JDRF REQUESTS EXPRESSIONS OF INTEREST FOR:

THE DEVELOPMENT OF TOOLS AND TECHNOLOGIES FOR SITE-TARGETED DELIVERY OF THERAPEUTICS TO PANCREATIC ISLET ENDOCRINE CELL TYPES- TARGET IDENTIFICATION AND VALIDATION

BACKGROUND & PURPOSE
The ability to target delivery of small molecules, cellular therapeutics, or imaging agents specifically to cell types within the pancreatic islet would be an enabling technology with a major impact on translational diabetes research. For example, research to develop interventional strategies to recapitulate pancreatic beta cell development and maturation in the adult, promote beta-cell replication, block apoptosis, or develop tolerance against autoimmune attack often employ pathways and targets that are not unique to the pancreatic islet. The therapeutic strategy of reprogramming other endocrine cell types to become beta cells would require specific delivery of agents to other cell types. The ability to restrict activity to the site affected in diabetes could potentially broaden the list of viable interventional strategies, whether through therapeutic mechanism of action (e.g., prodrugs, local functionality), delivery to specific cell types, or a combination thereof. Likewise, the development of approaches to non-invasively quantify beta cell mass, function, and health would be accelerated by the identification of specific beta cell ligands for imaging studies.

Site-targeted delivery for T1D is a long term, high-risk, high-reward goal. Without a specific therapeutic candidate currently with which to develop a customized solution, JDRF will cast a wide net for the identification and validation of specific targets on cells in the islet. JDRF plans to encourage efforts towards this goal by supporting early-stage programs to identify and validate potential islet cell specific targets with the understanding that the knowledge gained through funded programs will be creatively applied to other aspects of diabetology.

While this RFA will support discovery research towards site-targeted delivery, the interim goal of identification of islet cell-specific proteins is not novel; applications must take previous work in the field into consideration and highlight improvements over previous strategies. The following guidance may be helpful in planning your proposal:

Tissue selection The quality and purity of starting tissue will be essential to success. Starting with isolated primary human islet cell types would be ideal. Primary human islets are by nature unpredictable in supply, expensive, and precious; a relationship with a reliable program or collaboration with an existing transplantation program will be essential. Human pancreatic cell lines

Note that citations are meant as examples and not intended to be comprehensive


3 NIH/NIIDK supported IIDP program http://iidp.coh.org/
http://www.jdrf.org/files/General_Files/Get_Involved/Website_announcement_for_islets_for_research.pdf
are a viable option; understanding their biology, limitations, and the differences, if any, from primary human islet cells will be important in the development of the program\(^4\). Preference will be given for proposals utilizing human tissue, as there are known differences in the endocrine cell characteristics and architecture of rodent, pig, primate, and human islets. However, applications utilizing non-human models will be considered if there is a compelling rationale for their use and a plan for extension of findings into human studies.

**Islet biology expertise** Human islets are notoriously difficult to culture once isolated. Applicants who have not worked with human islet biology previously are strongly encouraged to seek collaborators. JDRF can provide assistance with introductions if desired.

**Disease modeling** While no in vitro model of disease can completely recapitulate physiological complexity, previous studies of “normal” vs. “diseased” islets have utilized cytokine stimulation. Different surface molecules will be expressed in different situations (e.g. nutrient, hormonal, disease states) and some discussion of this should be included. The identification of pathological conditions that will particularly benefit from this active targeting strategy is a consideration.

**Islet targeting** The endocrine cells of the islet are intimately connected with a specialized vascular bed and set of autonomic nerves. Approaches for endocrine cell-specific targeting might take advantage of the unique features of the islet vasculature\(^5\). Likewise, aspects of the nervous system of the islet may be considered for a targeting strategy, though it should be noted that amyloid deposition has not been correlated with the natural progression of Type 1 Diabetes and would not be a priority for development under this RFA\(^6\). The cell-specific targeting of islets to be transplanted is outside the scope of this RFA.

**Previous screens** No “optimal” beta-cell specific cell surface molecule has been identified for the development of agents to non-invasively image beta cell mass and function, though many groups have performed screens towards this goal. Previous approaches include antibody development against islets\(^7\), phage display\(^8\), and aptamer, small molecule, proteomics, and metabolomics screens. As the parameters for hit selection differ depending upon the desired utility, it may be worthwhile to revisit existing data from previous screens. For novel untargeted screen proposals, knowledge of islet biology, including islet relationships with the neural and vascular systems, should inform library selection and the assessment of potential targets. A description of how proposed screens differ from those previously performed should be included in the application.

**Known candidates** Applications proposing work with known candidates will require islet biology expertise. For beta-cell targeting efforts, applicants are advised to consider publications assessing various targets and candidate agents for the development of non-invasive imaging modalities for the

\(^{4}\) A genetically engineered human pancreatic β cell line exhibiting glucose-inducible insulin secretion
assessment of beta-cell mass for background on the range of acceptable binding kinetics to the beta cell surface. Targeting to other endocrine cell types should likewise consider potential binding criteria. Additionally, many specific candidate cell-surface proteins have been investigated, including their cell localization, internalization, and changes in expression during development, physiology, and disease. Endocrine/exocrine volume markers, and exocrine dysfunction i.e chronic pancreatitis might also be considered. The use of targeted long-circulating nanoparticles may be part of this strategy.

JDRF convened a workshop in November 2011 to bring together key opinion leaders from academia and industry to survey the current landscape and brainstorm approaches for the future. Summary recommendations are provided in an appendix for consideration in planning your applications.

OBJECTIVES
JDRF is soliciting Expressions of Interest (EOI)s for projects addressing the need for validated islet endocrine cell specific targets that could ultimately be useful in the development of cell site-targeted delivery for therapeutics or imaging.

To achieve this, JDRF is soliciting EOIs for proposals addressing aspects of the following:

- Unbiased identification and validation of islet-cell specific targets for therapeutic delivery, including, but not limited to,  
  o Genomics  
  o Proteomics  
  o Antibody screens  
  o Aptamer screens  
  o Peptoid screens

The EOIs must address the rationale behind the screen as well as the approach that will be taken to prioritize potential hits. The novel analysis of existing datasets will also be considered under this call.

- Further confirmation of suitability of known candidate islet endocrine-cell specific targets with in vitro and animal models of diabetes (i.e. physiology, pathology, impact of binding/perturbations on beta cell/islet/pancreas biology.

Pancreatic islet biology expertise or collaborations with appropriate scientists is required. JDRF can provide assistance with introductions.

The following topics are outside the scope of this RFA:

- Combining potential islet cell site specific targeting with either small molecules of interest or potential cell therapeutics. Note that the goal of applications to the present RFA should be to have one or more potential approaches ready for this transition by the end of the granting period.

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Advances in molecular imaging of pancreatic beta cells
Mai Lin et al Frontiers in Bioscience 13, 4558-4575, May 1, 2008


Wu Z, Kandeel F.
The development of strategies for targeted delivery to cells or tissues in the immune system
Cell-specificity of action via the development of prodrugs and/or local functionality

Applicants are strongly encouraged to use human tissue and/or cells for initial screening and validation. These resources may be helpful:
- In the USA: Integrated Islet Distribution Program (IIDP) http://iidp.coh.org/
- In Europe: JDRF-supported islets for basic research program
  http://www.jdrf.org/files/General_Files/Get_Involved/Website_announcement_for_islets_for_research.pdf
- JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD), http://www.jdrfnpod.org/
  (Note that permission will be required to access samples)

ELIGIBILITY
Applicants must hold an M.D., D.M.D., D.V.M., Ph.D., or equivalent academic degree and a faculty position or equivalent at a college, university, medical school, or comparable institution.

Applications may be submitted by domestic or foreign, public or private, non-profit or for-profit organizations. There are no citizenship requirements.

LEVELS OF FUNDING AND GRANT MECHANISM
Projects may request up to $250,000 USD per year for 3 years.
- Note that the third year of funding will be dependent upon the achievement of predetermined milestones by the end of year 2.

Indirect costs may not exceed 10% of the direct costs.

In the full application. applicants must provide:
- Projected timelines on a quarterly basis for each specific aim
- Projected deliverables for each year

These will be reviewed and may be modified as work progresses during the course of the research program in discussion with the JDRF Program Scientist.

JDRF ADVISORY PANEL
JDRF will convene a panel to provide a feedback on the overall program and individual funded projects in year 2 under CDA.

EXPRESSIONS OF INTEREST
EOIs should adhere to instructions printed in the template, which may be accessed on proposalCENTRAL. It should include, where applicable, the following information:
- Name, title, institution, and contact information of principal investigator, co-investigator and / or key collaborator
- Identification of team members with necessary beta cell biology expertise
- Name and participation level of any subcontractors
- Brief details of approach proposed, including rationale and references to published or preliminary data (preliminary data need not be presented in detail) (3 page limit)
- Short and long-term development goals set forth as milestones, as well deliverables
- Biosketch for each PI and co-PI
- Total estimated budget and project duration (up to 3 years)
EOIs will be selected based on scientific merit and programmatic priorities. After review by the JDRF Scientific team, selected EOIs will be invited for full application submission. EOIs are accepted on a rolling basis up until the deadline (30 August, 2012). EOI submission prior to the deadline is highly recommended to give JDRF opportunity to give feedback and time for EOI revision.

An approved EOI is required prior for submission of a full proposal. Please see below for complete instructions.

**DEADLINES**
- **RFA Release Date**: 9 May 2012
- **Expression of Interest Deadline**: 30 August 2012
- **Application Deadline**: 16 November 2012
- **Response to Applicants**: March 2013
- **Earliest Anticipated Start Date**: April 2013

**INSTRUCTIONS**
Applicants must register as an applicant and submit both their EOI and application using the templates available at JDRF's on-line application system proposalCENTRAL (https://proposalcentral.altum.com/). The completed templates are to be submitted via the on-line application system.

Both academic and industry applicants are welcome. However, the indirect costs must not exceed 10% of the total direct costs. Applicants are strongly advised to consult with JDRF Program Scientist by 1 August, 2012 to discuss the responsiveness of their proposal to this program. Enquiries in this area should be referred to Adrianne Wong, Ph.D., awong@jdrf.org, +1-212-479-7642.

**CONTACTS**

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✉ pcsupport@altum.com
☎ (301)-916-4557 ext. 227, or toll free in the US, (800)-875-2562 ext. 227
Assistance can be obtained Monday through Friday between 8:30am and 5pm U.S. Eastern Time
APPENDIX I: WORKSHOP SUMMARY

Strategic considerations for the development of site-targeted delivery

- Delivery of therapeutics vs. imaging may have differing requirements.
- Specifically, therapeutic interventions might be small molecule or cellular, and contrast agent could be developed for assessing beta cell mass, function, inflammation, or apoptosis.
- The target might be beta cells, islets, or inflammation. Properties thereof might be an intrinsic (beta-cell mass, function, status) characteristic, or an extrinsic process (e.g. apoptosis, inflammation) that affects beta-cells. A discussion of cell surface vs. intracellular targets concluded that binding to the cell surface would be easier, and binding to the cell surface and subsequent internalization may be desirable for drug delivery. In addition, stoichiometric (receptor binding) vs. catalytic (enzymatic conversion) (small molecule) issues were discussed.
- The disease process will affect physiology, pathology, and the “context” of beta-cell/islet.
- The duration of treatment, dependent upon efficacy and safety, would affect the overall strategy, as would the
- Delivery logistics and the size of the modality.
- Combination of multiple approaches for therapeutics delivery/imaging with different strengths is more feasible than a single approach encompassing all desirable characteristics
- Both starting with an existing target and building it to fit a specific utility and build a program by starting with the therapeutic goals to provide the principles that guide targeting strategies have merit given the early state of the field.

Scientific considerations for the development of site-targeted delivery

- Define requirements for goal: What measurements to use to predict utility?; “Must-have” vs. “nice to have”; Do any existing reagents fulfill criteria?
- Considerations for imaging: Cell number/mass may not be indicative of cell function; How might newly developed technologies represent an improvement over currently utilized biomarkers?; Do not want to disturb biology of beta-cell or of immune system (including immunogenicity)
- Considerations for targeting for therapeutic delivery: Drug kinetics and specificity to/for target cell; Do you need to target every cell or is a subset good enough?; May be potential for multiplexing
- Not enough is currently known about the biology of the beta-cell during the disease processes: Expression levels, especially in human islets/beta cells need further exploration

Session 1: The search for beta-cell specific ligands

Unbiased approach to identify targets on beta cell

- Genomics screening and database search
  - What are functions of these targets?
  - Expression by other tissues
  - Screening methods
    - cell surface vs. intracellular targets
    - Approaches to avoid selection of non-specific probes
    - Can bias towards internalization e.g. NLS
  - Probe size limitations
  - Functional testing for outcome measurements

- Cell lines vs. primary islets
  - With the caveat of avoiding growth and metabolism, cell lines are indeed useful and meet practical demands of experiments
  - Iterative process shifting between in vivo and in vitro testing to select compounds – panning should be on human islets
New human beta cell line may be promising
Recommendation to start with primary tissue (not cells, unless hESC-derived progenitors)

**Session 2: Lessons from imaging studies**

**Nanoparticle imaging/delivery**
- Take advantage of increased permeability of capillaries – nonspecific delivery for inflammation, insulitis
  - Nano-containers can be functionalized with beta-cell specific ligands
  - Size of fenestrations in pancreatic islets, increase in size in disease

**Requirements for a successful beta cell contrast agent**
- High specificity/target affinity, sensitivity affinity and stability, rapid blood clearance, high tissue uptake, should not be phagocytosed, should not adversely affect function of beta-cell
- Developing contrast agents to measure b cell function non-invasively
  - May be able to use b cell imaging to monitor outcomes of intervention trials
- Toxicity/radioactivity of imaging agents should be considered
  - Can we redose for longitudinal studies, especially in children?

**Findings from imaging studies, considerations**
- Large inter-individual variability in beta-cell imaging signal – attributable to efficiency of binding to beta-cell/islet? Or detection of contrast agent?

**Session 3 - Modalities**
- New technologies for imaging and sensors
  - Second harmonic generation, avoiding limitations of fluorescence
  - OCT useful for vascular imaging up to 4mm depth
  - Advantage of MR + PET
  - Need to address quantitative detection (vs. resolution) and cost control
- IC2 diabodies targeting sphingomylin
  - Modification of IgM for enhanced labeling in pancreas vs. other organs in vivo and in vitro
  - Nanoparticles with IC2 diabodies for imaging and delivery
  - Fluorescence vs. luciferase for in vivo imaging
- PET preferred over SPECT for resolution and quantitation
  - Best PET agents now may be 18F-FB-DTBZ and IC2
  - DTBZ still need BCM correlation
  - IC2 requires modification (above)
- Cerenkov imaging – may be useful in the future
- Safety of repeated PET in T1D pts – once per year may be acceptable
  - Internalization – how do you know whether/how to get stuff into beta cell?
- Multistep specificity possible