General method of intrahepatic islet transplantation

Robertson RP. Diabetes 2010;59:1285-1291
## Indications for islet transplant

### Table 1. Indications for islet transplantation

<table>
<thead>
<tr>
<th>Kind of transplantation</th>
<th>Indications</th>
</tr>
</thead>
</table>
| Islet transplantation alone   | Type 1 diabetes, duration > 5 years  
Age > 18 years, weight < 90 kg, insulin requirement < 1.0 U/kg/day  
Absence of malignancy or untreated infection  
Ability to comply with immunosuppression and close follow-up  
Refractory hypoglycemia or lability despite:  
1. Optimal intensive insulin or insulin pump with appropriate monitoring  
2. Supervision by a diabetologist or endocrinologist  
3. Increased hypoglycemic risk, evidenced by at least one of the following criteria:  
   i) Clarke score ≥ 4  
   ii) HYPO score ≥ 1000  
   iii) Lability index (LI) ≥ 400  
   iv) Combined HYPO ≥ 400 and LI ≥ 300 |
| (ITA)                          |                                                                                                                                           |
| Islet after kidney (IAK)       | Type 1 diabetes, successful prior renal allograft  
Tolerating maintenance immunosuppression  
Prednisone ≤ 5 mg/day  
Absence of BK virus, or other active opportunistic infection  
Non-sensitized (PRA < 20%) |
| transplantation                |                                                                                                                                           |

Clarke score (0 = no hypoglycemia, ≥4 = hypoglycemia unawareness), HYPO score is composite measure based on 4 wks of records and a year historical review (median was 143, 25th to 75th interquartile range 46-423, and the 90th centile 1047. The lability index is based on changes in glucose levels over time – 4wks.

<table>
<thead>
<tr>
<th></th>
<th>Islet Transplant Alone (ITA)</th>
<th>Islet After Kidney or Simultaneous Islet-Kidney (IAK/SIK)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>North America</td>
</tr>
<tr>
<td>Recipients</td>
<td>686</td>
<td>461</td>
</tr>
<tr>
<td>Infusions</td>
<td>1,356</td>
<td>879</td>
</tr>
<tr>
<td>Donors</td>
<td>1,785</td>
<td>944</td>
</tr>
</tbody>
</table>
Comparison of rates of progress of alloislet transplantation versus pancreas transplantation as therapy for type 1 diabetes.

R. Paul Robertson Diabetes 2010;59:1285-1291
Islet transplant centers with 5-year insulin independence rates

<table>
<thead>
<tr>
<th>Center</th>
<th>Approach</th>
<th>5-year rate</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>Anti-CD3 + etanercept</td>
<td>70% (at 7 yr)*</td>
<td>2011</td>
</tr>
<tr>
<td>Minnesota CITR</td>
<td>T cell depletion + anti-TNF</td>
<td>50%</td>
<td>2012</td>
</tr>
<tr>
<td>Edmonton</td>
<td>Alemtuzumab + Tac + MMF + anakinra + etanercept</td>
<td>60%</td>
<td>2012</td>
</tr>
<tr>
<td>UCSF</td>
<td>ATG + efalizumab/belatacept+SRL or MMF</td>
<td>80% (at 4 yr)*</td>
<td>2012</td>
</tr>
<tr>
<td>UIC</td>
<td>Tac/SRL or MMF + exenatide + etanercept</td>
<td>60%*</td>
<td>2012</td>
</tr>
<tr>
<td>Lille, France</td>
<td>Tac/SRL</td>
<td>50%*</td>
<td>2012</td>
</tr>
<tr>
<td>Geneva, GRACIL</td>
<td>ATG + Tac/SRL</td>
<td>50%*</td>
<td>2012</td>
</tr>
</tbody>
</table>

Islet transplant centers utilizing single-donor islet protocols

<table>
<thead>
<tr>
<th>Center</th>
<th>Approach</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>Anti-CD3 + etanercept</td>
<td>2005</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Edmonton-like</td>
<td>2003</td>
</tr>
<tr>
<td>Emory</td>
<td>Efalizumab + MMF</td>
<td>2010</td>
</tr>
<tr>
<td>San Francisco</td>
<td>ATG + efalizumab + SRL or MMF</td>
<td>2010</td>
</tr>
<tr>
<td>San Francisco</td>
<td>ATG + belatacept + SRL or MMF</td>
<td>2010</td>
</tr>
<tr>
<td>Edmonton</td>
<td>Peritransplant insulin + heparin</td>
<td>2010</td>
</tr>
<tr>
<td>Kyoto</td>
<td>Living donor islet transplant</td>
<td>2005</td>
</tr>
<tr>
<td>Baylor</td>
<td>ATG + anakinra + etanercept</td>
<td>2011</td>
</tr>
<tr>
<td>Vancouver</td>
<td>Exenatide</td>
<td>2007</td>
</tr>
<tr>
<td>Miami</td>
<td>Exenatide</td>
<td>2009</td>
</tr>
<tr>
<td>UIC</td>
<td>Exenatide</td>
<td>2008</td>
</tr>
</tbody>
</table>

COH-002 Blood Glucose
Pre-Transplant → 1 Year Post ITA
(Tx#1 April 2004, Tx#2 June 2004)

Pre | Post 1st Tx. | First Year Post 2nd Islet Transplant
--- | --- | ---

TP 1 (Hu# 501); 322k IEQs
cumulative = 6127 IEQ/Kg

TP 2 (Hu# 509); 230k IEQs
cumulative = 11010 IEQ/Kg

Blood glucose (mg/dl)

Date


0.005 Hypo events/day (1.8/year)

Blood Glucose ▲ Hypoglycemic events
Glucose 1-mo POST
3rd Islet Transplant

Sensor Data (mg/dL)

<table>
<thead>
<tr>
<th>Tue Sep 1</th>
<th>Wed Sep 2</th>
<th>Thu Sep 3</th>
<th>Fri Sep 4</th>
<th>Average</th>
</tr>
</thead>
</table>

Duration Distribution (hh:mm)

- Above 140: 11:50 (17%)
- Within (70 - 140): 59:35 (83%)
- Below 70: 0:00 (0%)
ITA Patient COH-016: Example of long-term benefits of islet transplant

- Insulin free (5 years, 26 days)

Blood Glucose (mg/dl)
COH-020 Blood Glucose
Pre- and Post-Transplants 1 & 2
(Tx 1: Feb 2011; Tx 2: July 2015)

Islet graft dysfunction/exhaustion
Blood Glucose Profile – COH20
(Last Tx: 2015-07-10)
City of Hope results: Islet transplantation eliminates hypoglycemia.

Average Hypoglycemic Episodes Per Week
Pre and 3, 6, 12, and 24 mo Post Transplant

- Pt 1
- Pt 2
- Pt 3
- Pt 4
- Pt 5
- Pt 7
- Pt 8
- Pt 9
- Pt 12
- Pt 13
- Pt 14
Islet transplantation reduces insulin resistance in type 1 diabetics

Comparison of intensive glucose management vs. islet transplantation in adult type 1 diabetics

36 type 1 diabetes patients with severe hypoglycaemia with follow up appointment

- 19 patients not optimised with conventional therapeutic strategies
- 17 patients optimised with conventional therapeutic strategies
- Decision to transplant in 10 patients
- 9 patients not optimised by conventional therapeutic options but not transplanted

Comparison of intensive glucose management vs. islet transplantation in adult type 1 diabetics

Islet injury and loss before and after transplantation in type 1 diabetics

Inset photograph shows human islets stained with dithizone red dye, indicative of a highly pure preparation.

Lower inset labels indicate the challenges involved with early islet damage post-transplant, and the factors leading to late islet graft loss—both of which must be addressed to maintain excellent long-term graft function.

Future Advancements in islet transplantation

1. Islet Quality Assessment:
   - LSC (Laser scanning cytometry)
   - OCR (Oxygen consumption rate)
   - IGS (Gene signature)
   - CARS & EIG (microfluidic single islet health assessment)

2. Monitoring transplanted islets:
   - MITRIS (Modeling insulin requirement after transplant)
   - BiPAP (SNP in Genome)
   - MSP (Methylation pattern)
   - Islet imaging

3. Longer-term Advancements:
   - Extrahepatic site for islets
   - Stem cell source of islets
Biomarkers In Islet Transplantation
Pre-transplant Islet Quality Assessment

- Laser Scanning Cytometry (LSC)
- Glucose-Responsive Oxygen Consumption Rate (OCR)
- Gene Signature of Islet Quality

Post-transplant In Vivo Monitoring of Islet Engraftment and Early Injury

- Bi-directional Pyrophosphorolysis – Activated Polymerization Assay (BiPAP)
- Methylation-Specific PCR (MSP)
**Laser scanning cytometry**

### Cellular Composition

- **A**
  - ins
  - amy

- **G**
  - glu
  - ppp

- **M**
  - som
  - ck19

### Cellular Health

- **Example:** Significant increase in expression of inflammation and cell death markers in beta cells with aging.

- **S**
  - % positive (whole preparation)
  - Islet markers: Ins, Glu, Som, PPP, CK19, Amy

- **T**
  - % positive (per islet)
  - Islet hormones: Ins, Glu, Som, Panc

### Graph

- Caspase 3
- BBC3
- Caspase 4
- TLR8
- IAPP

- **p**
  - *** p<0.0001
  - * p<0.05

**Todorov et al. 2010**
Glucose-responsive oxygen consumption rate (OCR)

Islet-specific

\[ \text{Human Islets} \]

\[ \text{Human Acinar} \]

Center-to-Center Reproducible

City of Hope v UWashington

Corr Coeff = 0.92

AUC = 0.793

Sweet et al. 2008
Establishing gene signatures of the highest quality islets for successful transplant outcome.
1. Donor specific markers were tracked in 103 sequential recipient plasma DNA samples from 11 transplant procedures.

2. Donor DNA marker detected in all patients immediately after transplantation for a mean duration of 22 days (15-28).

3. Sixteen donor DNA secondary signals were detected in four islet transplant patients, 13 of which (81%) were associated with events related to islet graft injury.
Human MSP assay was developed based on the methylation pattern of the insulin promoter.

Serum samples were collected pre- and post- transplantation (at days 1 and 14 post-TX) from 6 islet transplant patients. Genomic DNA from the samples was bisulfite-converted and used for nested qMSP and BSP assays. The data display the mean ± SEM of the Relative Unmethylation Ratio (RUR) calculations.

Model for effectiveness of Islet Therapy and Islet Scoring (MITRIS)

Post-Transplant Model for Insulin Therapy and Islet Scoring (MITRIS)
Department of Diabetes, Endocrinology and Metabolism

Patient input parameters

- Current weight? 95 lbs
- Metabolic parameters
  - 2h postprandial glucose (mg/dl)? 155.0
  - Glucagon stimulated c-peptide? 0.4 ng/ml
- Insulin
  - Baseline insulin intake (U/day)? 65.0
  - Current insulin intake (U/day)? 2

Patient visit data

- 2h PPG: 155.0
- Stimulated c-peptide: 0.4 (nmol/l)
- Baseline insulin intake: 0.684 (U/kg/day)

Total

- Graft Function Index: 2
- Predicted Insulin Requirements (PIR): 0.091 U/kg/day (8.6 U/day)
- Suggested increase in insulin intake: +6.6 U/day (minimum)

Orr et al. unpublished
Relationship of calculated insulin deficit (CID) with % HgbA1c and daily reported blood glucose

Orr et al. unpublished
Other problems with islet transplantation

- **Shortage of donor islets from cadaveric pancreata**
  - Large quantities of islets, typically from multiple (2-3) donors, have been needed to achieve insulin independence: larger initial infusion leads to larger mass of surviving beta cells
  - If lower islet masses from single donors could achieve insulin independence, islet transplantation could reach a wider audience and also reduce multiple exposure to HLA antigens
  - There is a compiling need for a renewable source of beta cells (from stem cells or xeno-islets)

- **Requirement for lifelong immunosuppression**
  - Further increases risks for cardiovascular disease, atherosclerosis, dyslipidemia, and cancer
  - Tacrolimus can cause neuro- and nephrotoxicity and is additionally toxic to beta cells; elimination of tacrolimus may improve metabolic function but increases risk of rejection
  - There is compiling need for immunomodulation to reduce dependence on immune suppression
Researching solutions for the future advancement of islet transplantation

*In vivo* islet expansion
Immune modulation
Islet expansion after transplantation

**Gastrin** is a strong promoter of islet neogenesis both *in vitro* and *in vivo* that may work synergistically with **GLP-1**. Therefore, administration of GLP and gastrin to islet recipients may induce *graft neogenesis* and *insulin independence with fewer islets*.

Growth factors (such as TGF-α, epidermal growth factor, and keratinocyte growth factor) and gastrointestinal peptides (such as glucagon-like peptide-1 and gastrin) could stimulate β-cell neogenesis.

**City of Hope** GMP-prepared, gastrin analogue (G17) is as active, but more stable than native gastrin. *In vivo* expansion with these growth factors could *maximize pancreas utilization* by reducing islet mass and number of transplants needed.

Transition Therapeutics, Inc.
In vivo islet expansion using gastrin

Gastrin treatment significantly increases beta cell mass in rats

Gastrin causes beta cell proliferation

![Graph showing % Beta cells](image)

![Graph showing % Alpha cells](image)

![Image of islet expansion](image)
In vivo and ex vivo imaging intraportally transplanted human islets expanded in mouse liver with $^{18}$F-TCE4-PET

A

Experimental Control Normal

B

Blood glucose

C

Liver uptake derived from ex vivo PET scans

D

Percent islet surface area in liver for slides positive for islet tissue

Wu, et al, manuscript in preparation
Immune modulation strategies: promoting regulation

*In vitro* expansion of T-reg
IAK Patient COH-022: Case Summary

Good response following two islet infusions given approx 1 mo apart, followed by rapid islet graft failure. Transient islet DSA & other evidence of alloresponse detected. Renal graft stable.

COH-022: Patient Management Profile (last transplant: 2011-03-10)
Natural history of the immunopathogenesis of T1DM

Nature Reviews Endocrinology 10, 229–242 (2014)
Questions

Q: Can Treg prevent islet or other allograft tissue rejection?
Treg improves the effectiveness of ATG, GM-CSF & Sirolimus in delaying GvHD in NSG mouse model

Healthy donor, n=1

- ATG: 40µg/mouse, i.p.
- Sirolimus: 1µg/g/d, 5ds
- GM-CSF: 2µg/d, 5ds

Expanded Tregs from a healthy donor doubled the time to death from GvHD (Green), whereas immune suppressants alone (a combination identical to that used in our active clinical islet protocol) had a marginal therapeutic effect (Blue).

Diabetic donor, n=2

Expanded Tregs from type 1 diabetics had the same beneficial effects (Green) as that of the healthy individual shown above. (significantly more prolonged survival than the immune suppressants alone; in purple). Using Tregs alone (black) also prolongs the survival rate when compared to other commercially available immunosuppressive drug (purple and pink) but to a lesser degree than the combination.

Unpublished data from our group
Upcoming Islet Cell Transplant Protocols (Near Starting)

1. *In vivo* islet expansion using Gastrin

2. *In vivo* Islet Imaging

3. Regulatory T-cell

4. Combine Islet/Kidney
Future of islet and stem cell transplantation research

- Currently, >80 trials of islet transplant are registered with Clinicaltrials.gov
- >1500 potentially enrollable subjects
- At an estimated cost of >$150,000 USD per subject, the burden on research organizations is enormous (>227 million USD) and limits trial progression
- Approval of islet transplantation as standard of care has been obtained in Canada, Europe and Australia but not in USA. Obtaining a biological license would make it reimbursable
- Stem cell transplant has recently begun in the USA, awaiting establishing its safety
More than 37 million islets were distributed to support 230+ research project at over 113 institutions.

Evidence of COH’s ability to produce high quality islets. Data provided by the IIDP Coordinating Center for islets distributed by COH during the first half of 2014 funding period. We have consistently produced the best islet for the IIDP.
City of Hope (SCIC) produces the highest islet quality of all NIH funded islet distribution programs in the country
(Data provided by the coordinating center for the first half of 2014 funding period)
Acknowledgments

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- Clinical Research Office
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- Clinical/IDS Pharmacy
- Pathology/Clinical Pathology
- Psychosocial Support
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- Many Other Throughout City of Hope and Collaborating Institutions

THANKS!
Patient experience with islet transplantation

http://losangeles.cbslocal.com/2015/10/05/experimental-treatment-at-city-of-hope-could-be-cure-to-type-1-diabetes/