Practical Ways to Achieve Targets in Diabetes Care

Advances in Beta Cell Imaging

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Duality of Interest Declaration

The research presented in this lecture was supported in part by grants from the Yale-Pfizer Bioimaging Alliance.
Imaging Objectives

• Monitor Disease Susceptibility & Progression of Beta Cell Mass Changes
  – Type 1 & Type 2 Diabetes

• Identify Therapies that Preserve, Restore, or Regenerate Beta Cell Mass
  – Immunomodulation & Anti-Inflammatory
  – Hormones & Growth Factors
  – Hypoglycemic agents acting directly & indirectly on beta cells

• Monitor Viability of Islet Transplantation and Stem Cell Therapies & Devices.
  – Regenerative Medicine

• Correlate Anatomical Measures with Functional Insulin Secretion & Biomarkers
## Imaging of β-cell function and Islet mass

<table>
<thead>
<tr>
<th></th>
<th>MRI</th>
<th>PET/SPECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>high +</td>
<td>low +/-</td>
</tr>
<tr>
<td>-Islet dia: 20-600 μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low</td>
<td>high (pmolar range)</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Prelabeling or Function</td>
<td>Receptor specific</td>
</tr>
<tr>
<td>Repeated measurements</td>
<td>Yes</td>
<td>Yes, dose limitation</td>
</tr>
</tbody>
</table>

### Magnetic Resonance Imaging

- Imaging of Transplanted Islet Mass
  - Superparamagnetic Iron-oxide nanoparticles
- Imaging β-cell function
  - Mn²⁺ uptake concurrent with Ca²⁺ uptake

### PET/SPECT:

- Imaging of Endogenous Islet β-cell Mass with Receptor-Specific Probes.
  - G-protein coupled receptors (GLP-1R)
  - Vesicular monoamine transporter-type 2 (VMAT2)
Magnetic Resonance Imaging of Islet Mass

• Dextran-coated Superparamagnetic iron oxide nanoparticles (~30nm dia)
• Islets incubated with nanoparticles,
• Endocytotic uptake by all islet cells (~2-12pg Fe/cell)
• SPIO shortens T2 and leads to reduction (darkening) in signal intensity.
• Change in T2 linear with number of islets
• Detection limit of 1 islet (in vitro) at 9.4T

Magnetic Resonance Imaging of Transplanted Islet Mass

- Labeled human islets (n=1000) transplanted under left kidney capsule
- Non-labeled human islets transplanted under right kidney capsule
- T2*-weighted MRI: darkening (shorter T2) of labeled islets
- Transplant stable for 188 days.
- Restored normoglycemia in STZ-treated NOD-SCID mice.

Transplanted Prelabeled Human Islets into Mouse Liver

- Labeled human islets (n=500) transplanted into liver by intraportal infusion
- Restored normoglycemia in STZ-treated NOD-SCID mice.

Monitoring of immune rejection of transplanted human islets

- Islets labeled by overnight incubation with Superparamagnetic iron oxide nanoparticles (Feridex, FDA-approved)
- Intraportal infusion of 1000 islets into mice: immunodeficient NOD-SCID, or immunocompetent Balb/C
- Marked increase in rate of loss of islet number in Balb/C vs. NOD-SID (associated with increased apoptosis in Balb/C mice)
- Fate of iron after cell death: internalization and clearance by Kupffer cells.

Noninvasive assessment of pancreatic β-cell function with manganese-enhanced MRI.

- The MR contrast agent Mn\textsuperscript{2+} labels glucose-stimulated beta cells by entering activated Ca\textsuperscript{2+} channels.
- Mn\textsuperscript{2+} increases the water proton longitudinal relaxation rate R1 proportionally with concentration.

MRI Mapping of Pancreatic Water Proton Longitudinal Relaxation Rate R1

- R1 mapping images acquired 1-hour after administering glc and Mn\(^{2+}\).
- Pulse sequence generates gradient echo images: intensity dependent on R1 and the inversion times.

- R1 mapping images depicting an axial slice through the mouse abdomen. White arrow denotes the Mn\(^{2+}\)-enhanced pancreas.

- Longitudinal magnetization relaxation curves are generated from images.
- Mn\(^{2+}\)-enhanced pancreatic R1 is calculated from curves.

Mn$^{2+}$-enhanced pancreatic R1 reflects functional beta cell mass

- Pixel-wise Mn$^{2+}$-enhanced pancreatic R1 maps
- Mn$^{2+}$-enhanced Pancreatic R1 is significantly lower in STZ-treated diabetic mice ($p < 0.05$, n=6 mice/group).

Detecting gradations in loss of functional beta cell mass

Used cytoxan-accelerated NOD-BDC2.5 transgenic T cell receptor mice, a model characterized by development of diabetes within a 7 day time period after injecting cytoxan.

- Mn$^{2+}$-enhanced pancreatic R1 decreased step-wise as Tg+ mice became diabetic.
- R1 remained constant in Tg- mice who do not develop diabetes within this window.
- Mn$^{2+}$-enhanced pancreatic R1 correlated well with pancreatic insulin.

1) Antkowiak PF et al. World Molecular Imaging Congress, 4th meeting. Accepted.
Molecular Imaging of \( \beta \)-cell mass by PET. Moving forward from validation to the clinic

- Development of receptor-specific probes
  - amenable to PET-isotope radiolabeling
  - safe for use in human investigation

- Validate correlation of quantitative PET image with BCM in animal models.

- Validate correlation of quantitative PET image with BCM in healthy and T1DM volunteers.

- Simplify infusion, scanning, and data analysis.
G-Protein Coupled Receptors as β-Cell Selective Imaging Targets

Pancreatic β-Cell

Database Mining of potential GPCR Targets
- GIP Receptor
- NPY2 Receptor
- mGluR5 Receptor
- PK1 Receptor
- GLP-1 Receptor
- GPR40 Receptor
- GPR109 Receptor
**Exendin-4-like fluorochrome, E4K12-Fl**

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Amino Acid sequence</th>
<th>Length (aa)</th>
<th>NIRF mod</th>
<th>MW (kDa)</th>
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<tbody>
<tr>
<td>Glucagon-like peptide-1</td>
<td>GLP1</td>
<td>HAEGTFTSDVSSYLEGQAAKEFIAWLWKGR</td>
<td>30</td>
<td>none</td>
<td>3299</td>
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<tr>
<td>Exendin-4</td>
<td>E4</td>
<td>HHEGFTSDLQKQQEAAEAVRLFIEWKLKGGPSSGAPPSSX</td>
<td>39</td>
<td>none</td>
<td>4188</td>
</tr>
<tr>
<td>Exendin-4(40-Pra)</td>
<td>E4&lt;sub&gt;40-Pra&lt;/sub&gt;</td>
<td>HHEGFTSDLQKQQEAAEAVRLFIEWKLKGGPSSGAPPSSX</td>
<td>40</td>
<td>none</td>
<td>4282</td>
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<tr>
<td>Exendin-4(40-Fl)</td>
<td>E4&lt;sub&gt;40-Fl&lt;/sub&gt;</td>
<td>HHEGFTSDLQKQQEAAEAVRLFIEWKLKGGPSSGAPPSSX(NIRF)</td>
<td>40</td>
<td>Pra40</td>
<td>5623</td>
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<tr>
<td>Exendin-4(K12Fl)</td>
<td>E4&lt;sub&gt;K12-Fl&lt;/sub&gt;</td>
<td>HHEGFTSDLQKQMEAAEAVRLFIEWKLKGGPSSGAPPSS</td>
<td>39</td>
<td>K12</td>
<td>5311</td>
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</table>

Molecular model of exendin-4-like fluorochrome, E4K12-Fl, based on NMR-structure

*Molecular* model of exendin-4-like NIR fluorochrome E4K12-Fl (amino acids 9-39) complexed with the extracellular domain of GLP-1R, based on the crystal structure 3C59 (30).

Reiner et al. Bioconjugate Chem., 2010
Immuno- and fluorescence histology of adjacent pancreas sections from a MIP-GFP mouse injected with E4K12-Fi

Reiner et al. Bioconjugate Chem. 2010
Internalization is key for in vivo imaging with radiolabeled GLP-1 analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>Kd (nM)</th>
<th>Bmax (receptors per cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Lys40(DTPA)]exendin-3</td>
<td>13.5 (9.9–18.5)</td>
<td>8.7 (7.6–10.1)</td>
<td>26 000 (24103 to 29103)</td>
</tr>
<tr>
<td>[Lys40(DTPA)]exendin-4</td>
<td>13.4 (10.5–17.0)</td>
<td>17.6 (14.2–23.3)</td>
<td>41 000 (35103 to 50103)</td>
</tr>
<tr>
<td>[Lys40(DTPA)]exendin(9–39)</td>
<td>14.4 (4.8–43.2)</td>
<td>15.1 (6.9–80.7)</td>
<td>37 000 (13103 to 60103)</td>
</tr>
</tbody>
</table>

• Similar in vitro binding of GLP-1 agonists and antagonist
• Reduced specific uptake of GLP-1 antagonist
• Biodistribution: Lower uptake and rapid washout of antagonist

SPECT-(GLP-1R) imaging of β-cell tumors with [Lys40(Ahx-DTPA-\(^{111}\)In)NH2]Exendin-4


PET-(GLP1R)- imaging of INS-1 tumor with [Lys\(^{40}(68\text{GA-DOTA})\)-Exendin-3
**VMAT-2 imaging**

**Brain:** VMAT2 transport of dopamine into vesicles for delivery to and release from presynaptic junction.

**β-cells:** VMAT2 transport of dopamine into insulin-containing vesicles. Dopamine binds to D2 receptors inhibiting insulin release.

Quantitative PET Imaging of pancreatic β-cell mass with $^{18}$F-FP-DTBZ in Control and T1DM humans.

- **MR session**: Region of interest (ROI) drawn in MR space. Labeled MR image. Non-linear registration. Co-registered PET & MR.
- **PET session**: PET image. Arterial blood. Extract TACs.
- **AST session**: C-peptide, insulin release. Correlate PET with AST results.
- **PET TACs**: PET TACs. Tracer binding parameters. Tracer binding correlates with beta cell function.
- **Kinetic modeling**: Tracer binding parameters.
Orthogonal View Images
(after bolus injection of 18F-FP-DTBZ)

- marrow
- myocardium
- pancreas head
- liver
- pancreas body
- pancreas tail
- spleen
Imaging $\beta$-cell mass vs. individual islets

Quantitative imaging of BCM is an integrated signal, **NOT** the uptake per islet.

- HR+ has reconstructed resolution of $\sim 6$ mm
  - Islet diameter: 20-600 $\mu$m.
- Conservative ROI placement
  - $\leq 2$ voxels (5.1 mm) across in body and tail, $\leq 3$ voxels (7.7 mm) in head
  - Pancreas diameter $\sim 12-20$ mm in head, 9-14 mm in body and tail
- Given spatial resolution, effective size of pancreas, and exclusion of edge voxels, errors due to Partial Volume Effects should be minor
  - Simulation: hot islets, warm pancreas, cold background blurred to 8mm FWHM

See also discussion in Ichise and Harris, *JNM* 52(3):494-5, 2011
Challenges for Pancreatic Imaging

Respiratory motion

Control subject
mCT PET/CT scanner
with ANZAI belt
4 hr respiratory gating scan
(8 segments)

Gated image (0-10 min)

Bolus + constant infusion
($k_{bol}=360\text{min}$)

Effect of respiration

Phase 1

Phase 6

Gated PET (Bolus+Infusion, 210-240 min)
Effect of respiration

Phase 1

Phase 6

Gated PET (Bolus+Infusion, 210-240 min)

Size of ROI on pancreas (whole)

78 mm$^3$
Effect of respiration

Phase 1

Phase 6

Gated PET (Bolus+Infusion, 210-240 min)

Size of ROI on pancreas (whole)

49 mm$^3$

78 mm$^3$
Effect of respiration

Gated PET (Bolus+Infusion, 210-240 min)

Size of ROI on pancreas (whole)
- Phase 1: 29 mm$^3$
- Phase 6: 49 mm$^3$
- Other: 78 mm$^3$

Naganawa M., et al. 
Region of Interest Placement

- ROIs drawn on summed PET images using MRI to guide and confirm localization

- Conservative ROI placement along central axis, excluding edge voxels susceptible to signal spill-out
Tissue Time Activity Curves

$[^{18}F]$FP-DTBZ pancreatic uptake is reduced in T1DM

Standardized Uptake Value [unitless]
$[^{18}\text{F}]\text{FP-}(+)\text{-DTBZ}$ Pancreatic Uptake Reduced in Type 1 Diabetes Patients

Healthy control (HC) subjects

Type 1 diabetes (T1DM) patients

All images summed 0-90 min post-injection (0-20 SUV)
[\textsuperscript{18}F]FP-(-)+-DTBZ Pancreatic Uptake is Reduced in Type 1 Diabetes Patients

Healthy control (HC) subjects vs. Type 1 diabetes (T1DM) patients

Normandin et al., J Nucl Med 2012

0-90 min post-injection (0-20 SUV)
\[ ^{18}\text{F}\text{FP}-(+)-\text{DTBZ} \] Pancreatic Uptake Correlates with $\beta$-cell Function.

Healthy control (HC) subjects vs. Type 1 diabetes (T1DM) patients

$R^2=0.60 \quad P=0.0007$

$R^2=0.31 \quad P=0.048$

$R^2=0.59 \quad P=0.0023$

Normandin et al., J Nucl Med 2012

All images summed 0-90 min post-injection (0-20 SUV)
Summary

- $[^{18}\text{F}]\text{FP}-(+)-\text{DTBZ}$ PET showed marked qualitative differences between controls and T1DM, which were quantified by kinetic modeling with arterial input functions.

- Before accounting for pancreas size, binding density correlated significantly with insulin secretion capacity.

- Accounting for pancreas volume enhanced group differences in $[^{18}\text{F}]\text{FP}-(+)-\text{DTBZ}$ binding and strengthened correlations between tracer binding and $\beta$-cell function.
Molecular Imaging of $\beta$-cell mass.
Targeting VMAT2 with Dihydrotetrabenazine (DTBZ) tracers: Moving to the clinic

1. Validate correlation of quantitative PET image with BCM in healthy and T1DM volunteers.
2. For Clinical Research:
   - Reduce scan time and standardize time for image acquisition
     - use of optimal infusion protocol
     - bolus vs. bolus-constant infusion
   - Standardize Modeling
     - one-tissue (1T)
     - two-tissue (2T) compartment model
     - multilinear analysis (MA1) with various $t^*$ (5 to 50 min)
   - Use a validated Reference Region (e.g., kidney, spleen, etc)
     - simplified reference tissue model (SRTM)
       - Non-specific binding ~same as pancreas exocrine
       - eliminate or minimize blood draws
Scan Duration

≥ 180 min (MA1)  BP_{ND}  

≥ 90 min (SRTM)  BP_{ND}  

Mean ± SEM
**BP_{ND} (SRTM)**

\[ t_{max} = 90\text{min} \]

- Pancreas whole: 20% underestimation of BP_{ND}
- Mean ± SEM

Pancreas whole:
- □: HC
- ●: T1DM

Good correlation:
- \[ y = 0.83x \]
- \[ R^2 = 0.81 \]

20% underestimation of BP_{ND}
Acknowledgments
Faculty and staff at Yale PET Center

Yale
• Richard E. Carson, Ph.D.
• Kitt-Falk Petersen, M.D.
• Marc D. Normandin, Ph.D.
• Mika Naganawa, Ph.D.

Pfizer
• Judith L. Treadway, Ph.D.
• Roberto Calle, Ph.D.
• Tim McCarthy, Ph.D.

Support
• Yale-Pfizer Bioimaging Alliance
• Juvenile Diabetes Foundation
Pancreas Volume Decreased in T1DM (MRI)

Pancreas volume (cm$^3$)  Pancreas volume index (cm$^3$ / m$^2$)

<table>
<thead>
<tr>
<th></th>
<th>Ctl</th>
<th>T1DM</th>
<th></th>
<th>Ctl</th>
<th>T1DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>32%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P = 0.002

P = 0.001
[\textsuperscript{18}F]FP-DTBZ Pancreatic Uptake Binding Parameters (Total \(\beta\)-cell mass: corrected for pancreas volume)

- Standardized Uptake Value (SUV\textsubscript{60-90}) [cm\textsuperscript{3}]
  - \(P < 0.005\)
  - Control
  - T1DM

- Volume of Distribution (\(V_T\)) [ml]
  - \(P < 0.01\)

- Binding Potential (\(BP_{ND}\)) [cm\textsuperscript{3}]
  - \(P < 0.005\)
  - 59%

Source: Normandin et al., J Nucl Med 2012
$[^{18}F]FP$-DTBZ Pancreatic Uptake Binding Parameters:
Correlation with $\beta$-cell function

$\beta$-cell density
(independent of pancreas volume)

$R^2 = 0.59$
$P = 0.0023$

Total $\beta$-cell mass

$R^2 = 0.78$
$P = 0.0001$

**Control**

**T1DM**

Normandin et al., *J Nucl Med* 2012