Presenter Disclosure Information

Alberto Pugliese, MD

Nothing to disclose
New Insights to Human Type 1 Diabetes from nPOD

- A tissue bank
- A data cloud resource
- An open, collaborative research project focused on key questions about human diabetes
Mission

- Obtain tissues from organ donors with T1D (diagnosed or sub-clinical)
- Distribute tissues to research projects (>100 since 2007)
- Promote tissue and data sharing, collaboration, manage project interactions
- Promote a comprehensive understanding of human T1D and identify new therapeutic targets
How nPOD works

Potential Organ Donor → OPO → TRANSPLANT → RESEARCH → nPOD

nPOD-T

Pancreas Transplant Recipients

T1D AAb+ T2D

Investigators

Apply with project

nPOD TPC

Tissue Prioritization Committee

nPOD OPPC

Organ Processing & Pathology Core

Organs recovered

Organs processed & distributed

Specimens distributed to approved investigators

DataShare

Publication

New Therapies

nPOD Investigators & Working Groups
nPOD supported projects by main research areas

88 active as of June 2013 (from >100 since 2007)

<table>
<thead>
<tr>
<th>RESEARCH AREA</th>
<th>NUMBER OF PROJECTS</th>
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<tr>
<td>Beta Cell Development, Differentiation &amp; Regeneration</td>
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<td>Immunology</td>
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<td>18</td>
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<td>Pathology</td>
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<td>Type 1 Diabetes Etiology &amp; Environment</td>
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</table>

- Organic pollutants
- peri-capsular basement membrane
- Progenitor cells
- Bone marrow
- Proteomics
- 12/15 Lipoxygenase
- Electron microscopy
- Beta cell dedifferentiation
- Transcriptome
- Stem cells
- Microbiota
- High throughput DNA sequencing
- Cytokines
- Maternal microchimerism
- Inflammation
- Imaging
- Insulitis
- E-Cadherin
- Cell conversion
- Extrathymic Aire-Expressing Cells
- Heparane sulfate
- RNAseq
- Mass spec
- TCR sequences
- IL-18R
- Glucokinase
- FasL
- B cells
- Autoreactive T cells
- Virus
- Epigenetics
- VGEF
- ER Stress
- Regulatory T cells
- Chemokines
- Beta cell vascularization
- Degranulated beta cells
- Glucose Transporters
- Adhesion molecules
## nPOD Recoveries as of June 2013

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<tr>
<th>Donor Type</th>
<th>Number</th>
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<th>Age (StDev)</th>
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<th>Maximum</th>
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<td><strong>38</strong></td>
<td><strong>50</strong></td>
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Beta cell mass versus T1D duration

Roberto Gianani

Percentage of control mean BCM in gms
T1D donors with residual beta cell mass

*Roberto Gianani*

<table>
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<tr>
<th>Case #</th>
<th>Age of onset</th>
<th>DM Duration in years</th>
<th>Antibody status</th>
<th>Insulitis</th>
<th>HLA-DR</th>
<th>Residual BCM (% of control mean)</th>
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Longer Duration T1D – 8 years

6046
18 years old
8 year duration
Caucasian Female

AutoAb: IA2A+ZnT8+

C peptide: <0.05 ng/ml
BMI: 25.2

Histopathology: Ins+ islets in some lobules, other lobules/entire blocks ins-/gluc+ islets. Insulitis +. CD3+ or CD45+ used. Also infiltrates are mainly acinar/extra-acinar. Mild acinar atrophy and adipose infiltration.

HLA: A*0201/0301
B*1501/3901
DRB1*0101/0401
DQA1*0101/0301
DQB1*0302/0501
Formation of a Human β-Cell Population within Pancreatic Islets Is Set Early in Life

Brigid E. Gregg, Patrick C. Moore, Damien Demozay, Ben A. Hall, Mei Li, Aliya Husain, Amy J. Wright, Mark A. Atkinson, and Christopher J. Rhodes

The Journal of Clinical Endocrinology & Metabolism
2012;97:3197-3206
β-cell persist in some T1D pancreata without evidence of replication nor insulin-glucagon co-expression
Replication is actually reduced in adult T1D

Matthew Rankin and Jake Kushner

Beta Cell Replication in Adults (19-50 yrs)

- Controls, n=11, age=34.6 ± 3, 45 out of 58,484
- T1DM, n=20, age=33.7 ± 2.3, 2 out of 15,861

Almost None Detected

Copositives

- Controls, n=7, age=29.9 ± 6.5, 0 out of 67,398
- T1DM, n=5, age=27.6 ± 7.7, 0 out of 28,518

None Detected
Double-hormone positive islet cells found in T2D donors treated with incretin therapies

Butler et al. Diabetes 2013
6052
12 years old (1 year duration) Male AA
AutoAb: IA2A+mIAA+
C peptide: 0.18 ng/ml
Image Analysis - β cell Quantification
Cell numbers, Ki67+ colocalization (replication)

Martha Campbell-Thompson

6052
12 years old (1 year duration) Male AA
2 days on ventilator!

~30% of Ki67+ cells were beta cells
Unusual but it can occur!
**nPOD Donors with Insulitis**
(15 as of June 2013)

<table>
<thead>
<tr>
<th>Case</th>
<th>Group</th>
<th>AutoAb Status</th>
<th>Age (years)</th>
<th>Duration (years)</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>C-peptide (ng/ml)</th>
<th>Insulitis (%)</th>
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<td>GADA+ IA2A+</td>
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<td>0</td>
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<td>0.1</td>
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<td>6209</td>
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<td>0.25</td>
<td>Female</td>
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<tr>
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<tr>
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<td>8</td>
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<td>C</td>
<td>&lt;0.05</td>
<td>2.6</td>
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Insulitis: ≥6 CD3 T cells adjacent or within islets (n ≥ 3 islets/section) with pseudoatrophic (insulin deficient) islets within pancreas (M. Campbell-Thompson)
Overview of results from systematic staining and in situ tetramer assay


[Diagram showing percentage distributions for different conditions and comparisons between non-diabetic (Islet Abs) and T1D (duration in years) groups.]

JEM
Immunohistochemical detection of islet antigen-reactive CD8 T cells within insulitic lesions of T1D patients


Both in recent onset and long standing diabetes
Young Donor with Recent Onset T1D – 0.25 years

6209
5 years old  **(0.25 year duration)** Female  
AutoAb: IA2A+ZnT8A+miAA+  
C peptide: 0.1ng/ml  
HighRes HLA: A*0101,0201; DRB1*0401,0301;  
DQA1*0301,0501, DQB1*0302,0201  
Histopathology: Ins+ (reduced)/Gluc+ islets with insulitis. Low Ki67 in all cell types. No pancreatitis.
The Peri-islet Basement Membrane, a Barrier to Infiltrating Leukocytes in Type 1 Diabetes in Mouse and Human

Éva Korpos,1 Nadir Kadri,2,3 Reinhild Kappelhoff,4 Jeannine Wegner,1 Christopher M. Overall,4,5 Ekkehard Weber,6 Dan Holmberg,7 Susanna Cardell,2 and Lydia Sorokin1

*Diabetes* 62:531–542, 2013
Co-localization of cathepsin S and W near CD45+ infiltrating cells and areas of basement membrane interruption
nPOD-3678 – Pancreas Transplant Recipient with Recurrent T1D
Memory T cells are present in the insulitis

Francesco Vendrame

**Insulitis: Frequency of memory T cells**

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nPOD-3678 – Pancreas Transplant Recipient with Recurrent T1D
Only a proportion of islets are affected near diagnosis

Francesco Vendrame

<table>
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<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>Mean</td>
<td>SD</td>
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INs
CD8
40X
SPK Patient nPOD-3678

MMTT

MMTT WAS PERFORMED 11 DAYS PRIOR TO BIOPSY; HbA1c WAS 10.7%
Lower Expression of GCK & ATP Synthase in the Insulin Positive Cells of SPK Patients with T1D Recurrence

Through nPOD collaboration: Clayton Mathews, University of Florida
Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes

I. Marhfour · X. M. Lopez · D. Lefkaditis · I. Salmon ·
F. Allagnat · S. J. Richardson · N. G. Morgan ·
D. L. Eizirik

Expression of the enteroviral capsid protein VP1 in the islet cells of patients with type 1 diabetes is associated with induction of protein kinase R and downregulation of Mcl-1

S. J. Richardson · P. Leete · A. J. Bone · A. K. Foulis · N. G. Morgan

Fig. 1 Representative immunohistochemical images of enteroviral VP1 levels in the UK (donor D4) (a) and nPOD (donor 6052-01) cohorts (b). (c) The proportion of cases with islets staining intensely positive for VP1. White bars, non-diabetic controls (UK n = 50 [7]; nPOD n = 12); hatched bars, type 1 diabetes cases with ICIs (UK n = 72 [7]; nPOD n = 10); black bars, type 1 diabetes cases containing only IDIs (nPOD n = 7)

Fig. 4 Photomicrographs of representative islets from the nPOD (donor 6084-01; a, c, e) and UK (donor D4; b, d, f) cohorts reveals that VP1 (green; a, b) co-localises with PKR (red; c, d). Double-positive cells are stained yellow and are visible in (e) and (f). Nuclear DAPI staining is shown in blue in the merged images (e, f)
Overall aim is to develop a pipeline for comprehensive and integrated understanding of the role of enteroviruses in disease pathogenesis.
Is there a wider therapeutic window & multiple therapeutic targets?

• The T1D disease process does not end at diagnosis!
• Insulitis may be found for years after onset
• Autoreactive T cells were identified in insulitis
• Insulitis largely consists of memory cells in pancreas transplant patients with T1D recurrence
• Insulin-positive islets, detected for years after onset
• Beta cell replication appears rare, and dependent on age, but exceptions exist – triggers?
• Discrepancy between beta cell loss and impairment of insulin secretion – possible roles of inflammation and epigenetics on beta cell dysfunction
• Relevance of nPOD studies for clinical trials
nPOD Network for Pancreatic Organ Donors with Diabetes

- Mark Atkinson
- Martha Campbell-Thompson
- Suzy Ball
- Jayne Moraski
- Mingder Yang
- Irina Kusmarteva
- Clive Wasserfall
- Teresa Miller
- John Kaddis
- Roberto Gianani
- Patrick Rowe
- Des Schatz
- Les Jebson
- Tiffany Heiple
- Sandra Lawson
- Kim Young
- Francesco Vendrame

nPOD Meeting, Atlantic Beach, February 2013
FEBRUARY 23-26, 2014
ONE OCEAN RESORT Atlantic Beach, Florida

2014 JDRF nPOD ANNUAL SCIENTIFIC SYMPOSIUM
Dedicated to Collaborative Human Type 1 Diabetes Research

JDRF
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