What’s New at nPOD?

Annual Meeting Update
2012
OPPC Update

Martha Campbell-Thompson, D.V.M, Ph.D.,
nPOD OPPC Core Director

- Please see poster
  - (Dr. Irina Kusmartseva, Emily Montgomery)
- Advances in 2011
- Donors
Lay slices to the right and place in cassette in same orientation (label left facing).

**Pancreas:** head, body, and tail- bread loaf so paraffin and OCT are harvested in alternating sections:

1. Snap frozen vials with minced tissues and with or without RNAlater- 4 or more vials each from junctions of head and body and body and tail regions.
2. Paraffin blocks- 5-10 (OCT takes precedence over fixed when sample size is small)
3. OCT blocks- 5-10 all regions or as indicated by size

**PLN:**

1. OCT blocks – 3-5- as many as feasible depending on total numbers
2. Cells- place several dissected LN's in 15ml tubes containing sterile RPMI, hold at refrigeration until shipped or processed. 1-4 tubes depending on numbers
3. Frozen vials as for pancreas. Paraffin is optional.

**Spleen** and **NonPLN:** as for PLN. Paraffin is optional.

**Duodenal mucosa:** as for pancreas. Paraffin is optional.
Pancreas Reconstructions

Anatomical Orientation
“as in vivo”
Blue- anterior
Black- posterior

Campbell-Thompson et. al, J of Visual Experimentation, in press
Expanded Phenotyping

Campbell-Thompson et. al, J of Visual Experimentation, in press
Reconstructions

With-in blocks “catalogues”
bar-coded serial sections
slide scanner readable

nPOD
Network for Pancreatic Organ Donors with Diabetes
Expanded in-house Analyses

- Pancreas RNA- snap frozen and/or OCT thick sections (Dr. Kusmartseva)
- Cell prep FC- before cryopreservation (Dr. Todd Brusko)

RIN

```
2.4 4.7 4.6 6.7
```
In the absence of islet autoantibodies or histopathology can we determine whether an individual has Type 1a diabetes after death?

George Eisenbarth 2011
For nPOD to impact the underlying mechanisms leading to T1D and ultimately complications will require sufficient and appropriate cases early in disease process

• Antibody positive non-diabetic: multiple; younger
• Early (new-onset)
• Age matched controls: healthy; type 2
• Accurate phenotypic characterization: ideally before death
Challenge

• Long duration of disease in many cases
  (median 12, range 1-44 years)

• Incomplete information
  Information gathered from the terminal medical charts and OPO questionnaires; Type 1a vs Type 1b vs Type 2

• Exclude secondary causes eg steroids, medications, CFRD

• Exclude immediate potential co-morbidities which might impact subsequent findings
Current nPOD Data

- Prior to Histology (n= 68)
  - 52 Type 1 diabetes (13 1 Ab, 6 >1 Ab)
  - 16 Type 2 diabetes (1 Ab)

- Histology (UF)
  - Type 1 diabetes (52)
    - 40 - no insulin+ islets
    - 12 - insulin+ (8 reduced, 4 many, 3 amyloid, 3 C-peptide)
    - 8 - insulitis
  - Type 2 diabetes (16)
    - 1 - no insulin+ islets
    - 15 - insulin+ (7 reduced, 8 many - 5 have amyloid, 5 C-peptide)
    - 0 - insulitis
Conclusion

Thorough phenotypic characterization suggests most cases classified correctly despite low frequency of islet autoAb in longstanding diabetes

Histology needed in islet autoAb negative cases to distinguish type 1a vs type 1b
Antibody Screening Strategy Update

Clive Wasserfall, MS
nPOD Autoantibody QA/QC Director
Figure 2. Age-specific death rates: United States, preliminary 2009

Kronus Assay

- ELISA based on double antigen principle
- No radiation
- Fits into the capability of most screening laboratories
- Modified by nPOD to fit into a STAT format.
GADA and Age

GADA

IU

0 15 30 30 45

p=NS Kruskal-Wallis

Age (years)

n= 61 53 34 51 29

0-10 11-20 50-60 60-70 71-80
IA-2A and Age

![Graph showing IA-2A levels across different age groups.](Image)

- **Legend:**
  - p=NS Kruskal-Wallis

- **Data:**
  - n= 61 53 34 51 29

**Age (years):** 0-10, 11-20, 50-60, 60-70, 71-80

**IA-2A IU:** 0, 20, 40, 60, 80, 100, 120, 140
Screening Quality Control

Screened Kronus

- Positive Or Negative
  - Screening Sample Clive
    - Retest Kronus
    - RIA Denver
  - Recovery Sample OPPC
    - Test Kronus
    - RIA Denver

Not Screened
DASP

– GADA  93% Specificity, 86% Sensitivity
– IA-2A 99% Specificity , 60% Sensitivity

• Raising cutoff to 18 GADA and 60 IA-2A
  – GADA 98% Specificity, 80% Sensitivity
  – IA-2A 100% Specificity, 58% Sensitivity

• Double positive with higher cutoff
  – 100% specificity, 54% sensitivity
Moving Forward

• Changed format of KRONUS kits to double the number of screens / kit
• Added ZnT8 to format
• nPOD kit with simplified all in one kit
• On call personnel routinely checking HLA especially for single aab positive
• Extra screens to focus on younger donors/tissue donors.
Organ Procurement and Lab Relations

Jayne Moraski, MS
nPOD Assistant Director
Organ Procurement Partners

Key:
- Dark blue = direct partners
- Light blue = Screening labs
- Green = Other Key Partners
- Yellow = Shipping Partners
- Purple = potential screening and tissue donor partners

nPOD
Network for Pancreatic Organ Donors with Diabetes
## 2012 Aab Screening Projection

<table>
<thead>
<tr>
<th>Laboratory Location</th>
<th>Current Affiliated OPOs</th>
<th>Total Donor Estimate</th>
<th>Screening Start Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>LABS, Inc. (Denver)</td>
<td>Donor Alliance</td>
<td>65</td>
<td>July 2007</td>
</tr>
<tr>
<td>Miami</td>
<td>LAORA</td>
<td>30</td>
<td>August 2008</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1200 projected</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Summary:**
- Screened 778 donors in 2010
- Screened 1,090 through October of 2011 – projected increase of 68%
Goals

- Maintain relations and educational updates with labs and OPO partners
- Increase OPOs that screen by 25% for next year
- Identify new tissue donor programs
Finding New-Onset Cases: Expanding the Donor Pool through Innovative Collaborations

Suzanne Ball, RN, MHS
nPOD Director
The Needle in a Haystack

2,440,000 Total Deaths in U.S.
- 1,250,000 In-Hospital Deaths reported to OPOs
- 110,000 Potential Cornea Donors
- 50,000 Potential Tissue Donors
- 46,000 Cornea Donors recovered
- 25,000 Tissue Donors recovered
- 12,500 Potential Organ Donors (Medically Suitable)

7,994 Organ Donors recovered annually

70% consent for research → 5600 Potential PA Donors

nPOD
Network for Pancreatic Organ Donors with Diabetes
2012 Initiatives

• Partner with Tissue Agencies to recover asystolic donors outside the hospital
• Collaborate with American College of Emergency Physicians (ACEP) to identify and refer New Onset/DKA deaths in pediatric patients
• Pilot a project with an organ procurement organization (OPO) to develop teams for non-traditional donor recoveries
Webinar/Case Discussions

Amy Wright, MS, MBA
nPOD Investigator Relations Coordinator
Webinars/Case Discussions

• **Vision:** All nPOD investigators…
  – collaborate and move T1D research forward
  – make use of precious samples in the most resourceful manner possible

• **Purpose:** Provide a forum for nPOD investigators to share and discuss data gathered on nPOD tissues.
Webinars/Case Discussions

• **Actions:** Use web conferencing system to hold webinars to share images and engage in discussions
  – Follow-up with surveys/topic requests
• **Case 6052**
  – Over 40 participants
• **Viral Presence in nPOD Tissues**
  – Formed workgroup with over 30 participants
Webinars/Case Discussions

• **Goals:**
  – Provide a suitable forum for data sharing and collaboration
  – Create focused workgroups to further discuss and collectively answer questions
  – Choose topics that are relevant and garner interest in forming workgroups
Proposed Data Sharing Platform

• **PHASE I – 90 Days**
  - Information technology assessment
  - Data analysis
  - Hardware and software validation

• **PHASE II – 120 Days**
  - Prototype established
  - Assessment and testing on new platform
  - Identification of additional feature needs

• **PHASE III – 30 Days**
  - Address any training and orientation needs
  - Go-Live with new and improved Platform
nPOD Data Flow: Now

Data Storage
1. MS Access, Excel
2. SQL Server
3. Varied
4. None or MySQL
5. SQL Server
6. MS Excel, Word
7. MS Excel
8. MS Excel

*Outlines all paths taken, not just common routes of data exchange
nPOD Data Flow: The Future

Image used w/permission, Adam Rauch, LabKey Software™
Investigator Experience Survey

• **Who was surveyed?** 102

• **Number who completed survey:** 34 (33.3%), including 29 investigators (PI’s and Co-PI’s), and 5 post-docs or other staff

• **Last Updated:** 01/17/2012
New Investigator Application

- Process Simple
- Requirements Clear
- Data Entry Easy
- Sample Availability Complete

Strongly Disagree Disagree Neutral Agree Strongly Agree

0 5 10 15 20 25
Service Request Forms (SRFs)

- Sample Request Process Clear
- Sample Limits Clear
- Experiment Specific Needs Easily Conveyed
- SRF Sufficiently Detailed
- Timely Handling
- Prompt Shipments
Future Online Data Sharing System

- Streamlined Application, Tracking, and Feedback
- Choose level of data sharing
- Know who has viewed my data
- Real-Time Sample Availability
Other Comments (Total Respondents)

• **How do you share data (9)**: Discussions with colleagues/ publications/ meetings/ conferences/ nPOD webinars and meetings/ local lab

• **Data sharing concerns (14)**: How will shared data be handled for similar projects/ Publishing novel findings, data repositories – when and how/ none – trust nPOD with unpublished information/ will this prevent collaboration and lessen rigor of study, promoting other labs to publish for the sake of being first/ sharing of stained sections submitted for publication but not yet accepted

• **Desired security of nPOD online database (13)**: Restricted to nPOD data sharing investigators only/ restricted to registered members only/ high/ standard login/ protect data from being altered or corrupted/ disclaimer about unpublished data/ know who is accessing data

• **Other Comments/Suggestions (8)**: Update online information for older cases where tissue pieces are no longer available/ advice on restricted samples that can no longer be accessed/ advance warning when a user reaches his or her limit to donor samples
nPOD New Initiatives

• nPOD-Complications
  – Mark Atkinson
• nPOD-E
  – Carmen Retrum
• nPOD-T
  – Alberto Pugliese
The Natural History of Type 1 Diabetes

- Genetic susceptibility
- Inciting Event(s)
- “Silent” β Cell Loss
- Diabetes Onset
- Chronic Disease

- T2D
- Animal Models
- Lack of sample access (trials)
- Failure to listen to patients
- Complications heterogeneity
- Etc., etc., etc.

% Islet Cell Mass

Time (years)

nPOD
Network for Pancreatic Organ Donors with Diabetes
“Proposed” nPOD-C aims at collecting and studying human tissues from donors with and without complications from T1D.

Four areas of T1D complications:
- Cardiovascular disease
- Nephropathy
- Neuropathy
- Retinopathy
nPOD-C Group Members

- **Steering Committee:**
  - Martha Campbell-Thompson, Florida; Judy Hunt, JDRF; Stephen Rich, Virginia; Robert Levine, JDRF; John Malone, Southern Florida; Eva Feldman, Michigan; Tom Gardner, Penn State; Mike Mauer, Minnesota; Mike Steffes, Minnesota; Matthias Kretzler, Michigan; Dale Abel, Utah

- **Cardiovascular Task Force:**
  - **Chair:** Dale, Abel; **Members:** Ira Goldberg, Columbia; Dean Li, Utah; Christian Schulze, Columbia; Heinrich Taegtmeyer, Texas; Renu Virmani, Maryland

- **Nephropathy Task Force:**
  - **Co-chairs:** Mike Mauer, Matthias Kretzler; **Members:** Hanna Abboud, Texas; Ron Tilton, Texas; Erwin Bottinger, Mt. Sinai

- **Neuropathy Task Force:**
  - **Chair:** Eva Feldman; **Members:** Peter Nawroth, Heidelberg; Angelika Bierhaus, Heidelberg; Vera Bril, Toronto; Gordon Smith, Utah; Rayaz Malik, Manchester; Bill Kennedy, Minnesota; Jim Dyck, Mayo Clinic

- **Retinopathy Task Force:**
  - **Chair:** Tom Gardner; **Members:** Tim Kern, Case Western; Gerry Lutty, Johns Hopkins; Hans Peter Hammes, Heidelberg; Victor Elner, Michigan; Ron Klein, Wisconsin; Peter Compochiaro, Johns Hopkins
The Future - Pushing the Boundaries and Advocating for Complications Research in Type 1 Diabetes

- Approach existing networks (complications, clinical trials) to obtain more cases with well defined natural histories & medical records
- Obtain partial medical records of nPOD donors (in general population)
- Create data base for storage of:
  - Partial/medical history
  - Research data
- Serve as a model for other registries/biorepositories in T1D… encouraging openness
- Include analysis of those with T2D
nPOD-E
Europe

Carmen Retrum, M.S.
nPOD Coordinator of Special Projects
Why is nPOD-E Important?

• Main goals:

1. Expand organ collection (and distribution) by creating a network for organ recovery at qualified sites in European countries
2. Obtain immediate access to highly valuable stored samples
3. Provide the resources of nPOD tissues to European investigators, as well as those in the U.S.
Why is nPOD-E Important?

- **Main goals:**
  1. Expand organ collection (and distribution) by creating a network for organ recovery at qualified sites in European countries
  2. Obtain immediate access to highly valuable stored samples
  3. Provide nPOD tissues and resources to European investigators, as well as those in the U.S.
  4. Facilitate European distribution for perishable specimens
nPOD-E Overview

• Collaboration sites in Sweden & Finland
  – Working together to increase nPOD investigator access to tissue samples

• Individual sites established in Italy & Spain
  – Will screen, recover, store, and distribute
  – nPOD structural model utilized
Collaboration Site Progress

• **Sweden:**
  - Collaborating with Dr. Gun Frisk, at Uppsala University, to obtain retrospective and prospective tissue samples, specifically islets from AAb+ donors.
  - Have received **365 slides** from a pre-existing donor collection.

• **Finland:**
  - nPOD-E is collaborating with Dr. Hyöty, at the University of Tampere, and the PEVNET/Europod project by developing coordinated activities (specimen distribution, data, data management, other).
Established 2 New Sites in Europe

- **nPOD-E Italy:**
  - Established new nPOD-E site in Siena, Italy with Dr. Francesco Dotta.
  - Similar to nPOD, this project will screen and recover Aab+ and T1D donors from multiple sites and use all of nPOD’s SOP.

- **nPOD-E Spain:**
  - Established new nPOD-E site in Barcelona, Spain with Dr. Eduard Montanya.
  - This project will recover from AAb+ and T1D donors. And use nPOD SOP for its screening, recovery, case processing and distribution methods.

- Screening and recovery efforts for both to begin in February 2012.
Case Recovery & Processing (Per nPOD SOP)

Inbound Processes

Stored and managed at nPOD-E Site

To nPOD OPPC for immunopathology per SOP and scanned to Aperio

*Each site will manage their own case & specimen related data
Investigator Request

Outbound Processes

nPOD-E Site distributes tissue

TPC/OPPC review

*OPPC views tissue availability via database; site inputs distribution
## nPOD-E Projections

### Individual Site Expectations for 2012

<table>
<thead>
<tr>
<th></th>
<th>AAb Screens</th>
<th>AAb Cases</th>
<th>T1D Cases</th>
<th>Total Cases</th>
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<tbody>
<tr>
<td>Spain</td>
<td>70-100</td>
<td>1-3</td>
<td>0-3</td>
<td>2-5</td>
</tr>
<tr>
<td>Italy</td>
<td>400+</td>
<td>4-6</td>
<td>2-5</td>
<td>7-9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>500</td>
<td>6</td>
<td>6</td>
<td>12 projected</td>
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nPOD-T
Transplantation
Alberto Pugliese, MD
nPOD Co-Executive Director
The Type 1 Diabetes Spectrum

- Genetic Susceptibility/Protection & Environmental Factors
- Autoimmune Responses
- Autoimmune Reactivation, Persistence and/or Waves?

β-cell Mass/Insulin Secretion

Time

NORMAL RANGE

PREVENTION TRIAL
INTERVENTION TRIAL
TRANSPLANT

Waves of regeneration? Survival of some β-cells?
Patient 2 - SPK-3601

A. AUTOANTIBODIES

- Daclizumab - Thymoglobulin - Rituximab

B. C-PEPTIDE AND AUTOREACTIVE T CELLS

C. PANCREAS TRANSPLANT BIOPSY

D. AUTOREACTIVE T CELLS

Original Article

Recurrence of Type 1 Diabetes After Simultaneous Pancreas-Kidney Transplantation, Despite Immunosuppression, Is Associated With Autoantibodies and Pathogenic Autoreactive CD4 T-Cells

Francesco Vendrame,¹ Antonello Pileggi,¹,² Elsa Laughlin,³ Gloria Allende,¹ Ainhoa Martin-Pagola,¹ R. Damaris Molano,¹ Stavros Diamantopoulos,¹ Nathan Standifer,²,⁴ Kelly Geubtner,⁵ Ben A. Falk,⁵ Hirohito Ichii,¹,² Hidenori Takahashi,⁶ Isaac Snowhite,¹ Zhibin Chen,⁵ Armando Mendez,¹,⁶ Linda Chen,⁵ Junichiro Sageshima,² Phillip Ruiz,² Gaetano Ciancio,² Camillo Ricordi,¹,²,⁵,⁶ Helena Reijonen,⁶ Gerald T. Nepom,⁶ George W. Burke III,¹,² and Alberto Pugliese¹,⁵,⁶
Patient 2 (MSS), pancreas transplant biopsy

Left panel: a pancreatic islet stained for insulin (red), glucagon (light blue) and VP-1 (green).

Right panels: higher magnification of the inset from the left panel demonstrates colocalization of VP-1 and insulin. The image was selected from a Z-stack series acquired by confocal microscopy.

Vendrame et al. Diabetes 2010
Insulin protein and proliferation in ductal cells in the transplanted pancreas of patients with type 1 diabetes and recurrence of autoimmunity

A. Martin-Pagola · G. Sisino · G. Allende · J. Dominguez-Bendala · R. Gianani · H. Reijonen · G. T. Nepom · C. Ricordi · P. Ruiz · J. Sageshima · G. Ciancio · G. W. Burke · A. Pugliese

Received: 25 March 2008 / Accepted: 27 June 2008 / Published online: 12 August 2008
© Springer-Verlag 2008
Conversion of GAD & IA2 AAb ~4 years after Tx and conversion of ZnT8 AAb ~2 years later
The patient developed recurrent diabetes in July 2011 (about 9 years after Tx)
The patient was biopsied on 8/10/2011
SPK IM-203 HLA-A2 GAD CD8 T cells
Direct Pentamer Staining (H Reijonen, unpublished)

Positive in July, August (biopsy) and October 2011

GAD reactive, CD8 T cells in both naive and memory compartments;

These cells were shown in blood, Tx PLN and pancreas transplant

unpublished
### BLOOD GLUCOSE (mg/dl)

<table>
<thead>
<tr>
<th>Time</th>
<th>MMTT</th>
<th>OGTT</th>
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<tbody>
<tr>
<td>-10’</td>
<td>192</td>
<td>198</td>
</tr>
<tr>
<td>0’</td>
<td>nd</td>
<td>196</td>
</tr>
<tr>
<td>30’</td>
<td>265</td>
<td>273</td>
</tr>
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<td>60’</td>
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<tr>
<td>90’</td>
<td>302</td>
<td>453</td>
</tr>
<tr>
<td>120</td>
<td>294</td>
<td>477</td>
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### C-PEPTIDE (ng/ml)

<table>
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<th>Time</th>
<th>MMTT</th>
<th>OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10’</td>
<td>1.28</td>
<td>1.05</td>
</tr>
<tr>
<td>0’</td>
<td>1.31</td>
<td>0.99</td>
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<tr>
<td>30’</td>
<td>2.71</td>
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<tr>
<td>60’</td>
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<tr>
<td>90’</td>
<td>2.63</td>
<td>1.19</td>
</tr>
<tr>
<td>120</td>
<td>2.33</td>
<td>1.27</td>
</tr>
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</table>

**MMTT** 07/29/2011  HbA1c 10.7%

**BIOPSY** 08-10-2011

**OGTT** 10-4-2011  HbA1c 8.9%

*unpublished*
nPOD-T

nPOD-T aims at establishing feasibility of procuring human pancreatic tissue from transplanted T1D patients, when possible both transplanted and native pancreas.

This should allow for scientific discovery in relation to:

- recurrent disease, which mimics spontaneous disease development, correlating biopsy data with clinical data
- immunosuppression and potential regeneration
- rejection and other chronic changes
nPOD-T aims at collecting tissues in three different settings:

1. Organs/tissues from transplant recipients (post-mortem)

2. Biopsies of native and transplanted pancreas
   - at the time of transplantation
   - for recurrent disease and/or rejection, or hernia/other reasons

3. Archived biopsies
nPOD-T Organizational Diagram

**nPOD-T - Miami**
- **Functions**
  - Administration/coordination
  - Enrollment & Consenting (with Tx Centers)
  - Data management & information exchange with nPOD and Tx Centers
  - AAb testing
- **Structure**
  - PI (Pugliese)
  - Clinical Coordinator (IRB/consenting, medical records, archived specimen procurement, relations and nPOD and Tx Centers)
  - Research Associate for AAb testing

**nPOD - Gainesville**
- **Functions**
  - Donor tissue procurement, archival storage, sample distribution to investigators
  - Histology
  - Data management
- **Structure**
  - PI (Campbell-Thompson)
  - Pathology Staff
  - Administrative Staff (Atkinson)

**Transplant Centers**
- Indianapolis
- Miami
- Minneapolis

**nPOD Investigators**
- Consent forms, IRB protocols, AAb results, coordination
- Serum samples, medical data, documents
- Tissues from donors (via OPO), from biopsies, archived biopsy specimens
- Specimens, data

(results, data, samples, study coordination interactions)
Conclusion

Thank you to all of you.

Thank you JDRF.

Questions?