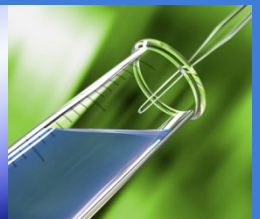
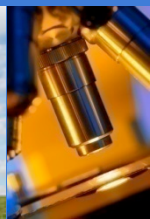
What's New at nPOD?

Annual Meeting Update
2012





nPOD
Network for Pancreatic Organ Donors with Diabetes

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

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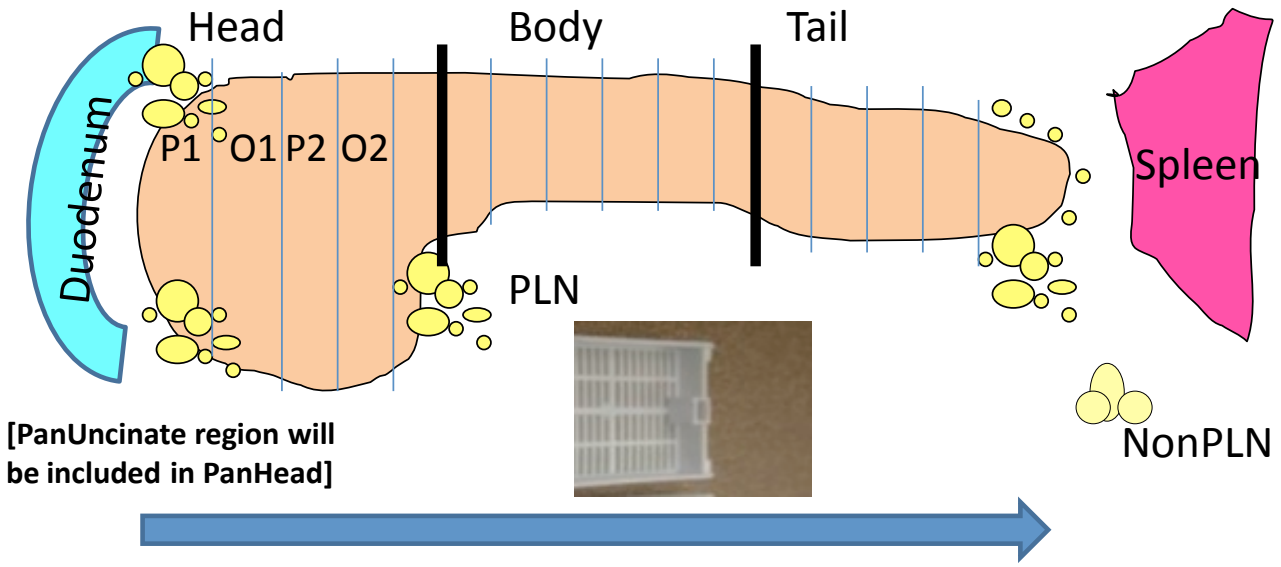


Lay slices to the right and place in cassette in same orientation (label left facing).

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 LNs in 15ml tubes containing sterile RPMI, hold at refrigeration until shipped or processed. 1-4 tubes depending on numbers

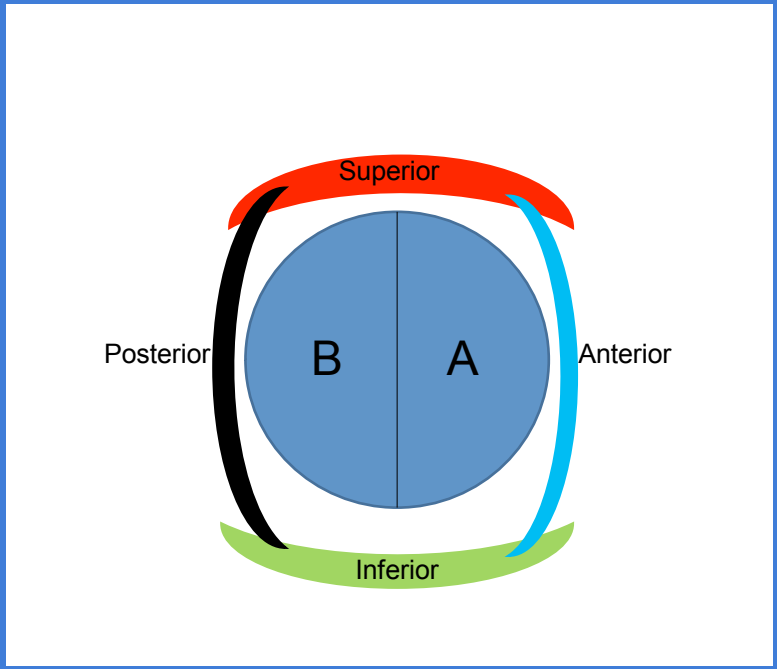
3. Frozen vials as for pancreas. Paraffin is optional.

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

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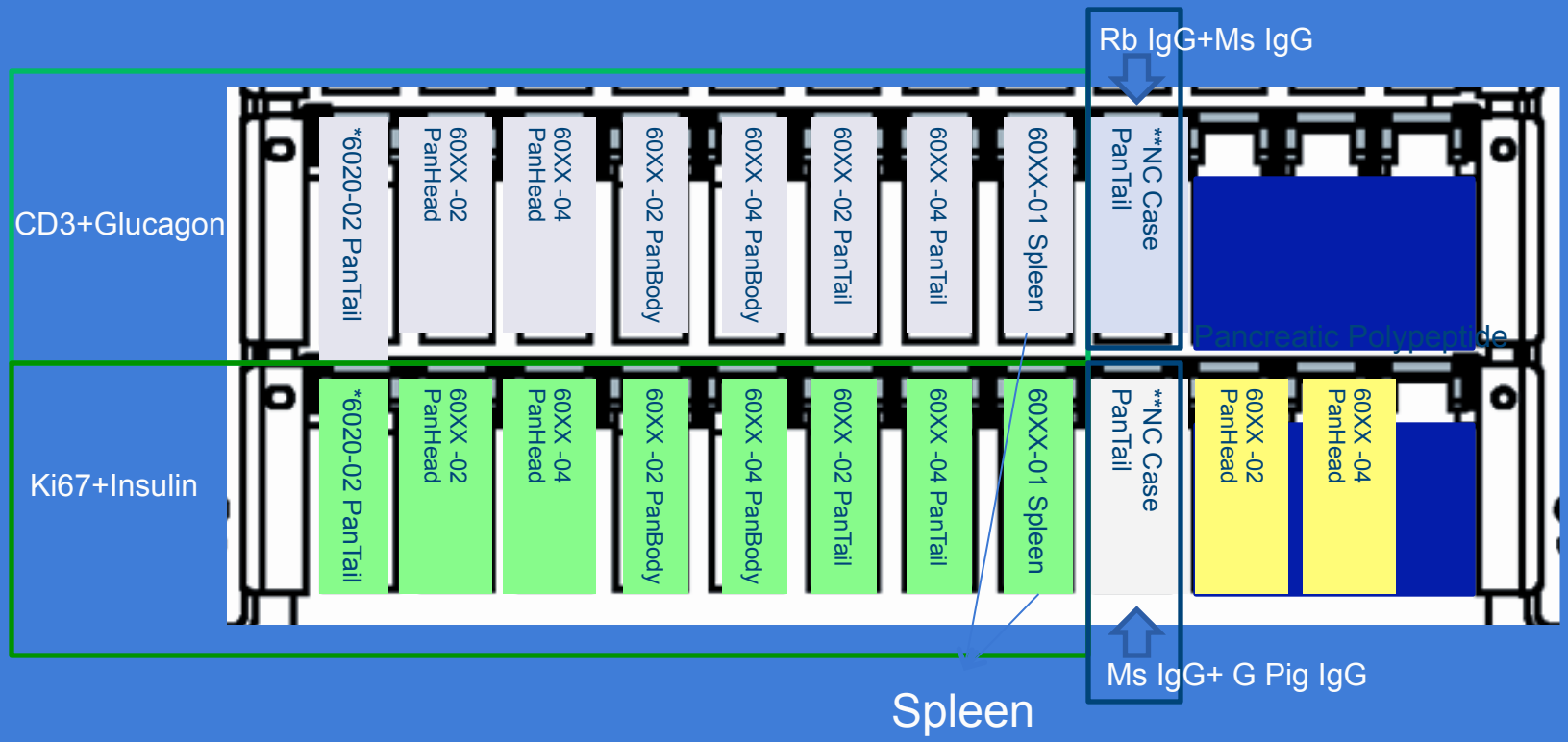
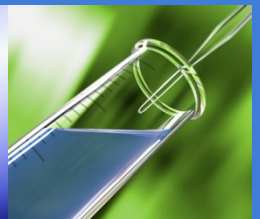
Expanded Phenotyping



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





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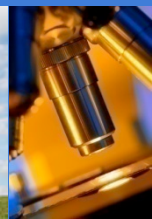

Reconstructions

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

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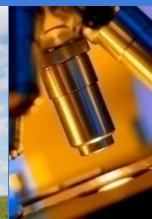
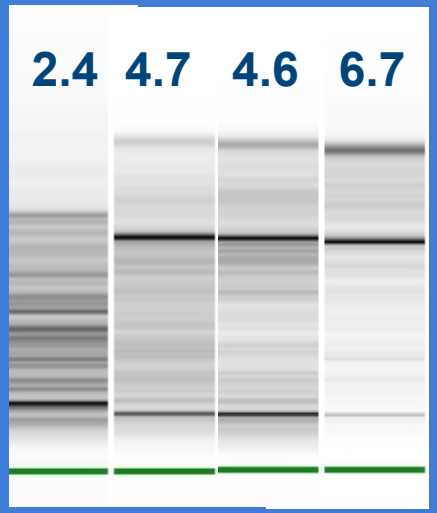


Expanded in-house Analyses

- Pancreas RNA- snap frozen and/or OCT thick sections (Dr. Kusmartseva)



- Cell prep FC-

before cryopreservation

(Dr. Todd Brusko)



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Donors


Donor Numbers
70
60
50
40
30
20
10
0
Transplant
Other
T2D
T1D Medalist
T1D
Autoab Pos
No diabetes
2007
2008
2009
2010
2011
2012 (planned)



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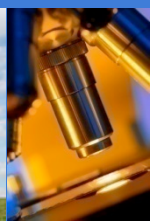

nPOD Case Classification

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

George Eisenbarth 2011



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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



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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




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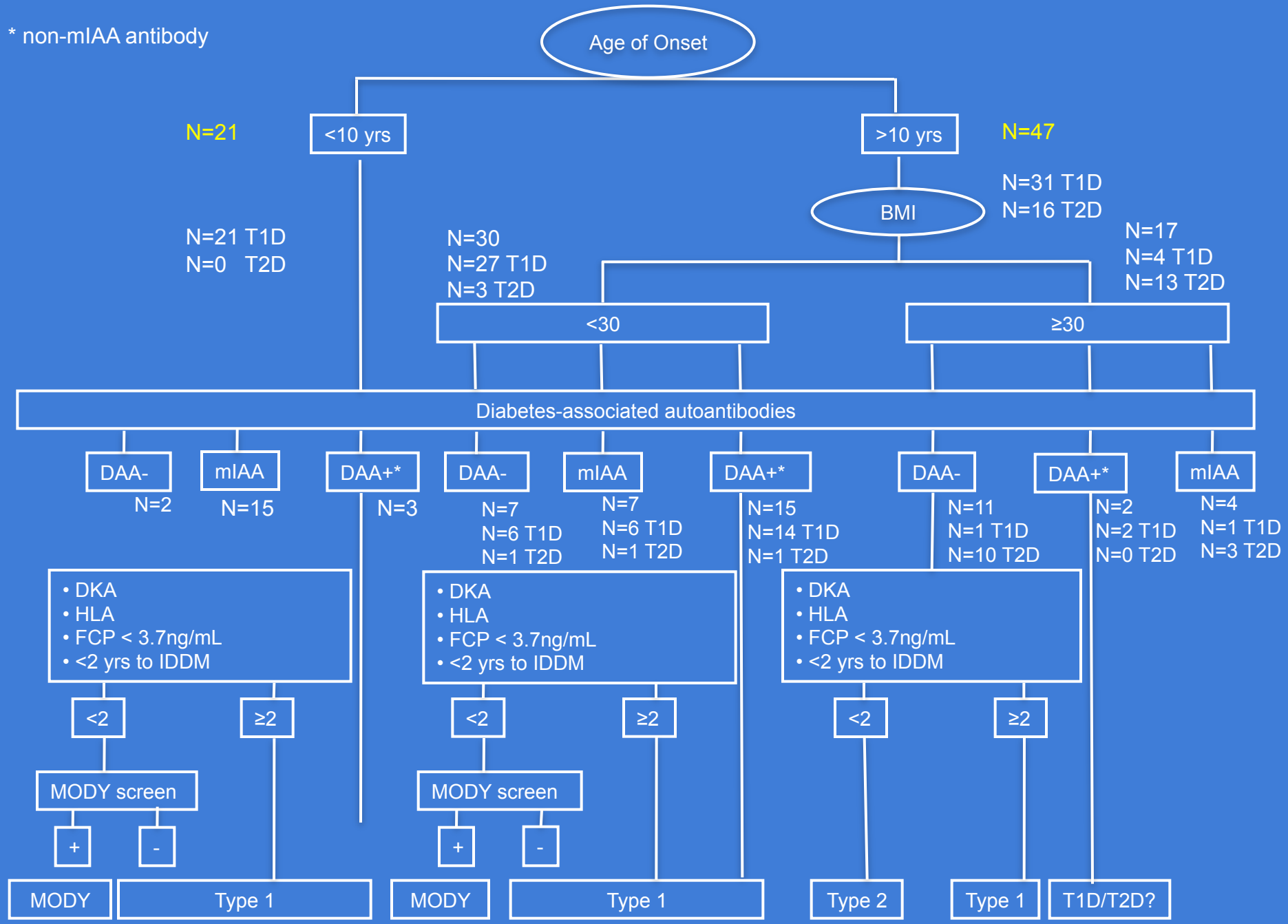
nPOD DIABETES CASES (n=68)



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

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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



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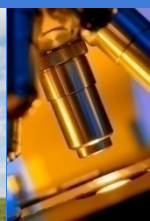

Antibody Screening Strategy Update

Clive Wasserfall, MS
nPOD Autoantibody QA/QC Director



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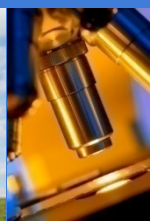

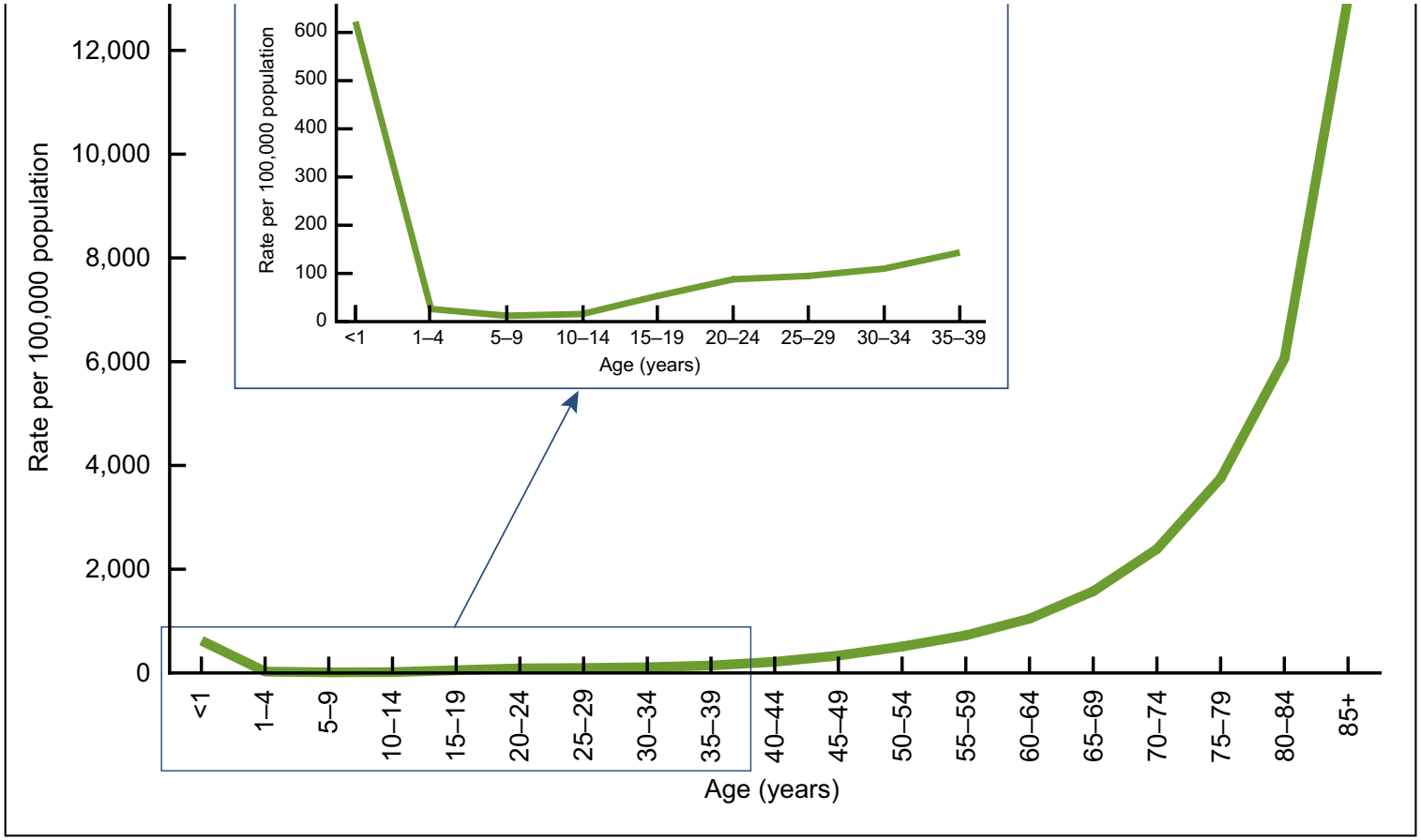

SOURCE: CDC/NCHS, National Vital Statistics System, Mortality.

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.



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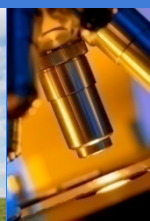
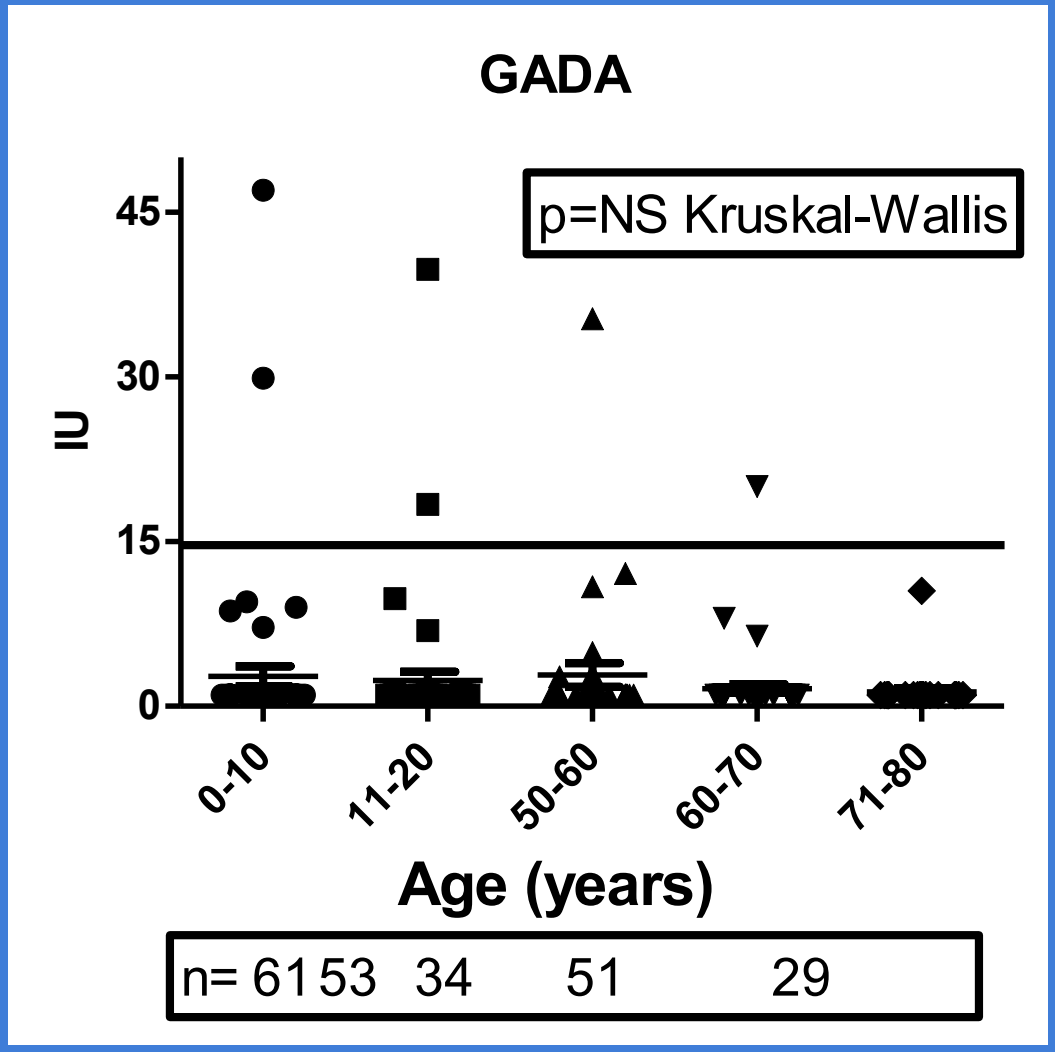


GADA and Age





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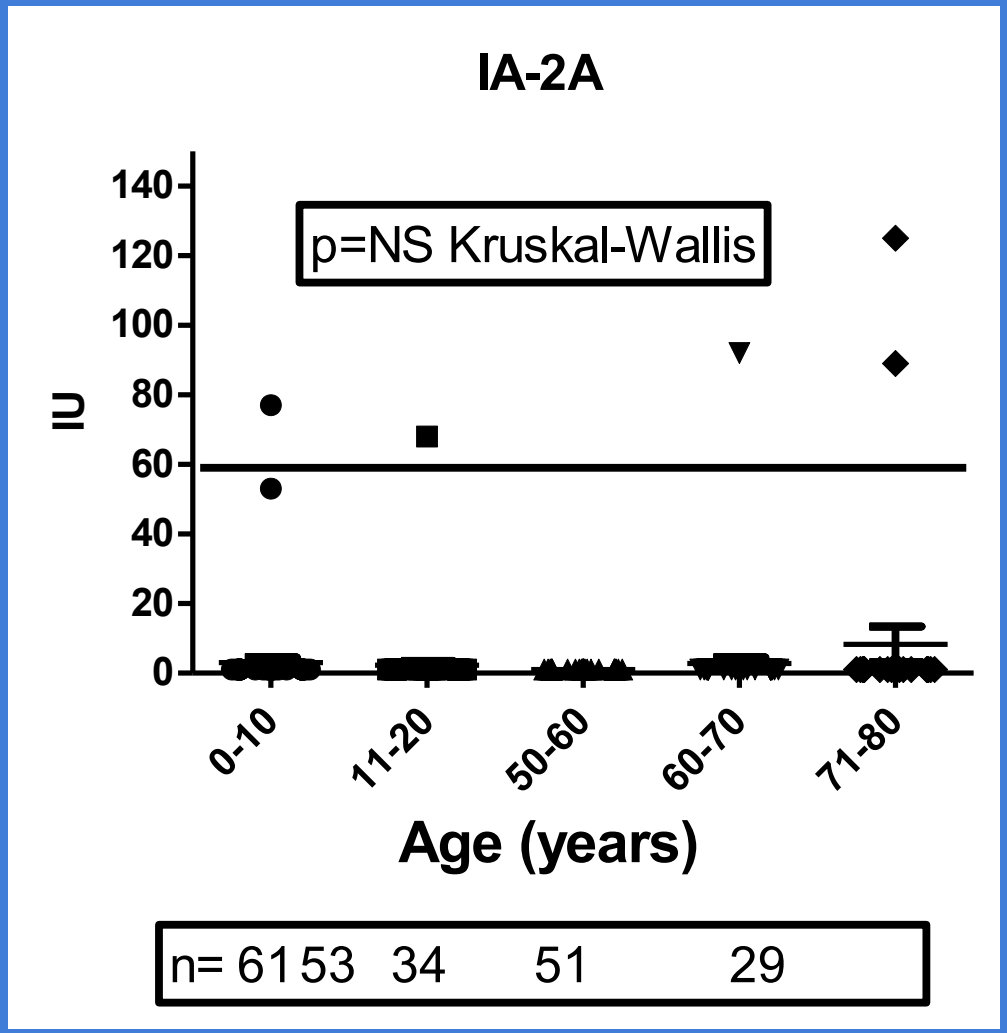
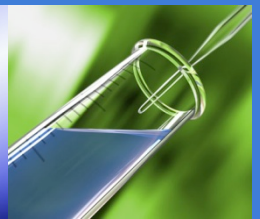
IA-2A and Age







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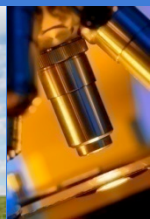
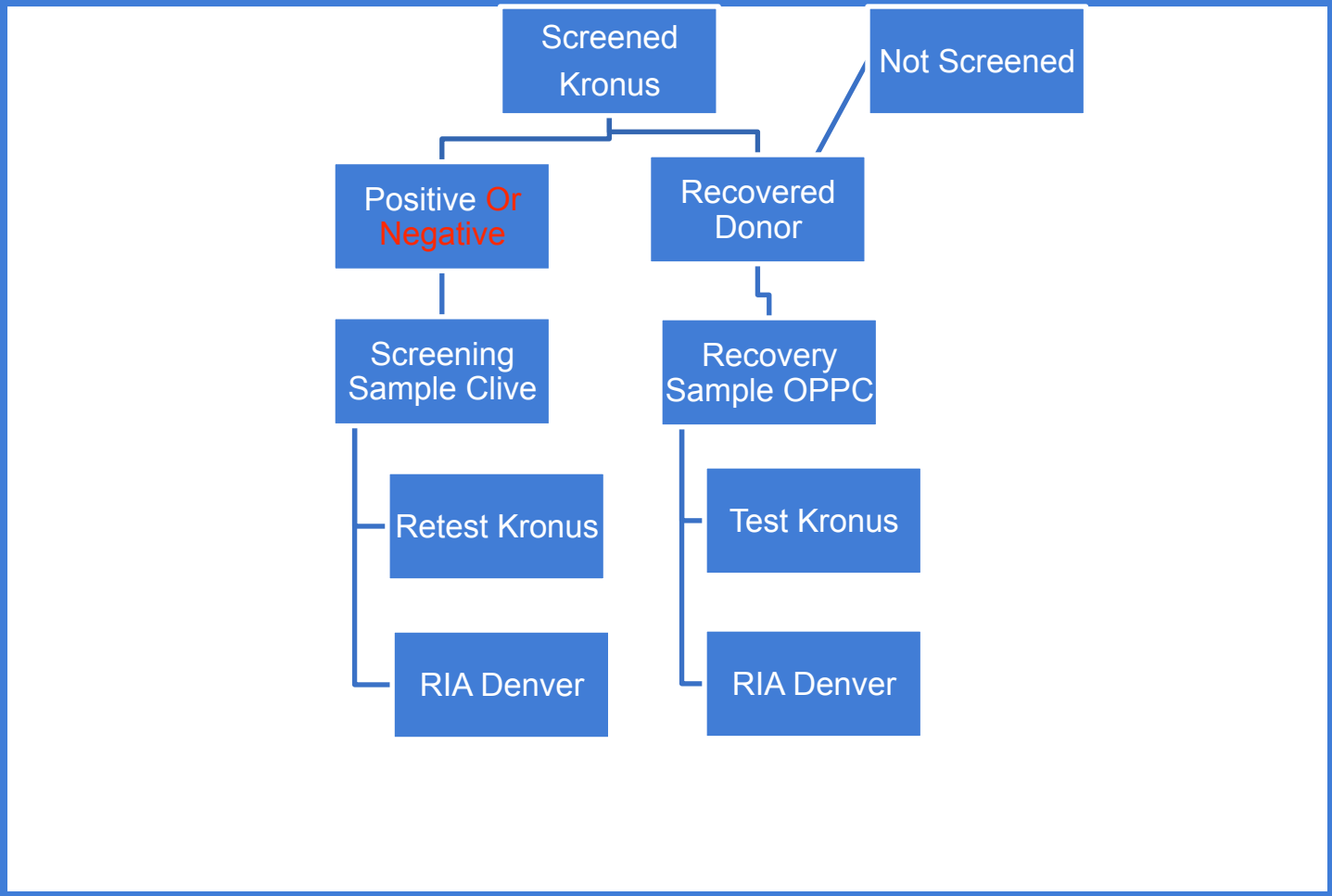
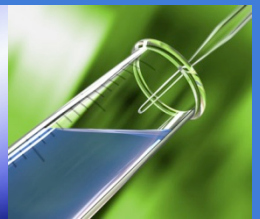


Screening Quality Control







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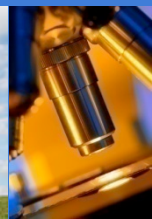

DASP

- 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




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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.




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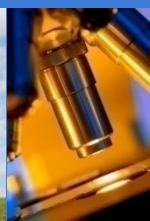

Organ Procurement and Lab Relations

Jayne Moraski, MS
nPOD Assistant Director




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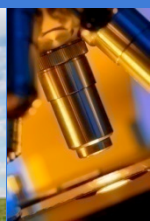
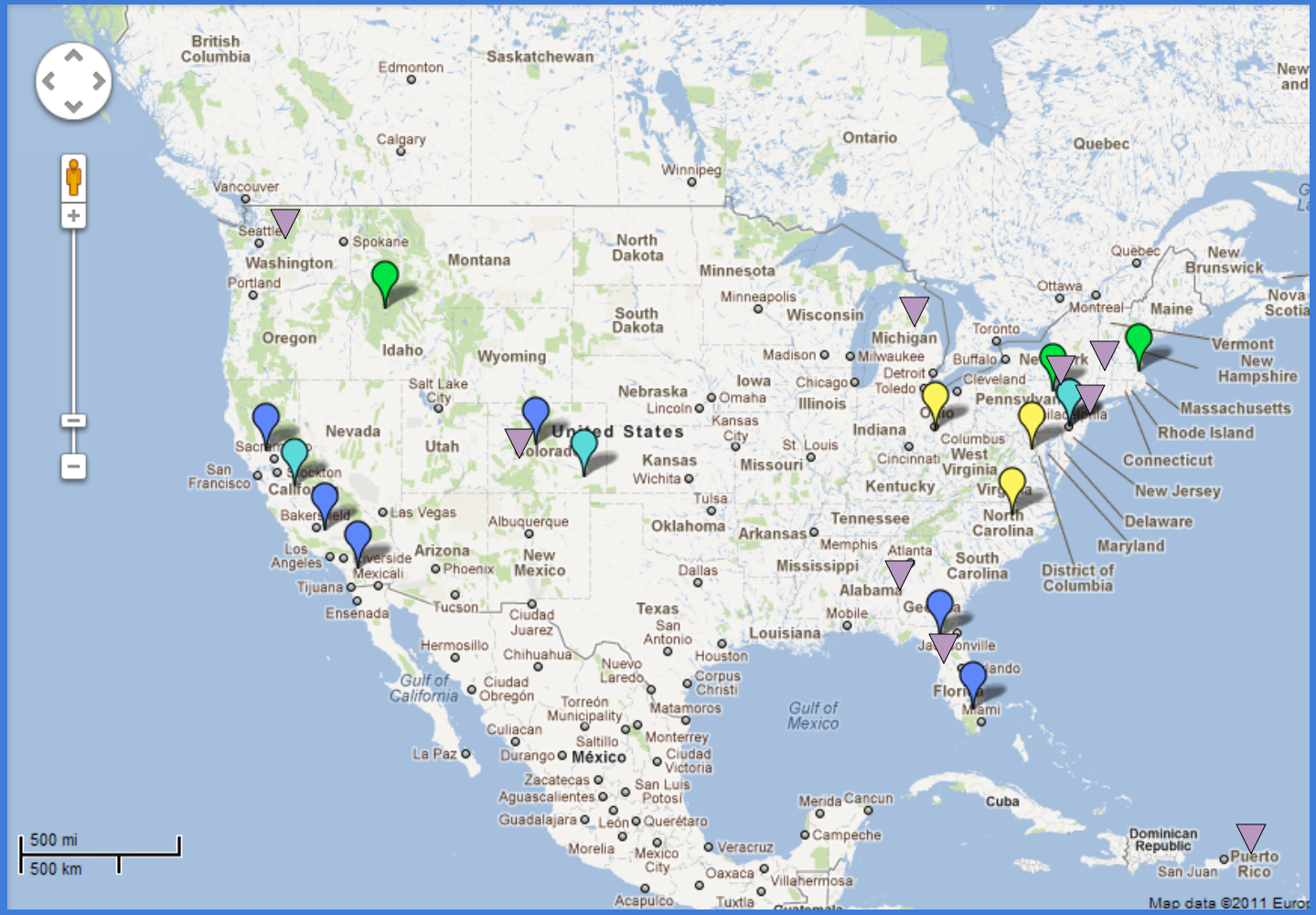


Organ Procurement Partners



Key:

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

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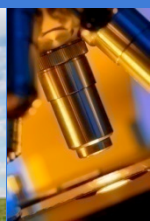

2012 Aab Screening Projection

Laboratory Location	Current Affiliated OPOs	Total Donor Estimate	Screening Start Date
MNIT (Los Angeles)	Golden State Lifesharing OneLegacy Intermountain CTDN (San Francisco) Nevada Donor Network	18 70 240 42 150 20	October 2009 August 2009 November 2009 July 2010 March 2011 March 2011
LABS, Inc.(Philadelphia)	Gift of Life LifeChoice (CT) NJSharing Network New York ODN	180 30 100 150	October 2009 August 2011 September 2011 December 2011
LABS, Inc. (Denver)	Donor Alliance	65	July 2007
Miami	LAORA	30	August 2008
	Total	**1200 projected**	

Summary:

Screened 778 donors in 2010

Screened 1,090 through October of 2011 – projected increase of 68%



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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




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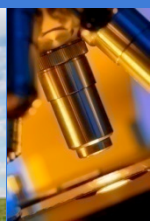

Finding New-Onset Cases: Expanding the Donor Pool through Innovative Collaborations

Suzanne Ball, RN, MHS

nPOD Director



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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



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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




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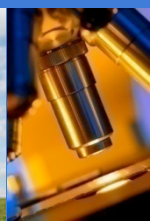

Webinar/Case Discussions

Amy Wright, MS, MBA

nPOD Investigator Relations Coordinator



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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.




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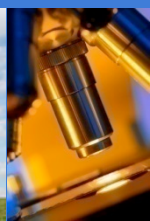
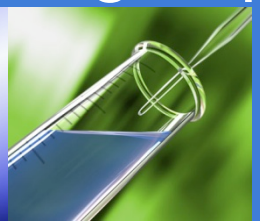


Webinars/Case Discussions

- 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

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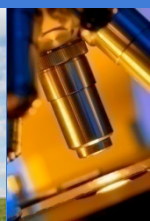

Webinars/Case Discussions

- 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




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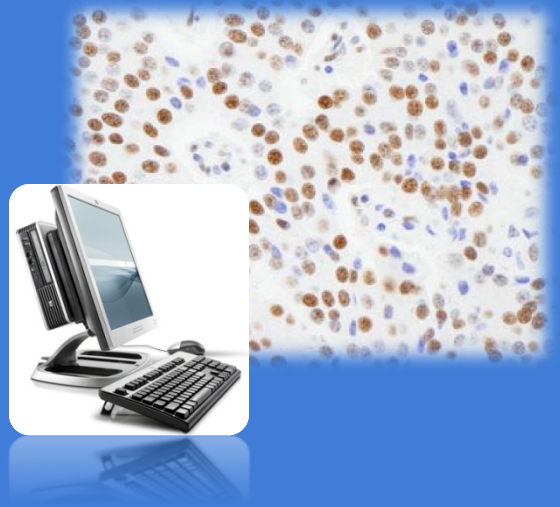
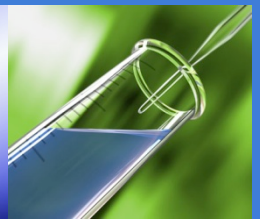


INFORMATION TECHNOLOGY UPDATE

JOHN KADDIS, Ph.D. City of Hope
LES JEBSON, M.H.A. University of Florida

January 17, 2012





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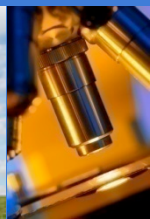
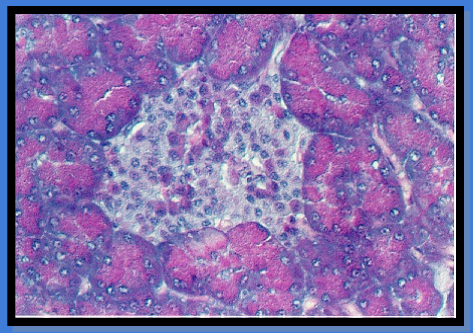


Proposed Data Sharing Platform

- **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



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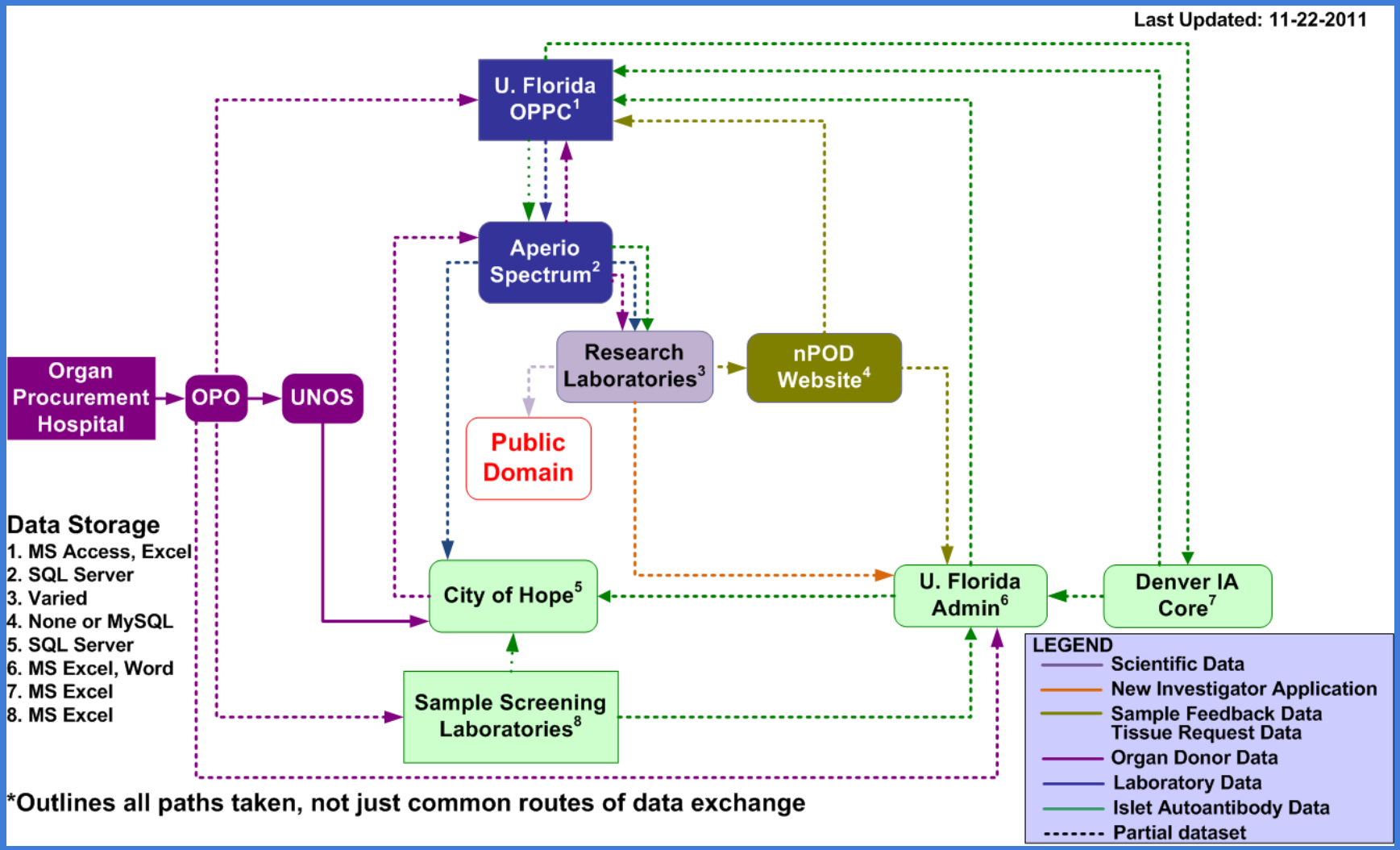
nPOD Data Flow: *Now*





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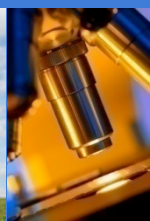

nPOD Data Flow: *The Future*

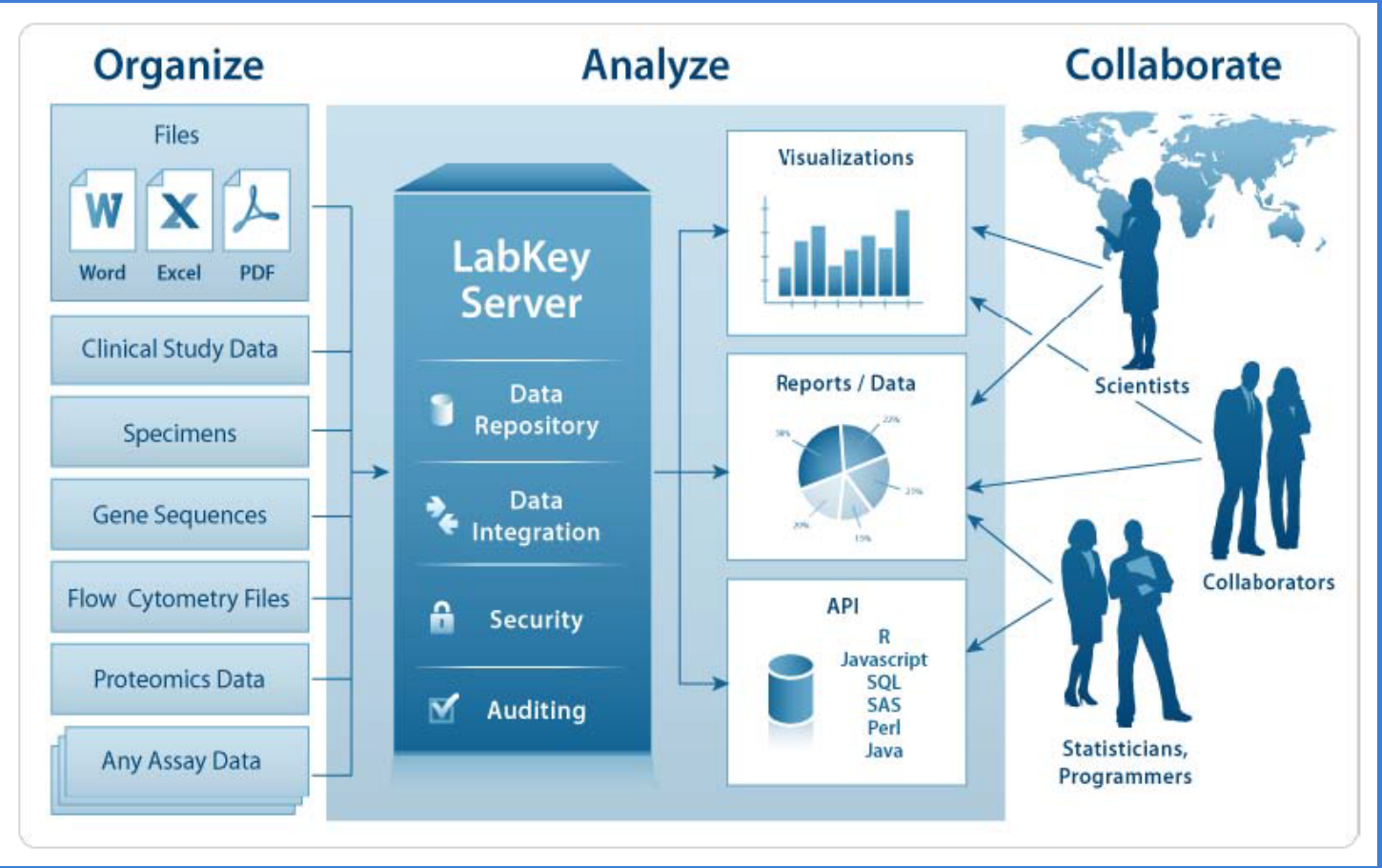

Image used w/permission, Adam Rauch, LabKey Software™



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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



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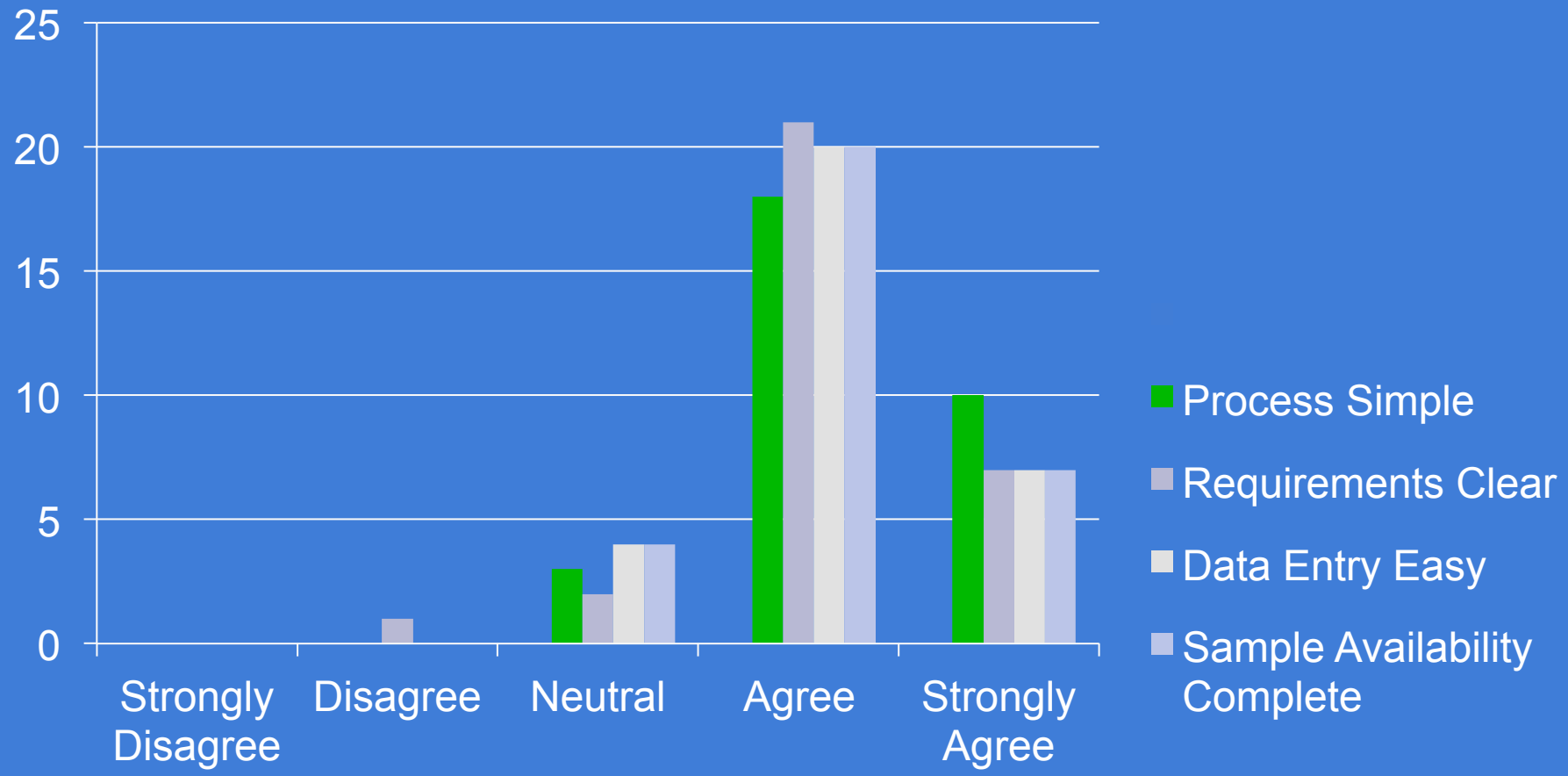
New Investigator Application



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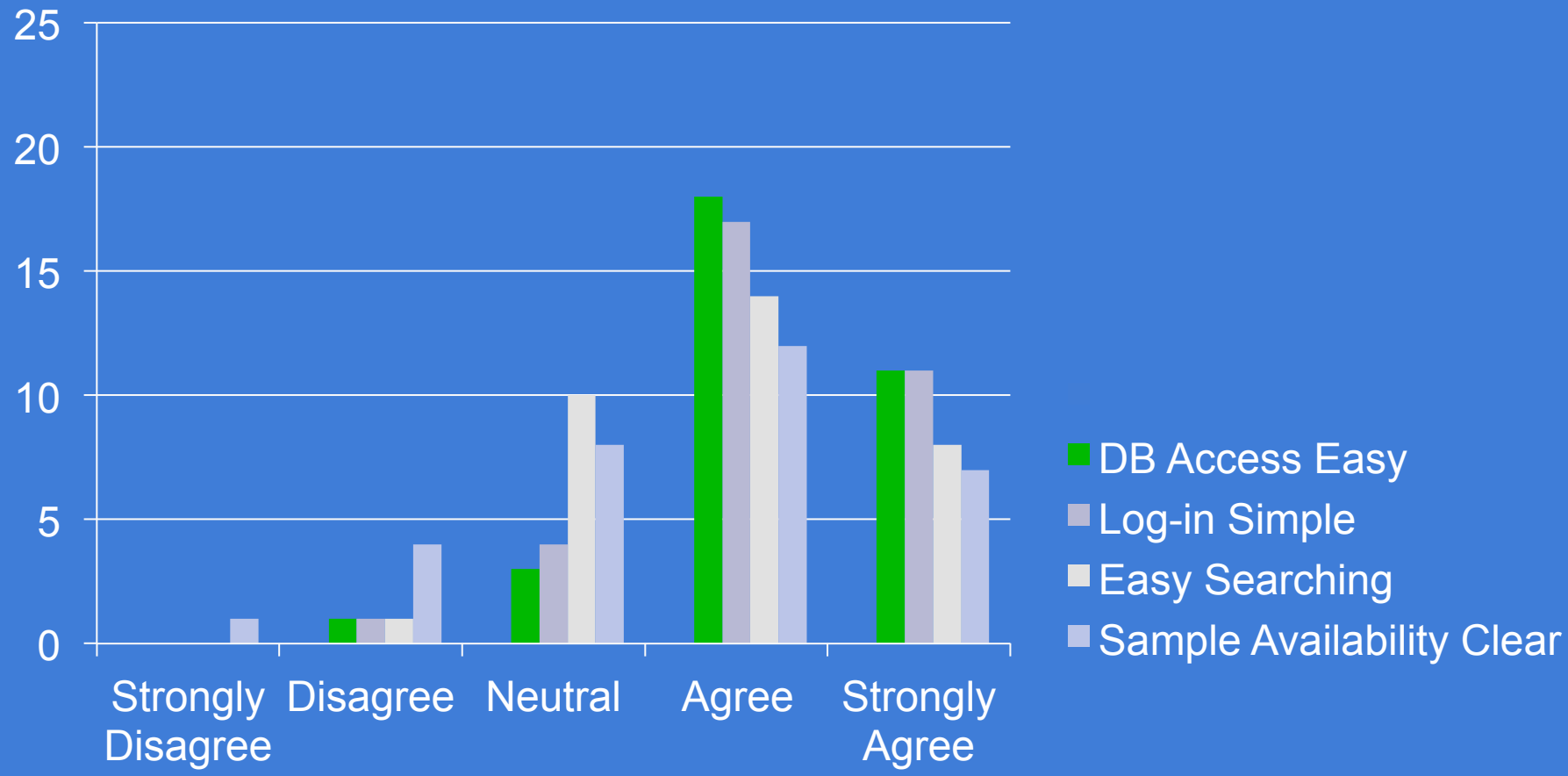
Online Pathology Database



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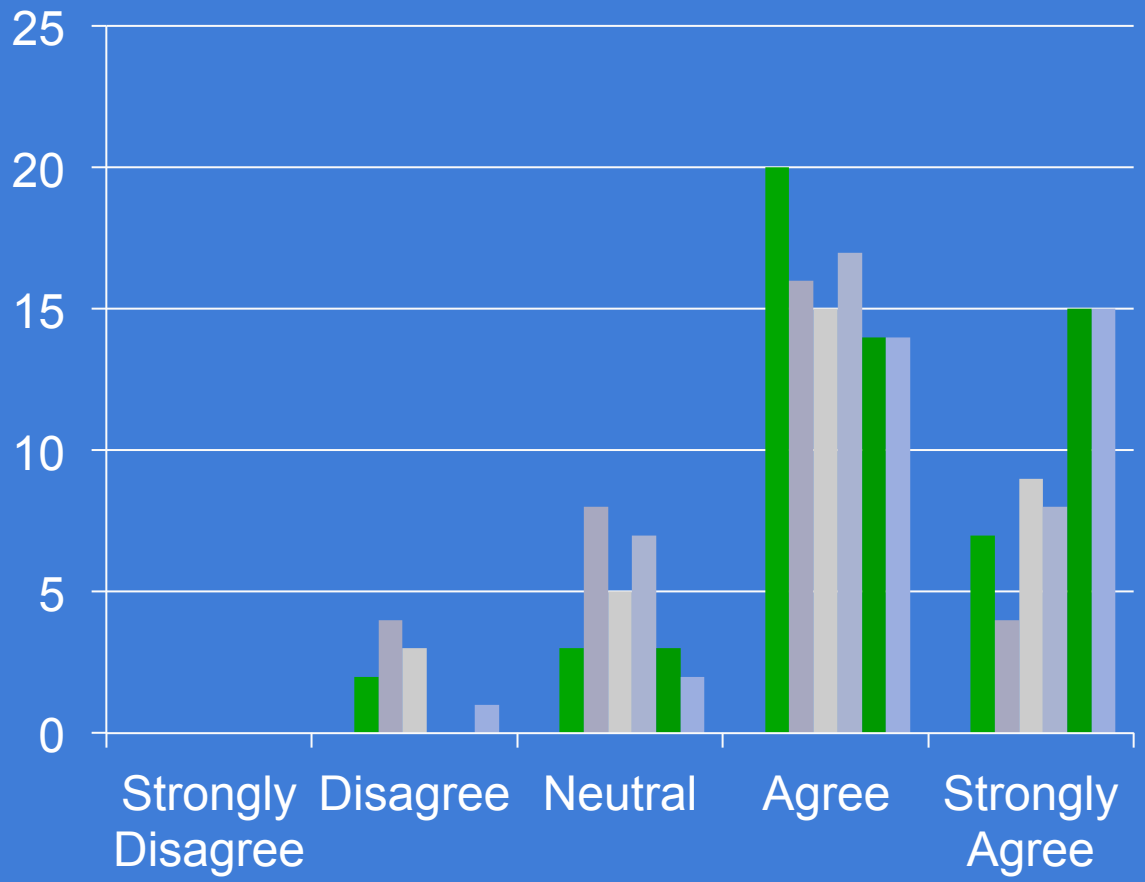
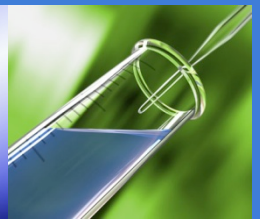
Network for Pancreatic Organ Donors with Diabetes

Service Request Forms (SRFs)





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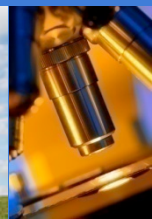
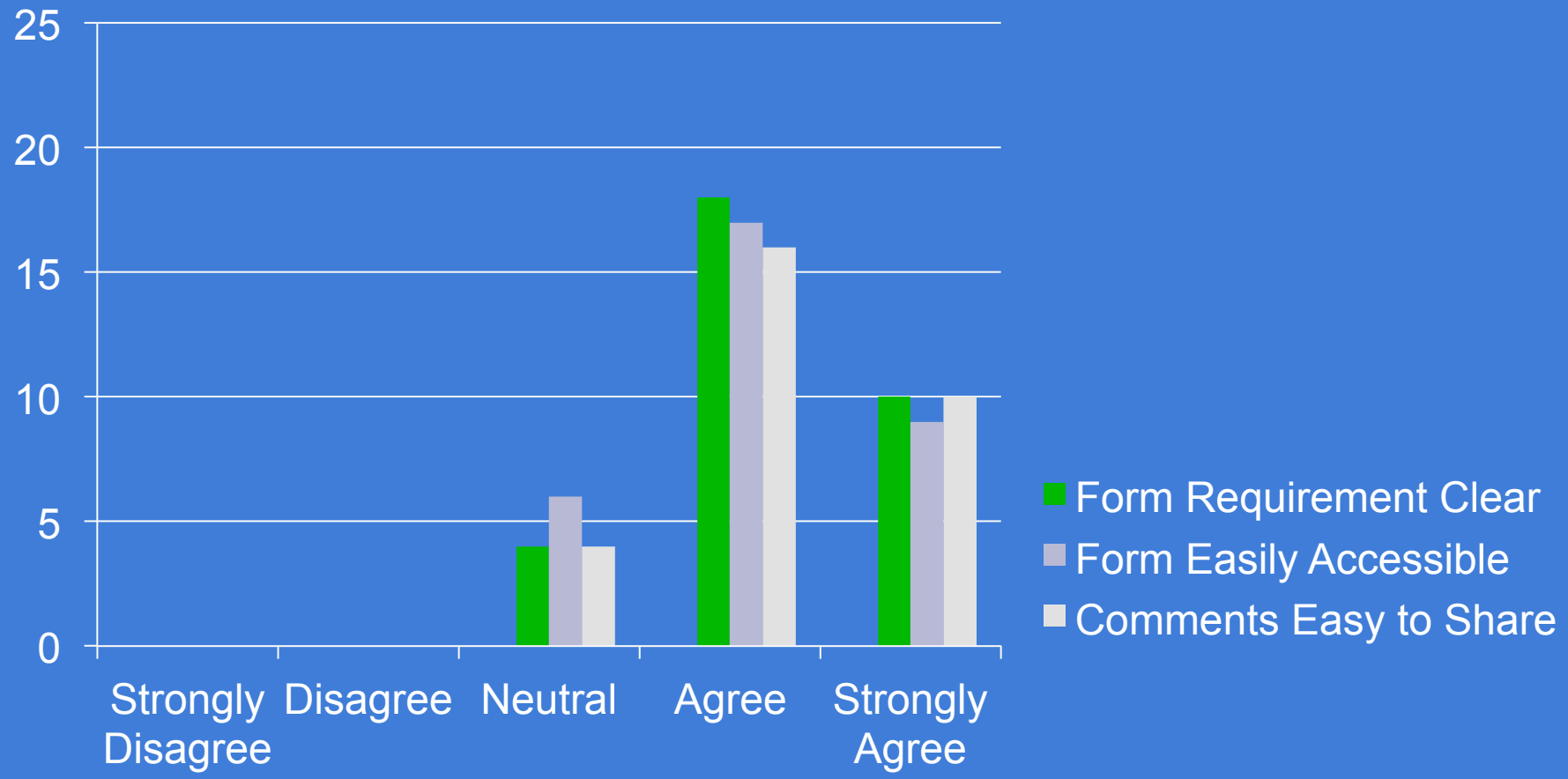
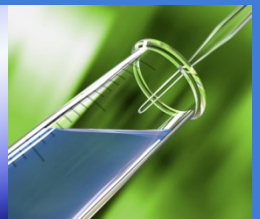


Investigator Feedback Forms





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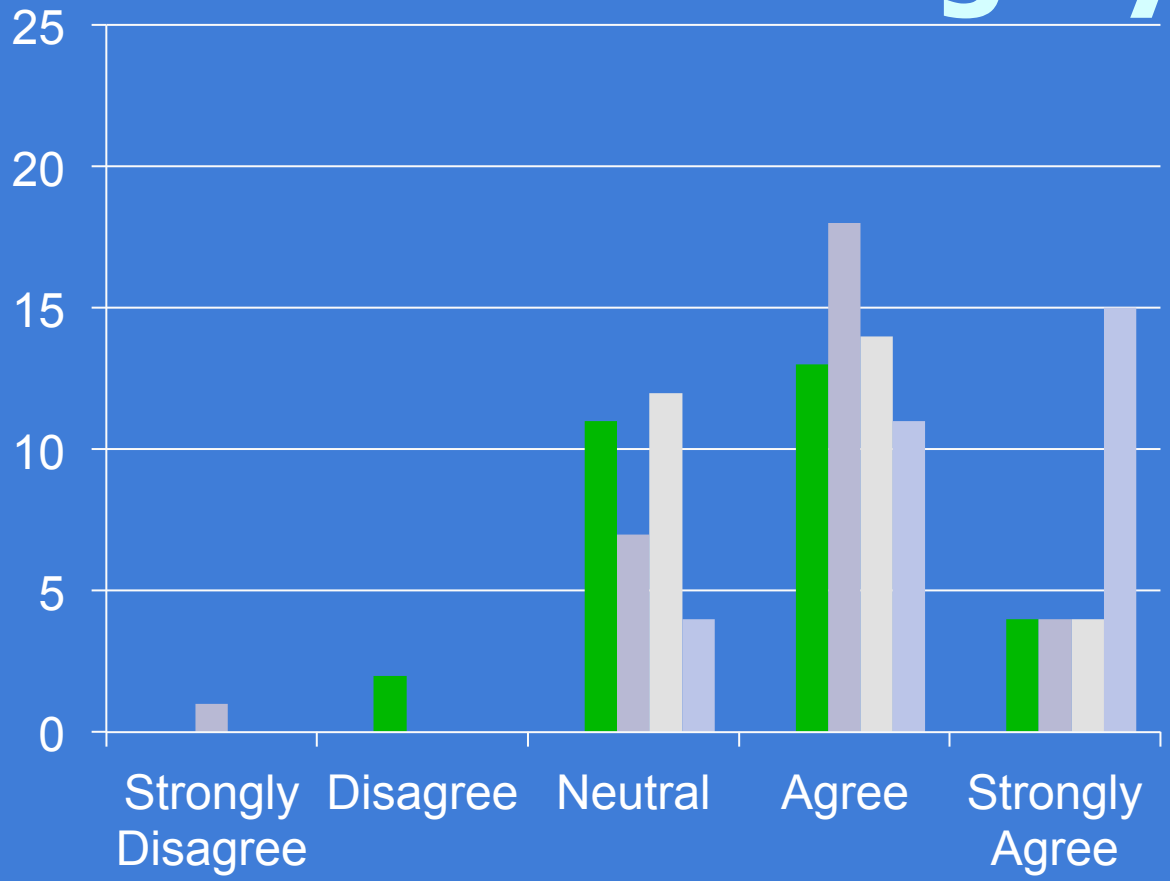
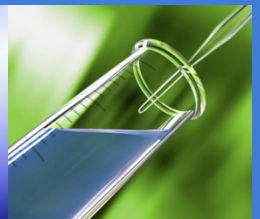
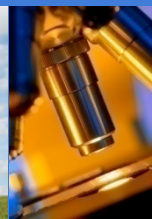
Future Online Data Sharing System





nPOD
Network for Pancreatic Organ Donors with Diabetes



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

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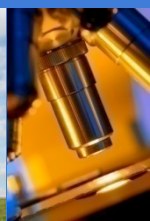

nPOD New Initiatives

- nPOD-Complications
  - Mark Atkinson
- nPOD-E
  - Carmen Retrum
- nPOD-T
  - Alberto Pugliese




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Network for Pancreatic Organ Donors with Diabetes

nPOD-c

Mark A. Atkinson



nPOD

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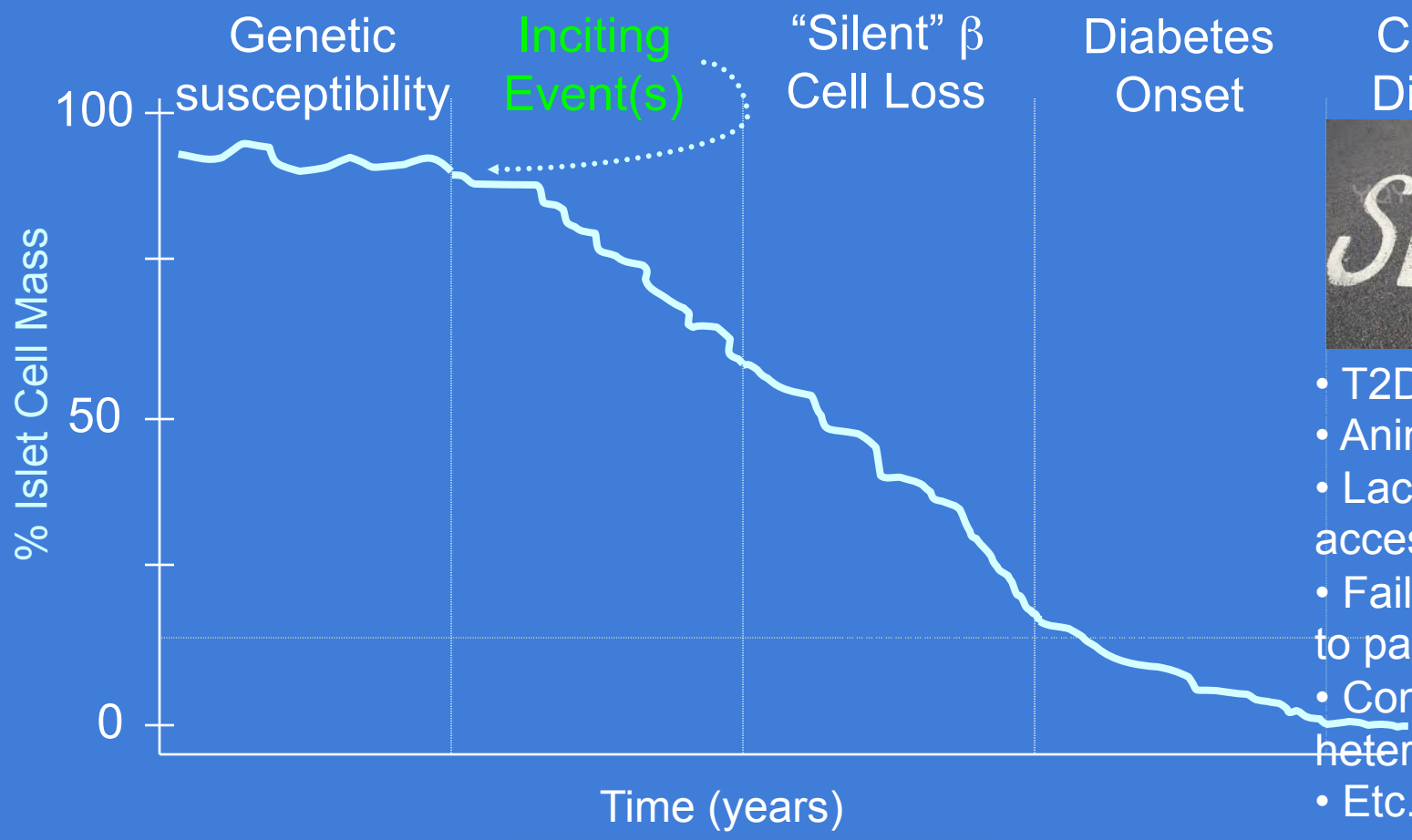
The Natural History of Type 1 Diabetes



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

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nPOD-C

"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




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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



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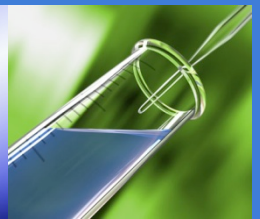
Network for Pancreatic Organ Donors with Diabetes

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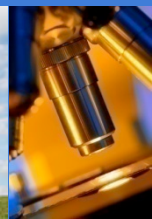
Network for Pancreatic Organ Donors with Diabetes



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:
  - PartialmMedical history
  - Research data
- Serve as a model for other registries/biorepositories in T1D… encouraging openness
- Include analysis of those with T2D

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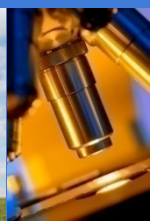
Network for Pancreatic Organ Donors with Diabetes

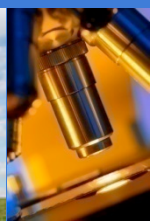
nPOD-E
Europe

Carmen Retrum, M.S.

nPOD Coordinator of Special Projects



nPOD
Network for Pancreatic Organ Donors with Diabetes

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.




nPOD
Network for Pancreatic Organ Donors with Diabetes

Why is nPOD-E Important?

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  3. Provide nPOD tissues and resources to European investigators, as well as those in the U.S.
  4. Facilitate European distribution for perishable specimens




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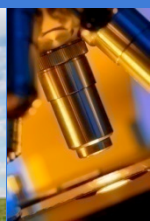

nPOD-E Overview

- 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




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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).




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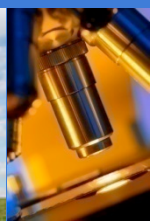

Established 2 New Sites in Europe

- **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.




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Inbound Processes

Case Recovery & Processing
(Per nPOD SOP)

All other tissues

Stored and managed at nPOD-E Site

Slides

To nPOD OPPC for immunopathology per SOP and scanned to Aperio

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



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Outbound Processes

Investigator Request

TPC/OPPC review

Notifies site

nPOD-E Site distributes tissue

*OPPC views tissue availability via database; site inputs distribution



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nPOD-E Projections

Individual Site Expectations for 2012

	AAb Screens	AAb Cases	T1D Cases	Total Cases
Spain	70-100	1-3	0-3	2-5
Italy	400+	4-6	2-5	7-9
TOTAL	500	6	6	**12 projected**




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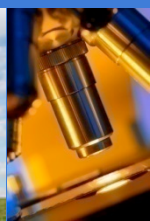

nPOD-T Transplantation

Alberto Pugliese, MD
nPOD Co-Executive Director



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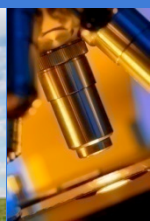
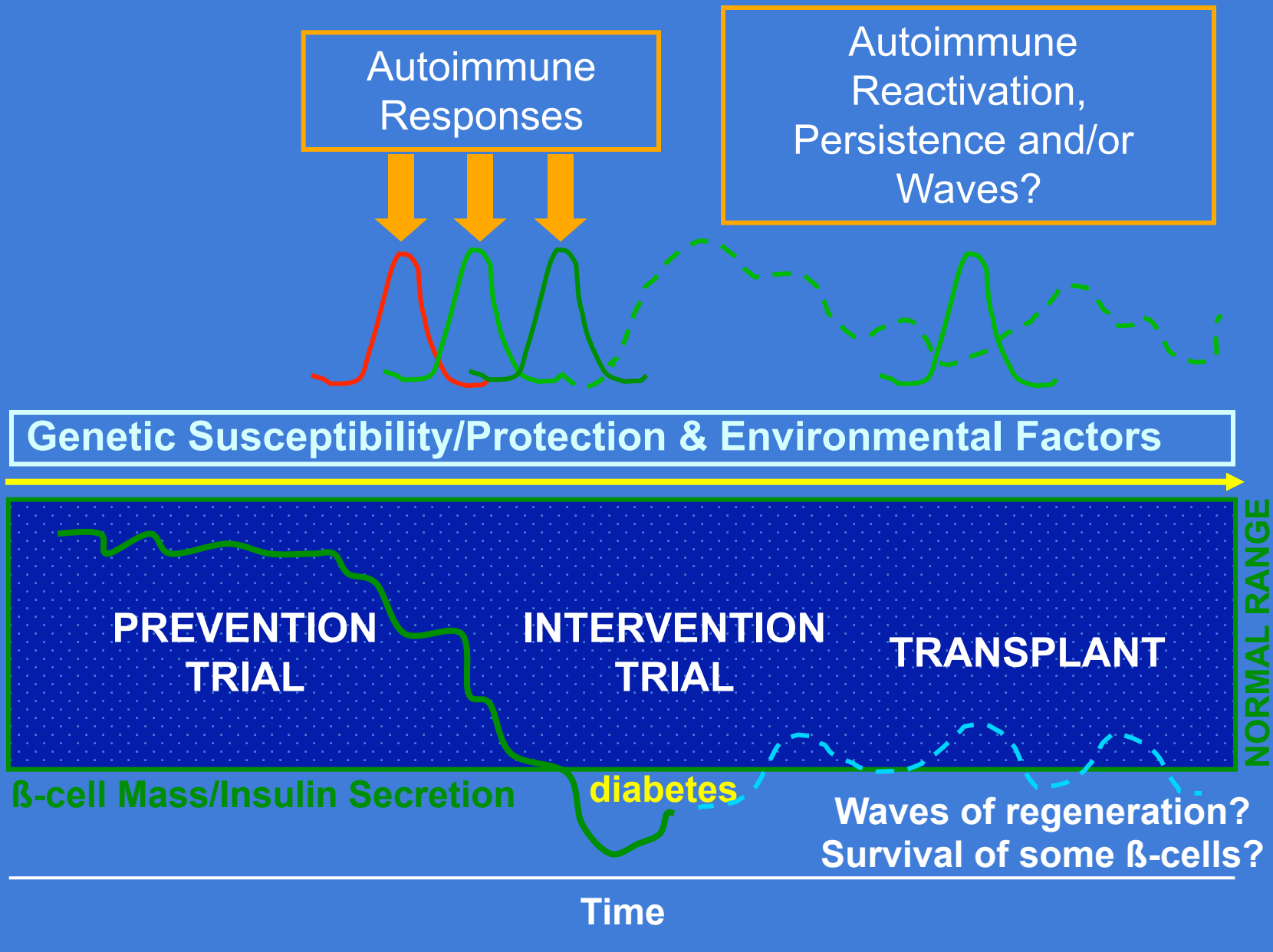
Network for Pancreatic Organ Donors with Diabetes

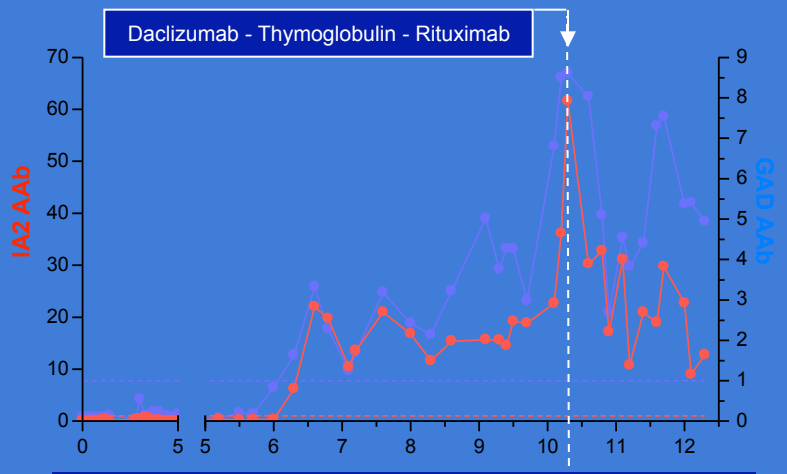



The Type 1 Diabetes Spectrum

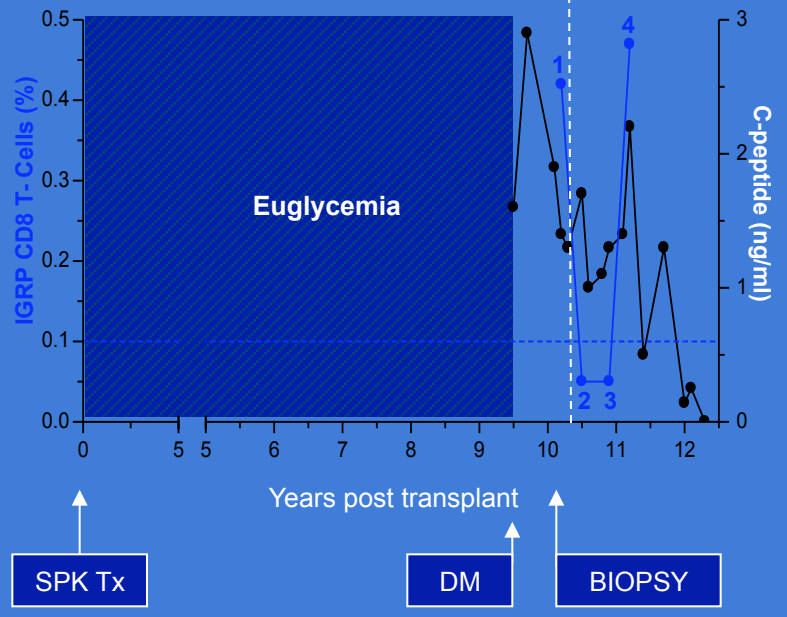


Patient 2 - SPK-3601

A. AUTOANTIBODIES



B. C-PEPTIDE AND AUTOREACTIVE T CELLS



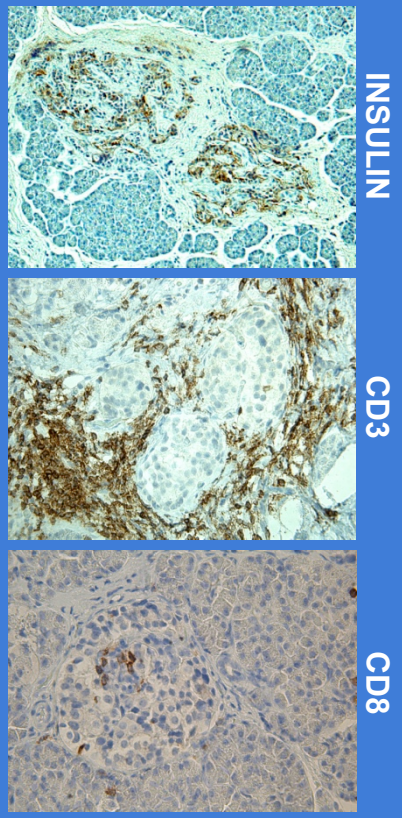
Diabetes 59:947–957, 2010

ORIGINAL ARTICLE

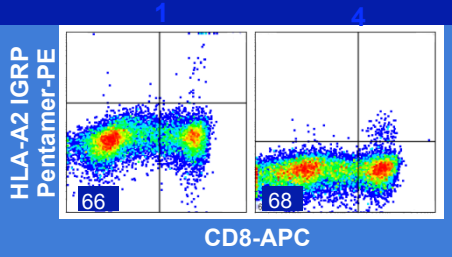
Recurrence of Type 1 Diabetes After Simultaneous Pancreas-Kidney Transplantation, Despite Immunosuppression, Is Associated With Autoantibodies and Pathogenic Autoreactive CD4 T-Cells

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,[3,4] Kelly Geubtner,[3] Ben A. Falk,[3] Hirohito Ichii,[1,2] Hidenori Takahashi,[2] Isaac Snowhite,[1] Zhibin Chen,[5] Armando Mendez,[1,6] Linda Chen,[2] Junichiro Sageshima,[2] Phillip Ruiz,[2] Gaetano Ciancio,[2] Camillo Ricordi,[1,2,5,6] Helena Reijonen,[3] Gerald T. Nepom,[3] George W. Burke III,[1,2] and Alberto Pugliese[1,5,6]

C. PANCREAS TRANSPLANT BIOPSY

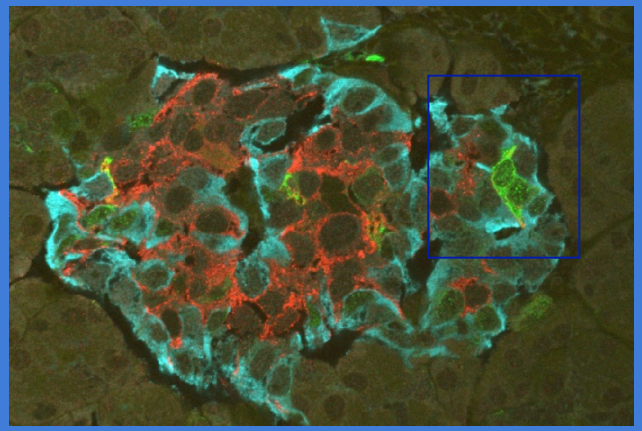


D. AUTOREACTIVE T CELLS

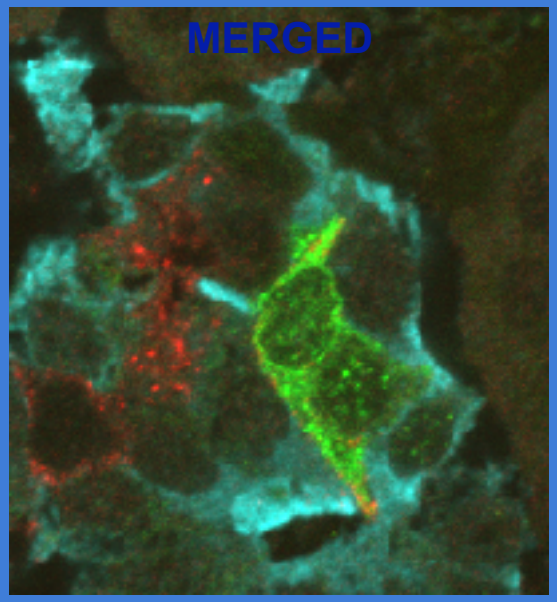


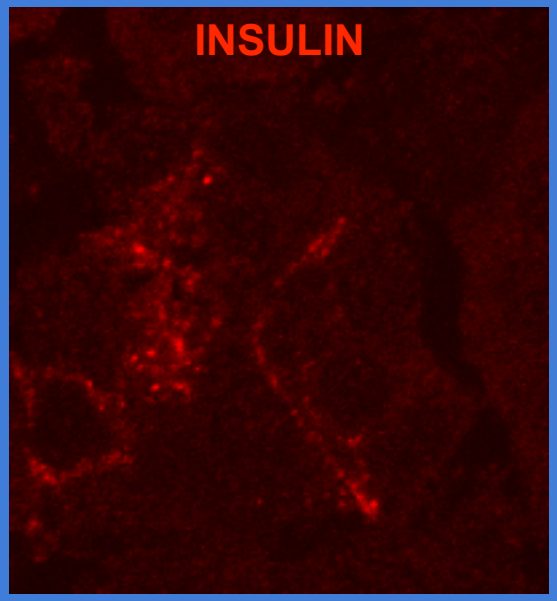
Patient 2 (MSS), pancreas transplant biopsy

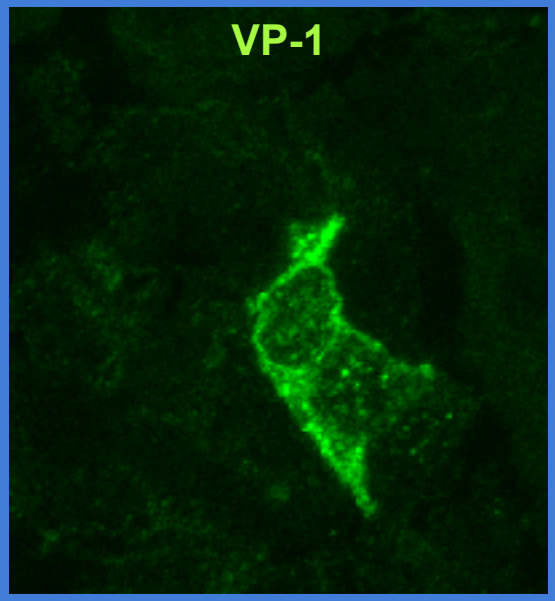
Left panel: a pancreatic islet stained for insulin (red), glucagon (light blue) and VP-1 (green).



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.





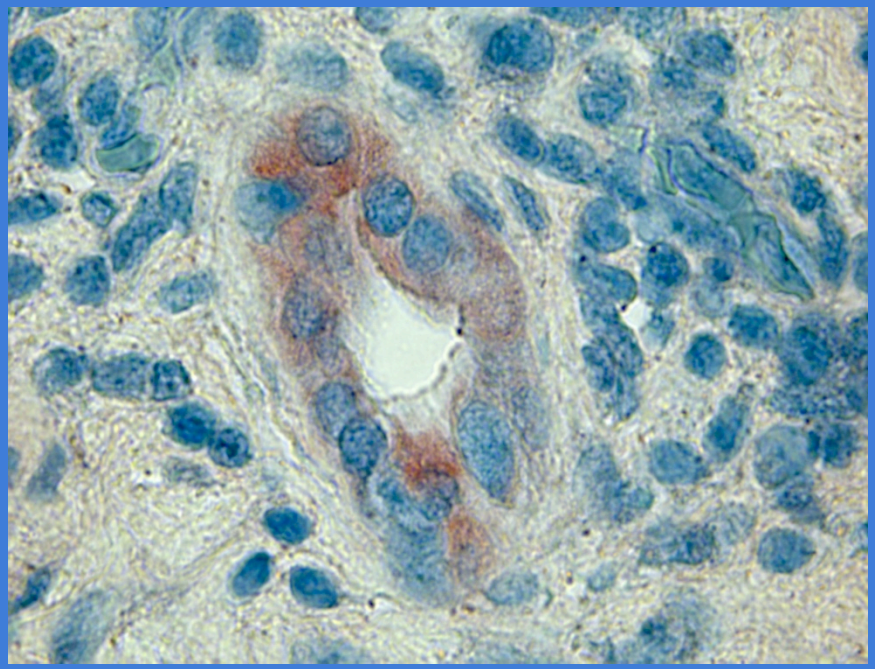


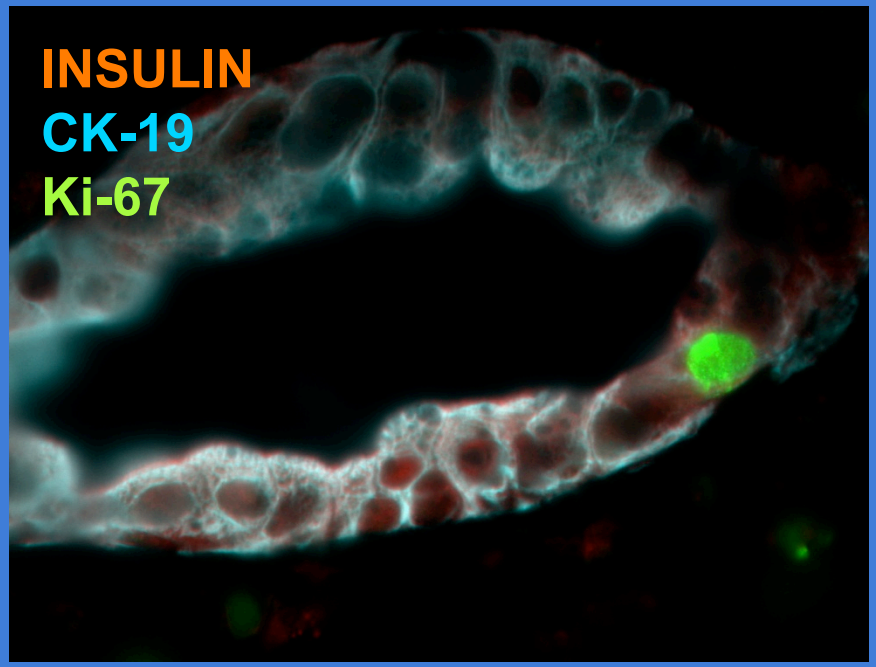
Vendrame et al. Diabetes 2010

Insulin protein and proliferation in ductal cells in the transplanted pancreas of patients with type 1 diabetes and recurrence of autoimmunity

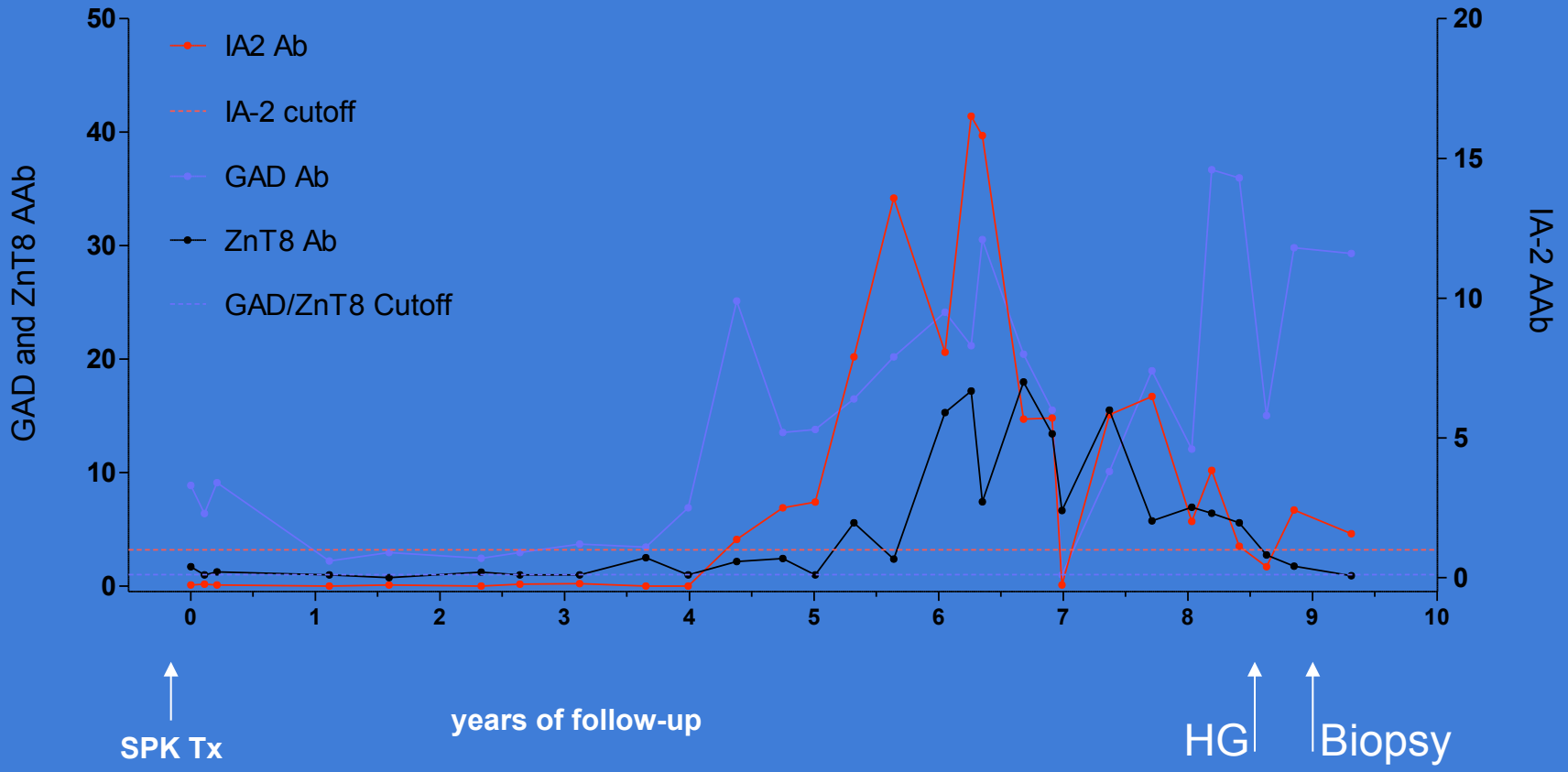
A. Martin-Pagola · G. Sisino · G. Allende · J. Dominguez-Bendala · R. Gianani · H. Reijonen · G. T. Nepom · C. Ricordi · P. Ruiz · J. Sageshima · G. Ciancio · G. W. Burke · A. Pugliese

Received: 25 March 2008 / Accepted: 27 June 2008 / Published online: 12 August 2008





SPK IM-203
HLA A2, B8, DR3; A26, B72, DR7



- 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

unpublished

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

PBMC

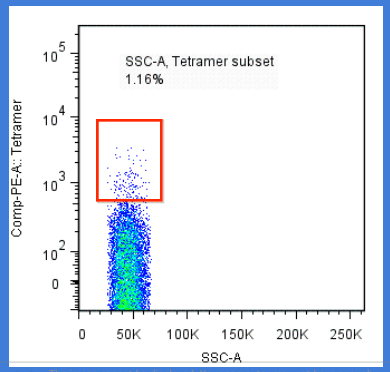
PLN

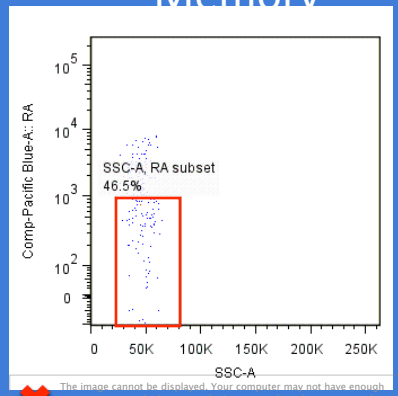
Pancreas

A2-GAD pentamer

CD45-RA

Memory





18.5%

39.1%

0.9%

61.2%

SSC

SSC

unpublished

SPK IM-203

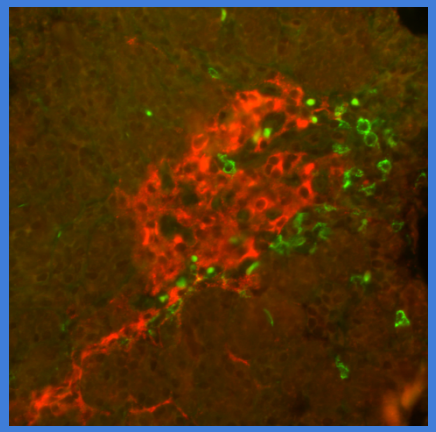
	BLOOD GLUCOSE (mg/dl)		C-PEPTIDE (ng/ml)	
	MMTT	OGTT	MMTT	OGTT
• -10’	192	198	1.28	1.05
• 0’	nd	196	1.31	0.99
• 30’	265	273	2.71	1.08
• 60’	280	410	2.99	1.17
• 90’	302	453	2.63	1.19
• 120	294	477	2.33	1.27

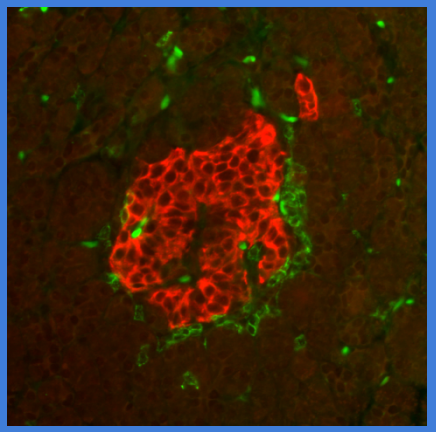
MMTT 07/29/2011 HbA1c 10.7%

BIOPSY 08-10-2011

OGTT 10-4-2011 HbA1c 8.9%

INS CD8





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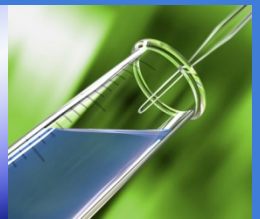
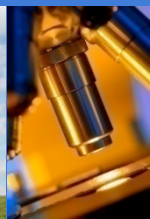


nPOD

Network for Pancreatic Organ Donors with Diabetes



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
Network for Pancreatic Organ Donors with Diabetes


nPOD-T

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
Network for Pancreatic Organ Donors with Diabetes

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

results, data, samples, study coordination interactions

nPOD - Gainesville

Functions
-Donor tissue procurement, archival storage, sample distribution to investigators
-Histology
-Data management

Structure
-PI (Campbell-Thompson)
-Pathology Staff
-Administrative Staff (Atkinson)

consent forms, IRB protocols, AAb results, coordination →

← Serum samples, medical data, documents

Tissues from donors (via OPO), from biopsies, archived biopsy specimens ←

Specimens, data ↔

Transplant Centers

- Indianapolis
- Miami
- Minneapolis

nPOD Investigators

Conclusion

Thank you to all of you.

Thank you JDRF.

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

Questions?



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