeutic product can be consistently prepared. This is usually accomplished by accumulating data from several non-clinical production runs, which shows that the product is of clinical grade; i.e. the manufacturing process is controlled, consistent and meets specifications for release.

Islet Characterization

▪ **Identity, Purity, Potency, Viability, Others**

Like any cellular and tissue-based product, you must be able to demonstrate that you can safely and reproducibly manufacture the therapeutic product. This is typically done by characterizing the product and establishing specifications for lot release. Lot release specifications for cell and tissue-based therapies includes demonstration of safety and assessments of several product characteristics such as identity, purity, viability and potency as well as others. This data is important in order to evaluate the manufacturing process and consistency of the product lots.

Identity

An islet equivalent (IE) is defined based on both insulin content and morphology/size. An insulin granule binding dye, such as diphenylthiocarbazone (DTZ) is commonly used to identify beta cells. Since beta cells are only one of several other cell types needed to constitute an islet, a morphological assessment, based upon a mean diameter of 150 um, is used in addition to staining by DTZ, to define an islet equivalent. However, at least two other cell types are found in islets; alpha cells, which secrete glucagon, and delta cells which secrete somatostatin. Should measurements of these molecules also be made? FDA recommends that lot release specifications for cellular and tissue-based therapies for identity include assessments to identify the specific *therapeutic* cells or tissues.

Purity - composition of islet preparations

Historical data in the ITR shows that functional transplants of islets have ranged in purity from <5% to >95%. For cell and tissue-based therapies, FDA recommends that lot release specifications for purity encompass quantitative measurements of *therapeutic* cells or tissues which are both viable and functional. It should also include measurements of "other" cell types including, non-viable cells which may be beneficial or detrimental to patients. For islet preparations, one example of "other" cells are exocrine cells which secrete hydrolytic enzymes. Most cell and tissue-based therapies, are complex mixtures of both desirable cell types and other impurities, typically other cells types which may be beneficial or detrimental to the patient. Assuming these impurities are not harmful to the patient, FDA frequently requests that investigators monitor the impurity profile as an additional means of demonstrating that the clinical product can be manufactured consistently from lot to lot. Significant variations in the impurities, from one lot to the next, can serve as a useful indicator that a manufacturing process is not controlled or consistent .

Potency

A suitable potency assay is one that demonstrates that the clinical product possesses the specific ability to give the desired clinical effect. To this end, investigators in islet transplantation use assays to measure the function of islets that possibly could be developed into lot release assays for potency. For example, in the glucose stimulated insulin release assays, islets *in vitro* are exposed to glucose and any insulin secreted as a result, is quantified. A more time-consuming functional assay involves implanting islets

into diabetic, immunodeficient mice to effect a cure, which can take several weeks. If investigators could validate these assays to demonstrate that a given number of islet equivalents will release a given amount of insulin *in vivo*, or some other suitable measure, one of these assays could potentially serve as a potency assay

Viability, number and size distribution of islets

There is no historical data available in the International Islet Transplant Registry (ITR) with regard to the viability of islet preparations, though it is clearly an important parameter for any cellular and tissue based therapy. For cell or tissue-based therapies, FDA recommends that initial, lot release specifications for viability of the *therapeutic* cells or tissues be established at 70%.

In addition, historical data in the ITR and published reports indicate that a minimum of 6,000 IE/kg need to be transplanted to increase the likelihood of a functional graft. There does not appear to be any conclusive data correlating the number of transplanted islet equivalents with those that actually engraft post-transplant. FDA recommends that for cellular and tissue-based therapies, lot release specifications for cell number include a specification for the minimum number of viable and functional cells necessary to confer the desired therapeutic effect.

Also, as a result of differences between donor pancreata, measurements of the size distribution of the islets obtained from each preparation are often made, since it is likely that this will be variable and could have an impact on engraftment, survival or function of the graft post transplant

Other issues

Multi-donor islet transplants

In general, FDA guidelines discourage the pooling of tissues from more than one donor. This is due in part to concerns about increasing the risk of transmission of adventitious agents to recipients, issues of immunogenicity and manufacturing concerns such as differences in potency and purity of islets from different pancreata. To minimize the concerns mentioned above, FDA recommends that islets from each donor pancreas should be processed apart from one another and meet all lot release specifications, with the exception of minimum number of IE.

Draft of Pancreatic Islet Cell CMC Questions (3-10-2000)

1. Organ Quality - Source Material for Islets

Based on data in the International Islet Transplant Registry (ITR), most investigators recommend that in addition to standard infectious disease screening, pancreata be excluded from use for clinical preparations of islets based on the following:

Donor age : <14 or > 60 years
Warm ischemia > 15 min
Cold ischemia > 8 hours
Presence of infection or malignancy
Methanol or carbon monoxide toxicity
History of diabetes
Serum lipase >500

- a. Please discuss and provide recommendations regarding the appropriateness of each of these exclusion criteria. For example, are restrictions on minimum and maximum donor age appropriate?
- b. Are there other diseases or conditions that should also be exclusionary?
- c. Are there other appropriate serum markers in addition to, or as an alternative to serum lipase (serum amylase, for example)?

2. Appropriate types of identity testing

An islet equivalent (IE) is defined based on both insulin content and morphology/size. However, a beta cell is only one of several other cell types needed to constitute an islet. For example, at least two other cell types are found in islets; alpha cells, which secrete glucagon, and delta cells which secrete somatostatin. Should measurements of these molecules also be made?

- a. Please discuss if additional assessments should be used to identify an islet equivalent.
- b. Please discuss whether assessment of these other cell types would be important in determining the quality of the product.

3. Viability, Number and Size distribution of Islet Preparations

There are no data available in the International Islet Transplant Registry (ITR) with regard to the viability of islet preparations, those this is a critical parameter for any cellular or tissue based therapy. In addition, data in the ITR and published reports indicate that a minimum of 6,000 IE/kg need to be transplanted to increase the likelihood of a functional graft. Also, islets from different donor pancreata may have different size distributions.

- a. Please discuss and provide recommendations for appropriate measures of viability for islets.
- b. Does the BRMAC recommend that in the absence of data supporting a lower viability specification, that an initial lot release specification for viability of 70% for islets is appropriate?
- c. For a single donor, allogeneic islet transplant, what recommendations does the BRMAC have regarding the minimum number viable and functional islet equivalents?
- d. Does the BRMAC have recommendations regarding the maximum dose of IE that should not be exceeded for portal vein infusions?
- e. Please discuss and provide recommendations about whether assessments should also include the size distribution of the IE to be infused? If so, is there a targeted standard size distribution for IE?

4. Purity - Composition of Islet Preparations

Historical data in the ITR reveals that functional transplants of islets ranged in purity from <5% to >95%.

- a. Please discuss the types of assessment that should be performed to determine the purity of islet preparations.
- b. Does the BRMAC have recommendations for the minimum acceptable purity level for islet preparations for clinical use?
- c. Please discuss and provide recommendations for monitoring the impurity profiles of "other" cell types in islet preparations?

5. Potency

A suitable potency assay is one that demonstrates that the clinical product possesses the specific ability to give the desired clinical effect.

- a. Please discuss and provide recommendations for suitable potency assays that are predictive of functional islets in humans.
- b. Please discuss and provide recommendations for the types of potency assays that can be performed prior to patient administration.
- c. Please discuss assays for measuring continued islet function in the patient, post-transplant.
- d. What recommendations does the BRMAC have for qualifying potency assays as they relate to clinical measures of islet function?

6. Demonstration of Control in Islet Processing

Data available from the ITR clearly indicate that producing high quality islet preparations consistently is a complex process which requires considerable expertise.

- a. Please discuss the need for investigators to demonstrate that high quality islet preparations can be consistently made prior to initiating clinical research studies in humans.
- b. Does the BRMAC have any recommendations about the types of data necessary for demonstration of adequate processing control of islets?

7. Multi-donor islet transplants

In general, FDA guidelines discourage the pooling of tissues from more than one donor.

- a. Please discuss pooling of islets from multi-donors for use in a single recipient. For example, if a given pancreas failed to yield a sufficient number of viable, functional IE, would it be appropriate to pool preparations from one or more other preparations to obtain the desired number.
- b. Please discuss appropriate time frames for completion of all infusions in situations where a recipient may receive islets from multiple donors, sequentially.