CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

itun institut transplantation urologie néphrologie INSERM - UMR 1064

UNIVERSITÉ DE NANTES

Sudden and Severe Hyperglycemia6 years after SPK

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ITUN, Nantes University Hospital, France

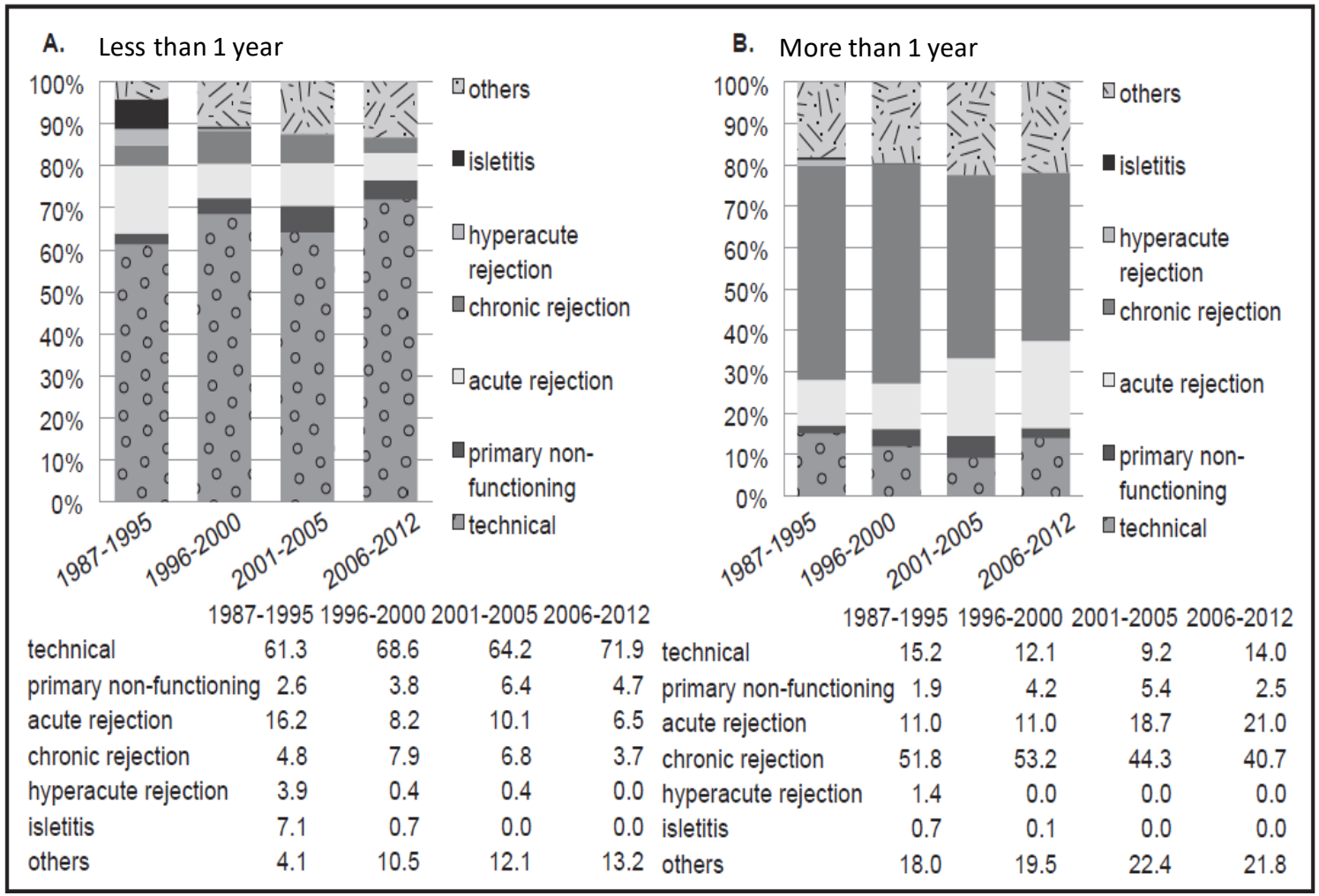
Barcelona, March 2016

INTRODUCTION

Type I Diabetes Mellitus

- Auto-immune disease
- Early onset in life
- Specific adaptative immunity against β-cell antigens
- Islet-specific T-cell attack and β-cell destruction
- Clinical manifestations occur after 90% of β-cell mass loss
- Auto-antigens:
  - anti-insulin
  - anti-IA2
  - anti-GAD 65
  - anti-ZnT8
- Vital need for therapy: insulin, transplantation

Causes of Graft Loss



	1987-1995	1996-2000	2001-2005	2006-2012
technical	61.3	68.6	64.2	71.9
primary non-functioning	2.6	3.8	6.4	4.7
acute rejection	16.2	8.2	10.1	6.5
chronic rejection	4.8	7.9	6.8	3.7
hyperacute rejection	3.9	0.4	0.4	0.0
isletitis	7.1	0.7	0.0	0.0
others	4.1	10.5	12.1	13.2

	1987-1995	1996-2000	2001-2005	2006-2012
technical	15.2	12.1	9.2	14.0
primary non-functioning	1.9	4.2	5.4	2.5
acute rejection	11.0	11.0	18.7	21.0
chronic rejection	51.8	53.2	44.3	40.7
hyperacute rejection	1.4	0.0	0.0	0.0
isletitis	0.7	0.1	0.0	0.0
others	18.0	19.5	22.4	21.8

Waki et al, Clin Transpl 2013

CASE REPORT

- Woman from Algeria, 35year-old
- Diagnosis of IDDM at 5 year-old
- No assessment of auto-antibodies
- Negative C-peptide
- Frequent, severe hypoglycemic events (6-month ICU stay due to hypoglycemic-induced coma)
- Macro-angiopathy: not advanced
- CKD stage 4 (créatinine 250 µmol/L; e-GFR 21 ml/min)
- Severe retinopathy (blind)
- Sensitive advanced neuropathy
- Asthma
- Hypertension
- Smoking
- Depression
- BMI of 23 (45 kg/151 cm)

SPK, August, 2010

Donor

- Deceased male donor, 23 year-old
- Cold ischemia 11 h (pancreas), 12 h (kidney)
- 5 HLA mismatches
- CMV D+/R+

	A		B		DR		DQ	
Recipient	01	02	41	49	04	07	02	08
Donor	01	/	37	57	07	15	03	01

Recipient

- No anti-HLA antibodies
- Negative LCT cross-match
- No auto-antibodies
- Duodeno-enteric anastomosis
- Portal diversion

Immunosuppression

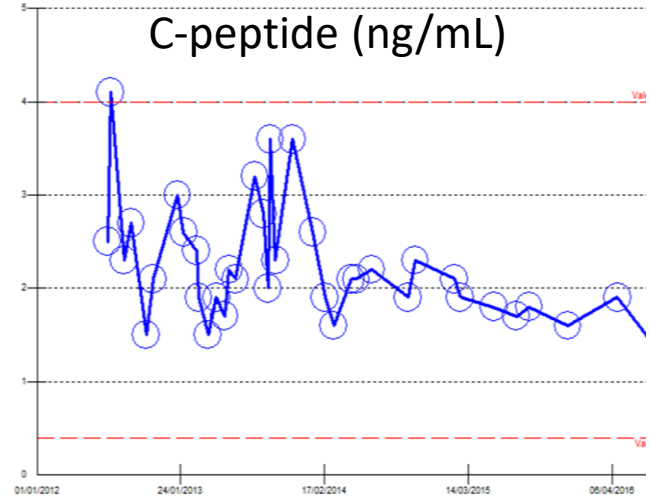
- Thymoglobulin induction
- Tacrolimus/Mycophenolate mofetil
- Steroid-free since Tx

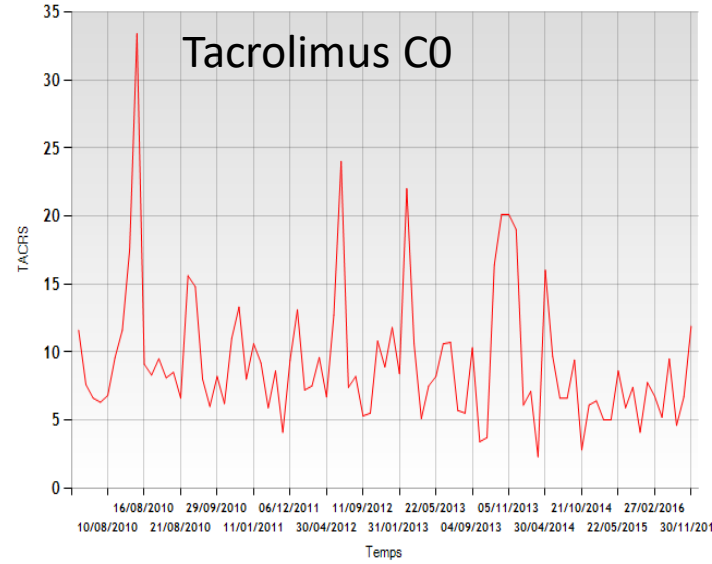
F/U

- No immediate complication, creatinine 77 µmol/L, no insulin need

Uncomplicated f/u

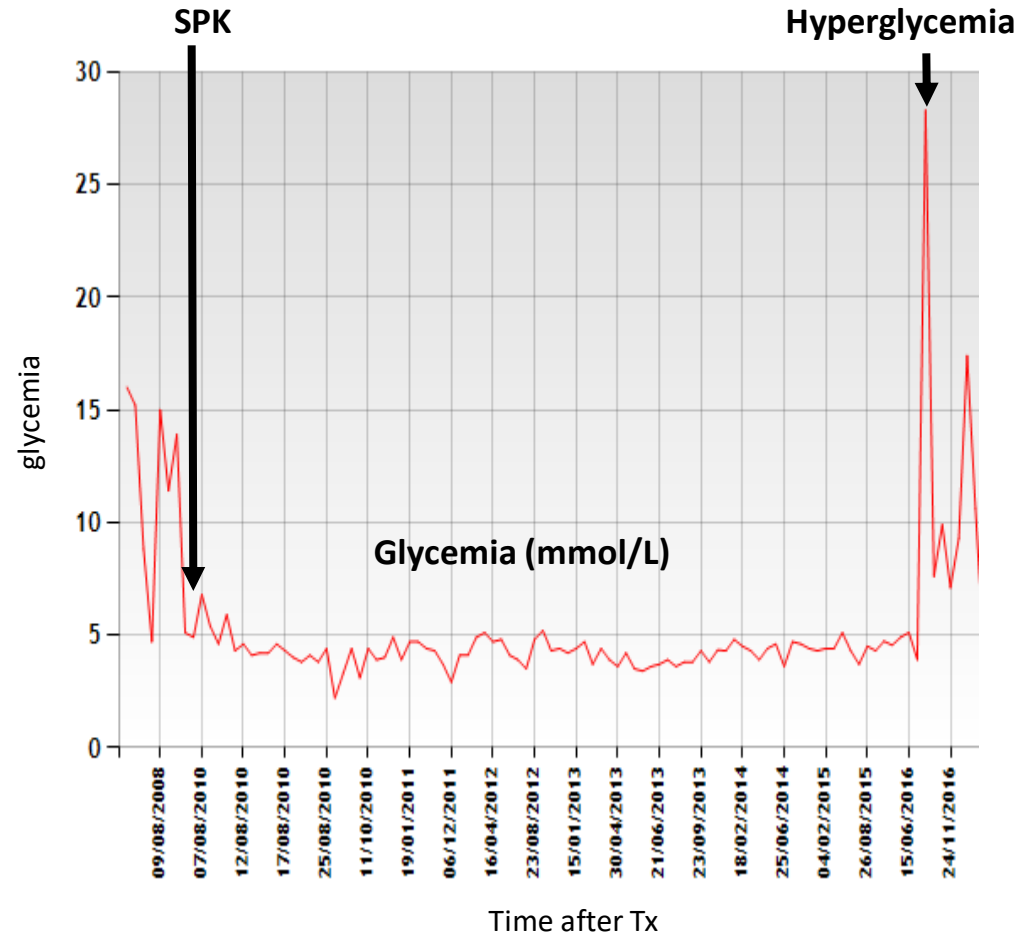
- normal A1C hemoglobin level
- C-peptide, 1.5-3.5 ng/ml
- normal OGTT every year
- normal renal function
- no proteinuria
- no hypertension
- single graft pyelonephritis
- good treatment adherence
- normal BMI (no weight increase during f/u)
- maintenance IS: Tac/MPA
- absence of acute rejection
- no renal histology
- **pregnancy without complication (mother and son) after 3 years**





Sudden Hyperglycemia (>4 g)

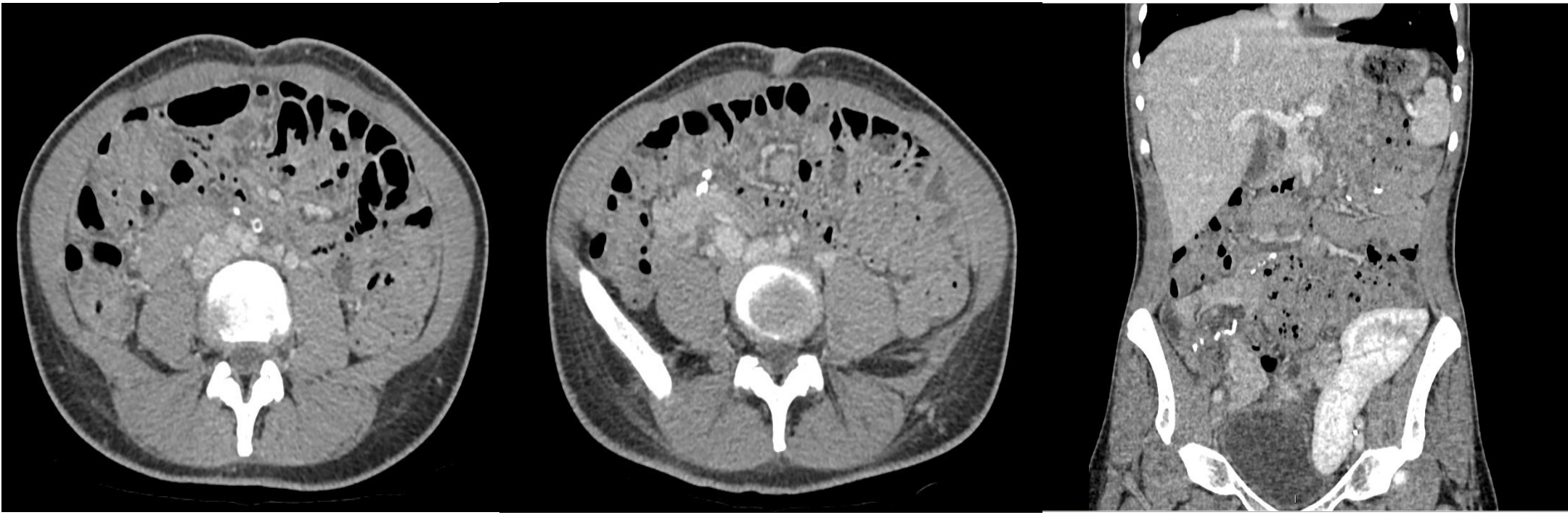
- onset november 2016 (6-yr after Tx)
- polyuria, polydypsia
- low C-peptide (0,8 ng/mL)
- normal lipase level
- normal renal function
- no proteinuria
- no anti-HLA antibodies (no DSA)
- good Tac levels



Sudden and Severe Hyperglycemia
6 yrs after SPK: Cause ?

- ? Vascular
- ? Rejection
- ? Tacrolimus toxicity
- ? Post-transplant diabetes
- ? T1 diabetes recurrence
- ? Others

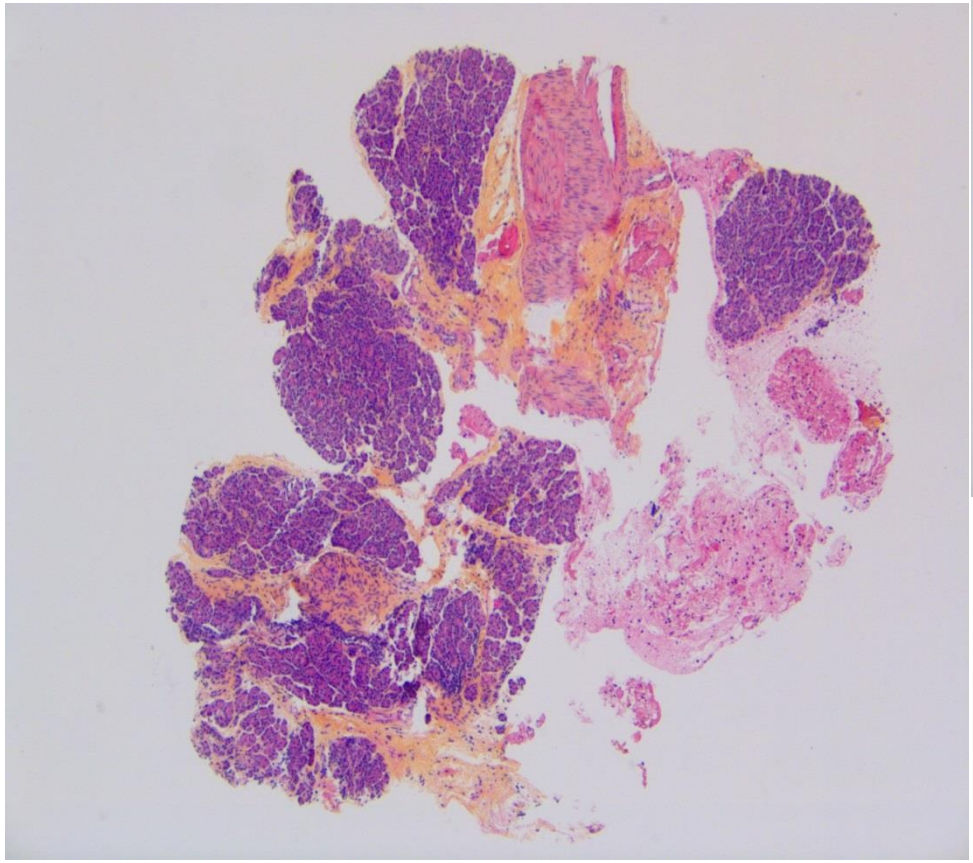
Contrasted CT Scan

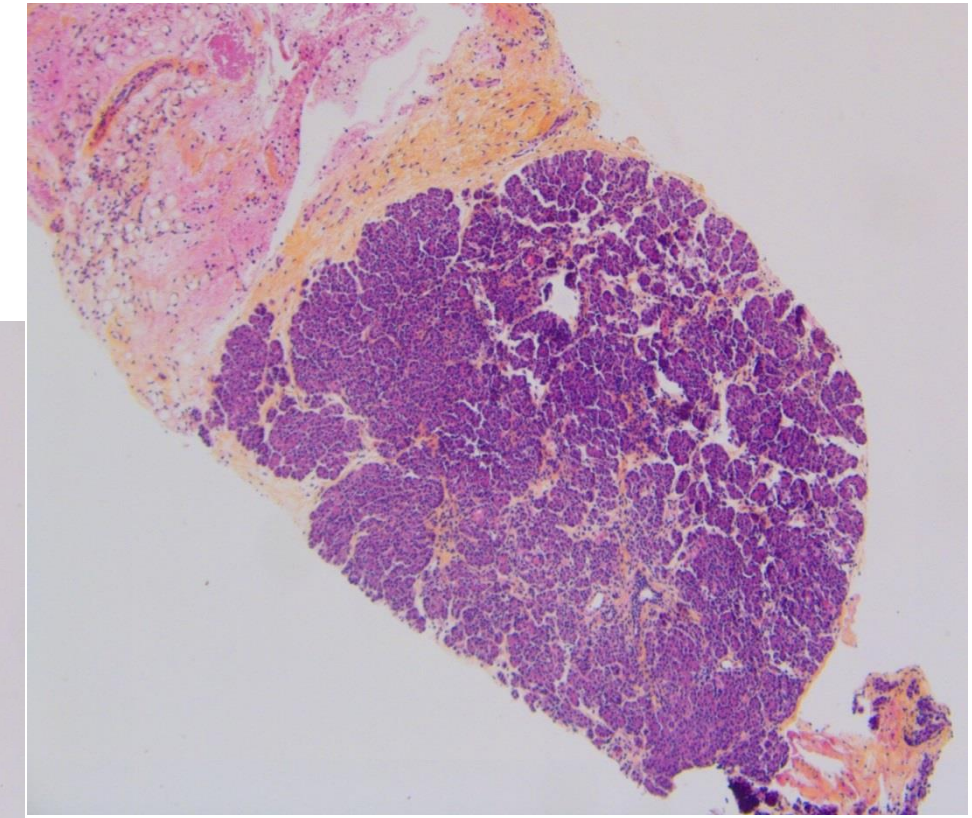


- No thrombosis
- Normal vascularisation
- No oedema

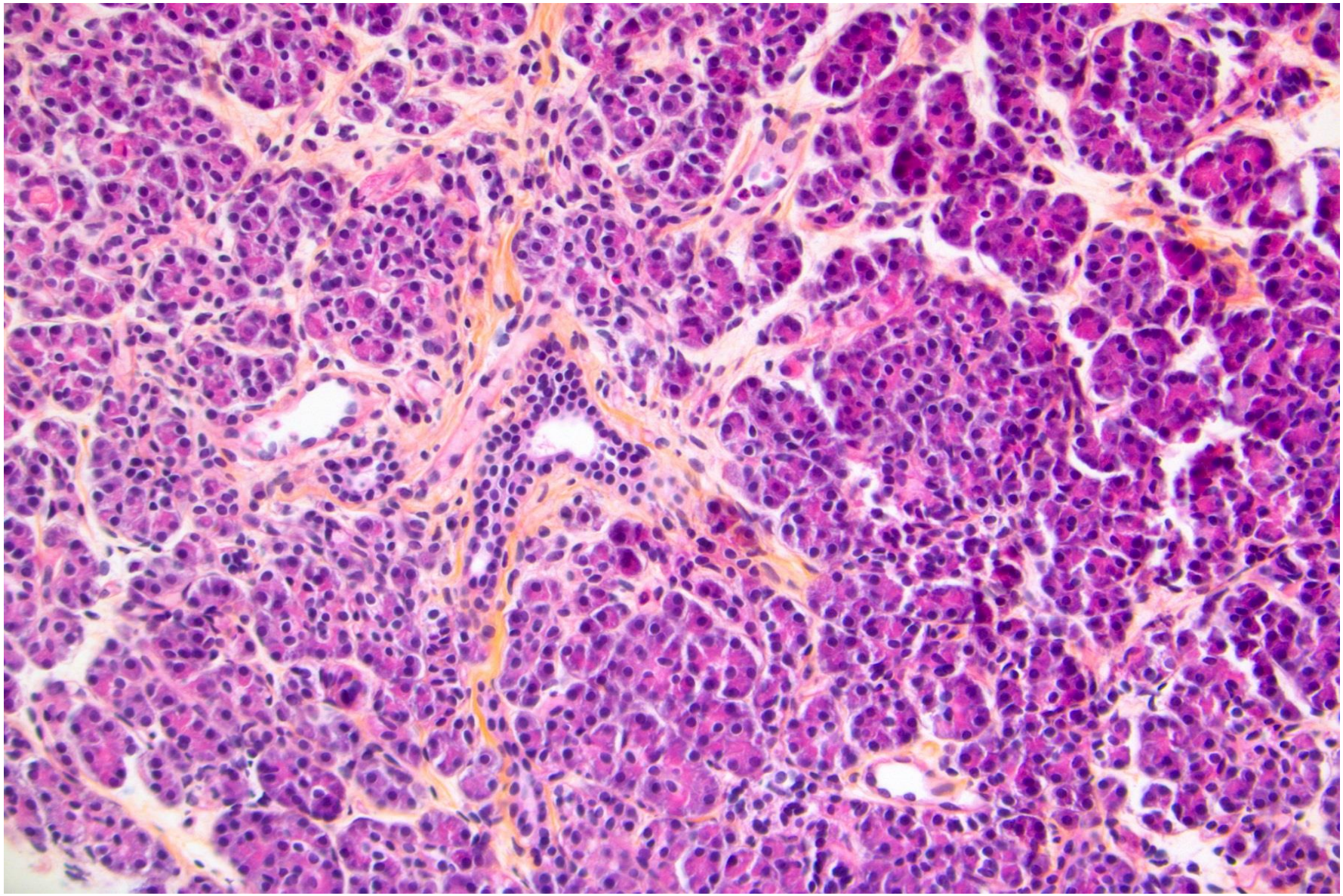
Percutaneous Pancreatic Biopsy

- no Rejection
- no Toxicity





- HES staining x 50
- fragmented specimen
- no significant lobular or septal inflammation
- no ductitis
- no arteritis
- no islet within these 2 pieces



HES staining x 200: no significant lobular or septal inflammation; no ductitis; no venulitis

Hypothesis

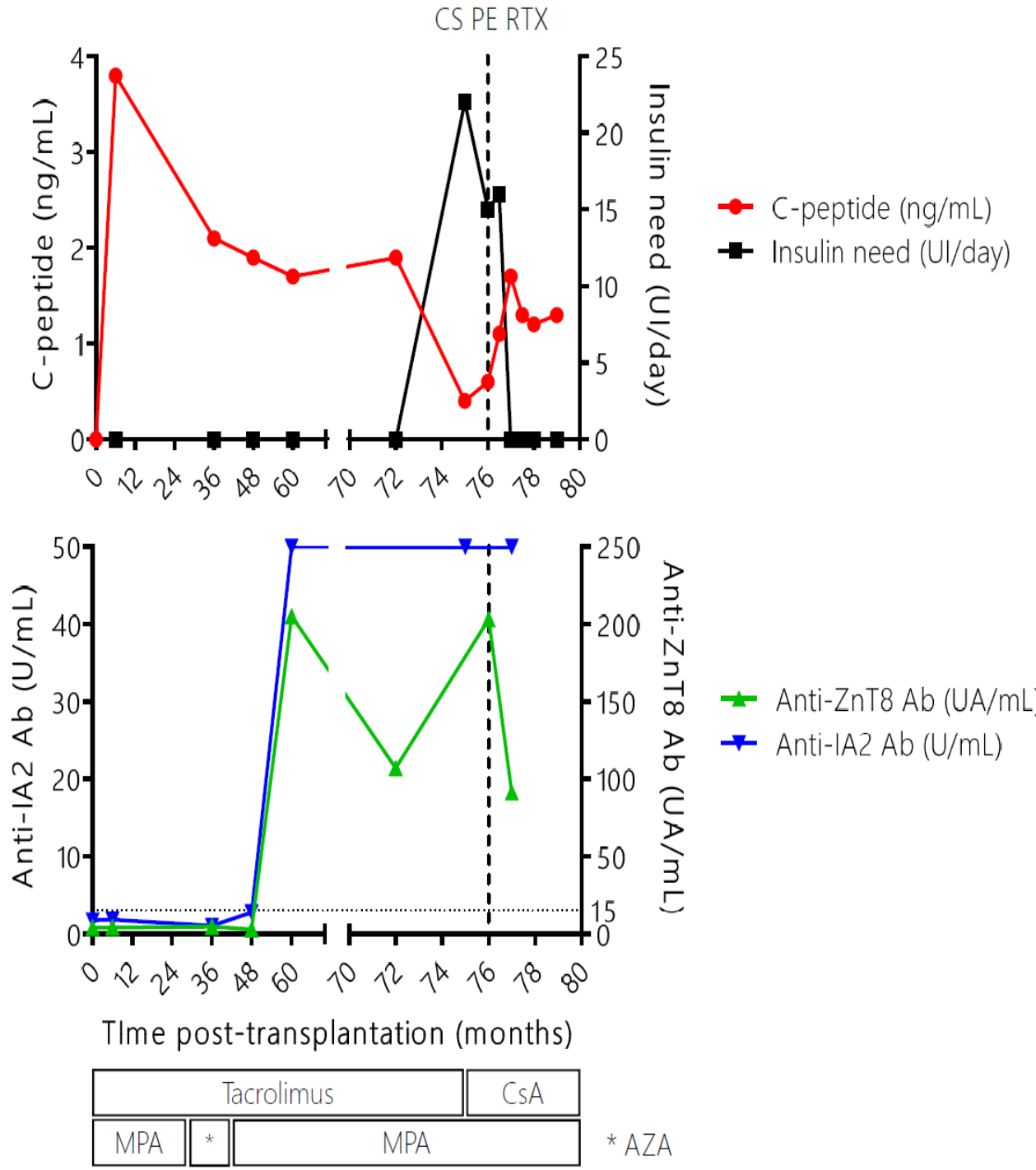
➢ ***? Tacrolimus toxicity***

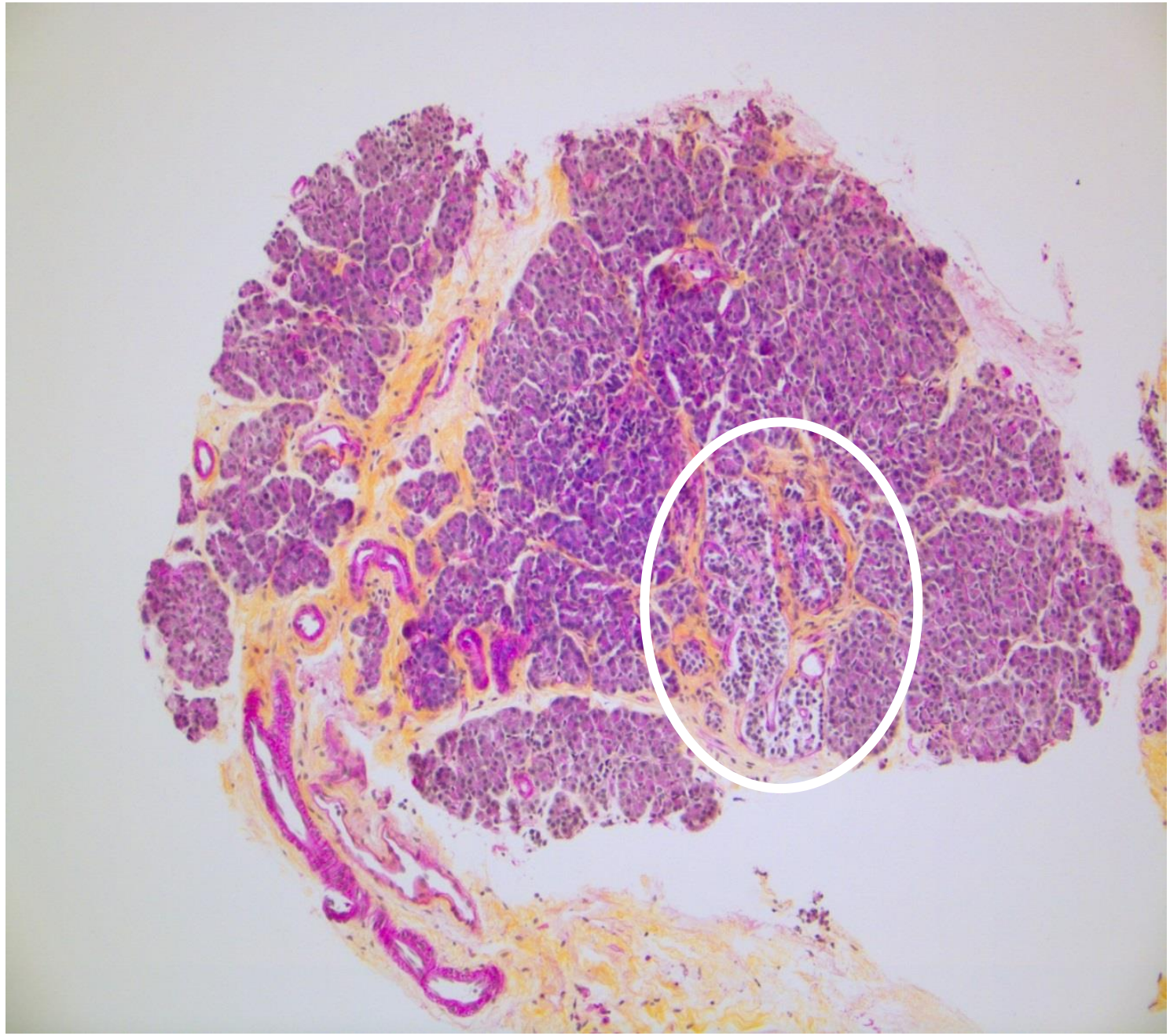
- Switch to Ciclosporine

➢ ***? Recurrence of type I diabetes***

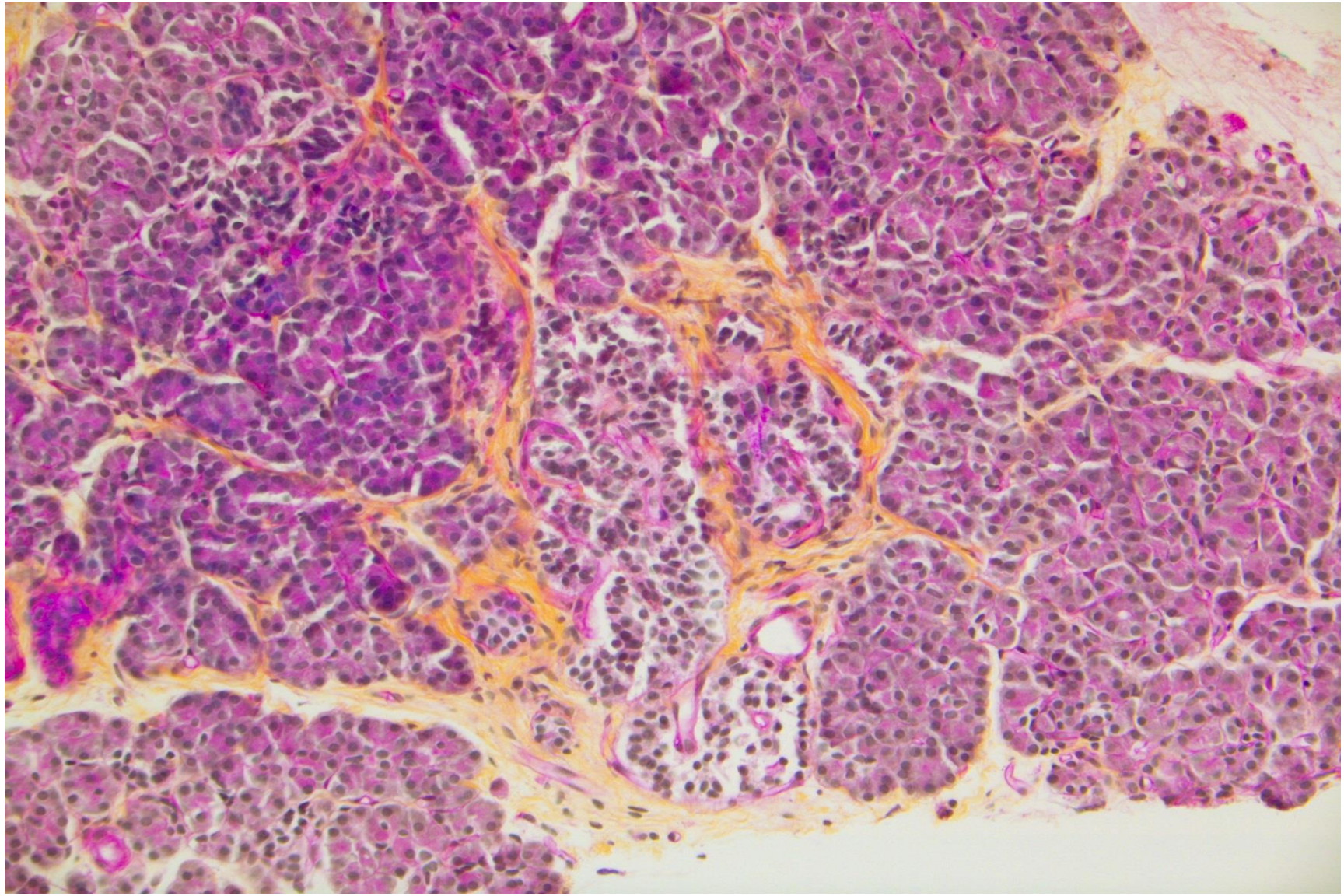
→ Positivity of anti-IA2, anti-ZnT8

- Steroid therapy: 3 boluses of 500 mg MP
- 3 PE
- Rituximab: 375mg/m² on D0 and D15
- IV Insulin and DPP-4 inhibitor (sitagliptin)

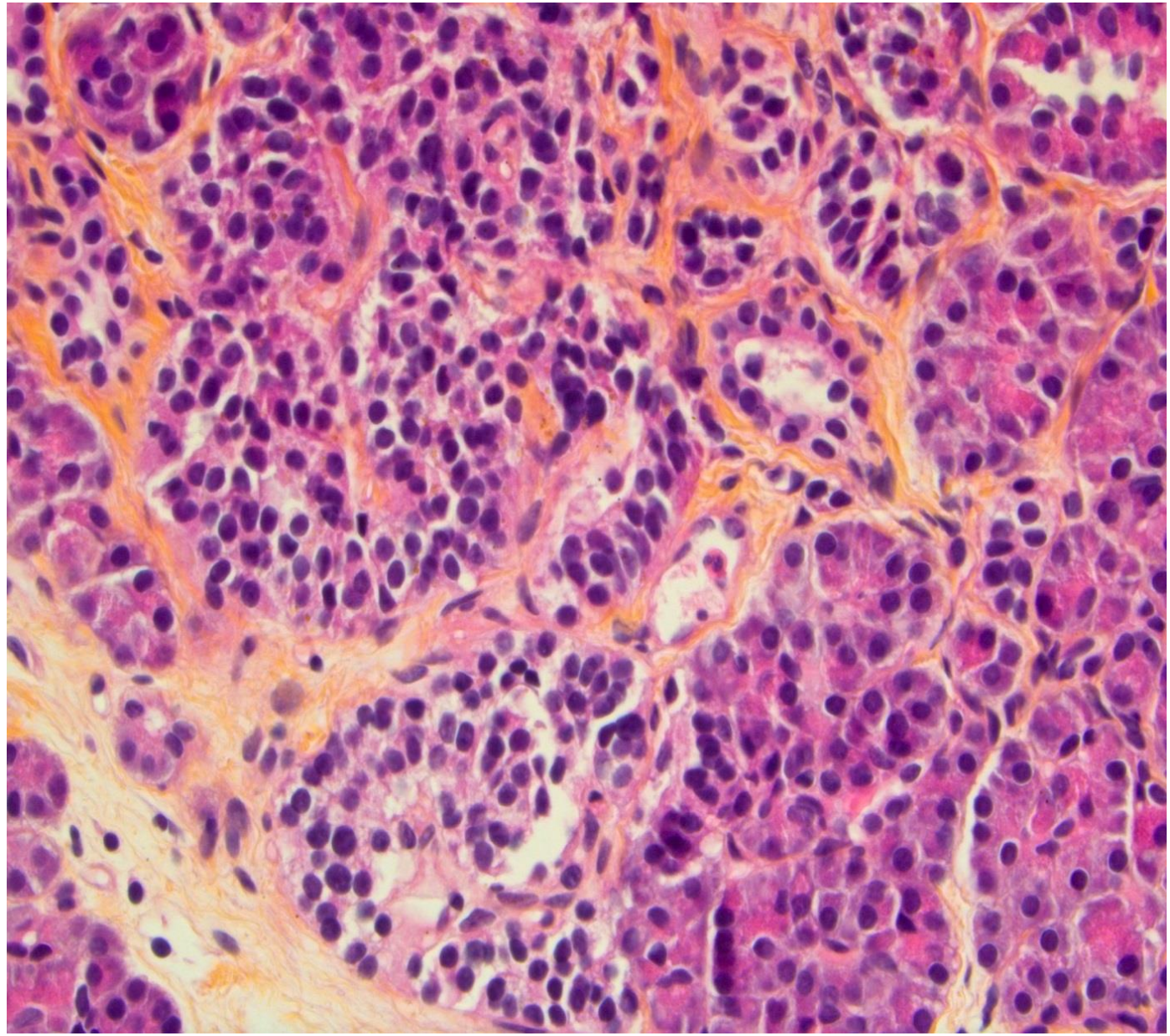

CS PE RTX
C-peptide (ng/mL)
Insulin need (UI/day)
C-peptide (ng/mL)
Insulin need (UI/day)
Anti-IA2 Ab (U/mL)
Anti-ZnT8 Ab (UA/mL)
Anti-ZnT8 Ab (UA/mL)
Anti-IA2 Ab (U/mL)
TIme post-transplantation (months)
Tacrolimus
CsA
MPA
*
MPA
* AZA



PAS staining X 100: one islet on this third piece, no significant lobular or septal inflammation, no arteritis, no capilaritis

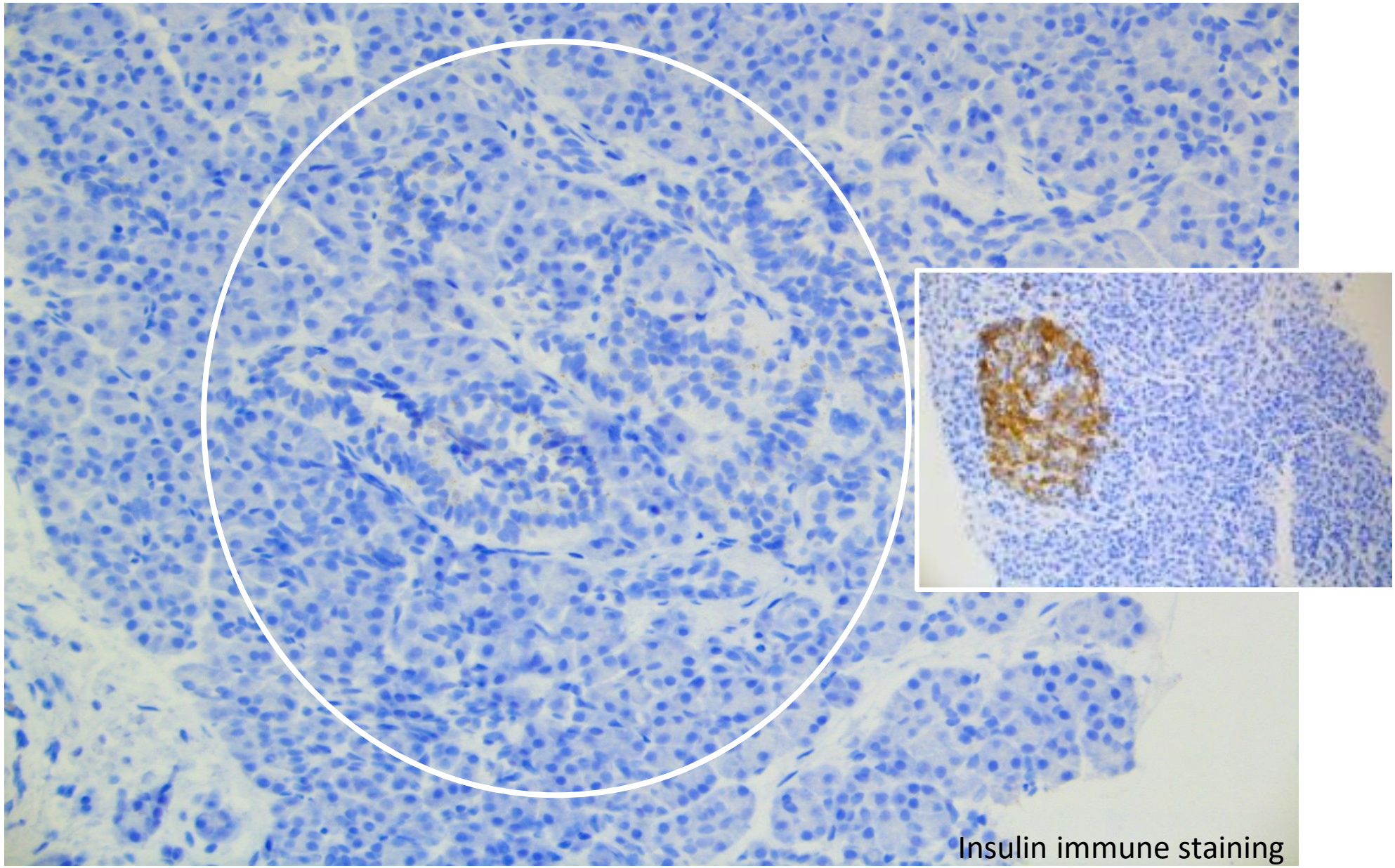


PAS x 200: absence of inflammation (no isletitis); presence of empty space, lacunae



Absence of inflammation (isletitis), presence of empty space (lacunae), minimal cellular vacuolization, no cellular drop-out, no apoptotic fragment

Absence of insulin producing Beta cells within the islet



Follow-up: last visit March 27

- no insulin (since 3 months)
- fasting glycemia: 5.4 mmol/L
- 2-hr OGTT glycemia: 5,5 mmol/L
- fasting C-peptide: 1,3 ng/mL
- 2-hr stimulated C-peptide: 3,8 ng/ml
- fasting insulin: 10,2 µUI/ml
- 2-hr stimulated insulin: 20,3 µUI/ml
- A1C hemoglobin level: 6,2%
- Creatinine: 84 µmol/L

- MPA, 360 mg b.i.d.
- Ciclosporine, C0 175 ng/mL (200 mg b.i.d.)
- Cotrimoxazole, 400 mg
- Valganciclovir, 450 mg b.i.d.
- Esomeprazole, 40 mg
- Sitagliptin, 50 mg (DPP-4 inhibitor): stopped March 27, 2017

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SERVICE : GREFFES RENAUX CONSULT.
Dr. : CANTAROVICH Diego

PATIENT : [redacted]
Nom de naissance : [redacted]
Né(e) le : 29/08/1981 SEXE:F
Enregistre le : 27/03/2017 08:49:38
Edite le : 27/03/2017 10:54:23

N° IPP: 016853454
N° Hosp.: 170188008

Ref: 170860463/ 10728506
Prlvt le: 27/03/2017 08:09:00

Informations Complementaires
Période: , Diurèse: , PRESCRIPTION COMPLETE ? (V): OUI

BIOCHIMIE PLASMATIQUE

ELECTROLYTES					
SODIUM	136	mmol/L	(N 136-145)	138	07/03/17
POTASSIUM	* 5,1	mmol/L	(N 3.4-4.5)	5,6	07/03/17
ECHANTILLON SANS HEMOLYSE APPARENTE					
CHLORE	100	mmol/L	(N 97-108)	103	07/03/17
BICARBONATES	24,2	mmol/L	(N 24-34)	21,7	07/03/17
CALCIUM TOTAL	2,32	mmol/L	(N 2.15-2.50)	2,44	07/03/17
PROTEINES TOTALES	71	g/L	(N 65-80)	76	07/03/17
Valeurs de référence déterminées sur serum.					
Pour le plasma hépariné : majoration due au fibrinogène, de 2 à 4 g/L chez l'adulte					
et de 1 à 3 g/L chez le nouveau-né.					
PHOSPHORE	0,96	mmol/L	(N 0.81-1.45)	1,14	07/03/17
SUBSTRATS					
GLUCOSE	5,4	mmol/L	(N 4.1-5.9)	7,1	07/03/17
UREE	6,4	mmol/L	(N 2.8-8.1)	8,4	07/03/17
CREATININE	* 82	µmol/L	(N 44-80)	103	07/03/17
ESTIM. DU DFG (CKDEPI) pour patient non afroaméricain					
	* 80	mL/min/	(N 90-140)	61	07/03/17
ESTIM. DU DFG (CKDEPI) pour patient afro-américain					
	92	mL/min/	(N 90-140)	70	07/03/17
Cette formule de calcul n'est applicable qu'à partir de 18 ans et sans limite supéri					
eure d'âge.					
Les équations d'estimation du DFG sont imprécises au delà de 75 ans					
ainsi que pour les poids extrêmes ou en cas de variation de la masse					
musculaire.					
ACIDE URIQUE	197	µmol/L	(N 140-340)	218	07/03/17
BILIRUBINE TOTALE	7	µmol/L	(N 0-21)	6	07/03/17
MESURE D'ACTIVITES ENZYMATIQUES A 37° C					
TGO (ASAT)	27,0	UI/L	(N 0-36)	26,0	07/03/17
Attention : changement d'unités à compter du 11/10/2016.					
Les résultats antérieurs au 11/10/2016 sont affichés en µkat/L (1 µkat/L= 60 UI/L).					
TGP (ALAT)	10,2	UI/L	(N 0-36)	11,9	07/03/17
Attention : changement d'unités à compter du 11/10/2016.					
Les résultats antérieurs au 11/10/2016 sont affichés en µkat/L (1 µkat/L= 60 UI/L).					
LIPASE	19,4	UI/L	(N 0-60)	17,7	07/03/17
Attention : changement d'unités à compter du 11/10/2016.					
Les résultats antérieurs au 11/10/2016 sont affichés en µkat/L (1 µkat/L= 60 UI/L).					
GAMMA GT	25,0	UI/L	(N 0-42)	35,0	07/03/17
Attention : changement d'unités à compter du 11/10/2016.					
Les résultats antérieurs au 11/10/2016 sont affichés en µkat/L (1 µkat/L= 60 UI/L).					

Biochimie générale Résultat d'une demande - BOULAY, Asma GHEDJATI (016853454)
Date/heure de résultat: 27/03/2017 08:49
Date/heure de prélèvement:27/03/2017 08:09
Date/heure d'impression: 27/03/2017 17:11:57

PHOSPHATASES ALCALINES	55,4	UI/L	(N 45-99)	68,3	07/03/17

Attention : changement d'unités à compter du 11/10/2016.
Les résultats antérieurs au 11/10/2016 sont affichés en µkat/L (1 µkat/L= 60 UI/L).

BIOCHIMIE URINAIRE

RESULTATS EN CONCENTRATION

CREATININE	5,6	mmol/L		9,9	07/03/17
PROTEINES TOTALES	0,10	g/L		0,09	07/03/17

Technique au rouge de pyrogallol - Roche diagnostics

Faux positif si perfusion de gelatine fluide modifiée
(type Gelofusine) dans les 48h avant le prélèvement.

Rapport Protéines/Créatinine	0,02	g/mmol	(N <0.05)
Soit :	0,16	g/g	(N <0.5)

Le seuil décisionnel affiché correspond à la définition de la protéinurie clinique.
Validé par: Pr Damien MASSON (BIOCH) Dr Elodie BOISSIER (HEMA) Anaïs INQUEL (interne)

Pharmacologie PHAR - LAB/LAB

NE PAS IMPRIMER : DOCUMENT SANS VALEUR LEGALE

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Edite le : 27/03/2017 12:34:53

N° IPP: 016853454
N° Hosp.: 170188008

Ref: 170860459/ 10728501
Prlvt le: 27/03/2017 08:00:00

DOSAGE DE MEDICAMENTS-TOXICOLOGIE
SANG

CICLOSPORINE A	175	ng/mL	156	07/03/17
Posologie Matin	200	mg	200	07/03/17
Posologie Soir	200	mg	200	07/03/17

Zone thérapeutique de la concentration résiduelle (transplantation rénale) : 150 - 300 ng/ml (phase aiguë) ; 75-150 ng/ml (phase de maintien)

Radio-immunologie RIA_ - LAB/LAB

NE PAS IMPRIMER : DOCUMENT SANS VALEUR LEGALE

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HORMONOLOGIE

FONCTION PANCREATIQUE
ANALYSES DE SANG

Peptide C	1,3	ng/mL	(N 0.4-4)	1,3	07/03/17

Valeurs de références à jeun et établies chez l'adulte

Validé par: Pr Damien MASSON (BIOCH) Dr Elodie BOISSIER (HEMA) Anaïs INQUEL (interne)

Sudden and Severe Hyperglycemia
6 yrs after SPK: Cause ?

- ? Vascular
- ? Rejection
- ? Tacrolimus toxicity
- ? Post-transplant diabetes
- ? T1 diabetes recurrence
- ? Others

Sudden and Severe Hyperglycemia
6 yrs after SPK: Cause ?

- ? Vascular
- ? Rejection
- ? Tacrolimus toxicity
- ? Post-transplant diabetes
- **? T1 diabetes recurrence**
- ? Others

Sudden and Severe Hyperglycemia
6 yrs after SPK: Why improvement ?

- ? Honey moon phase
- ? Swith to CsA
- ? Efficacity of MP boluses
- ? Efficacy of Rituximab
- ? Efficacy of plasma exchange
- All of them

Sudden and Severe Hyperglycemia 6 yrs after SPK:

Improvement ok but for how long ?

- Honey moon phase: 1 year ?
- Rituximab: 1 more year ?
- CsA/MPA sufficient to prevent CD4/CD8 autoreactive T-cells reappearcence ?
- Belatacept ?
- M-TORi ?
- Suggestions ?

Lab Invest. 1985 Aug;53(2):132-44.

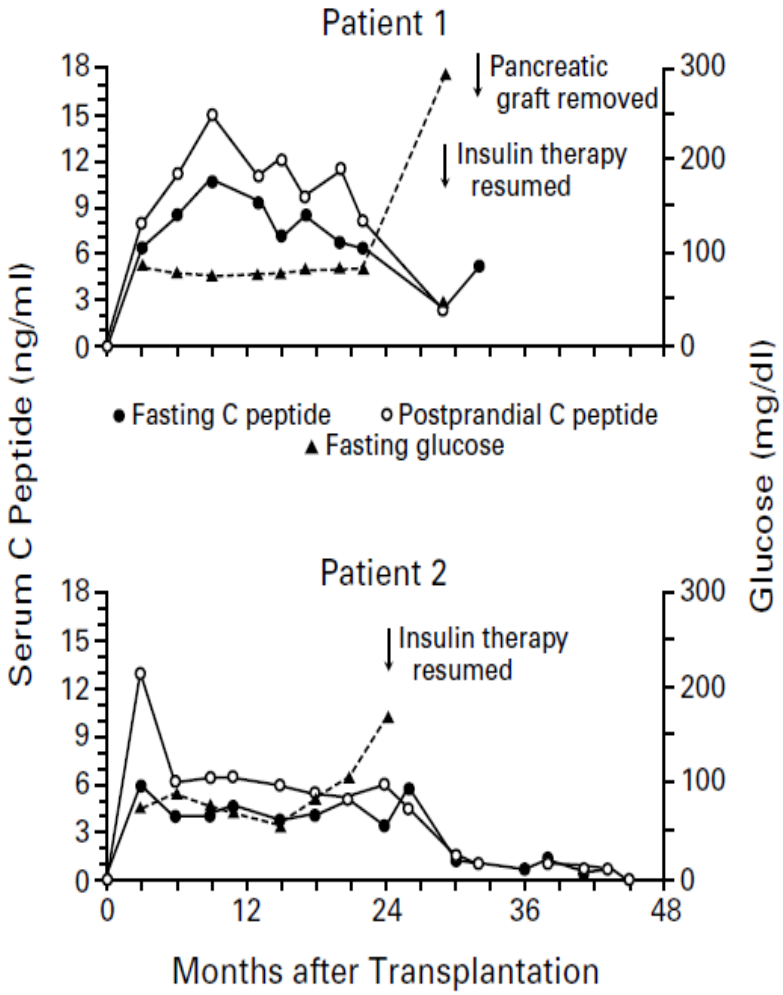
Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases.

Sibley RK, Sutherland DE, Goetz F, Michael AF.

Abstract

Four patients with type 1 diabetes mellitus received segmental pancreatic grafts. **The donors were HLA-identical twins in three patients and an HLA-identical sibling in one**. Each patient had normal glucose metabolism in the posttransplantation period but **impaired graft function developed after 6 to 12 weeks**. Complete loss of function developed in three patients. The fourth patient received immunosuppressive therapy but continues to require a low dose of insulin 15 months following transplantation. Pancreatic graft biopsy at the time of declining graft function in three patients revealed a mononuclear cell infiltrate centered upon islets consisting of variable numbers of T11 (pan T), OKT8 (suppressor-killer), OKT9 (transferrin receptor), OKT10 (activated), and HLA-DR-reactive mononuclear cells, as well as 63D3 and OKM1 reactive monocytes. Biopsies obtained following loss of graft function revealed resolution of the inflammatory process and selective destruction of all islet beta-cells in two patients, whereas graft biopsy in one patient demonstrated a mononuclear cell infiltrate in islets containing demonstrable beta-cells but no infiltrate in islets without beta-cells. Following immunosuppressive therapy the fourth patient showed resolution of the insulitis and destruction of beta-cells in 70% of the islets. The variable numbers of beta-cells observed in the remaining islets likely account for the relatively low amount of exogenous insulin required by this patient. There was no immunohistologic evidence of humoral mediated immune reaction in any of the biopsies. It is postulated that selective beta-cell destruction was a consequence of cell-mediated immunity leading to recurrent diabetes mellitus.

Type 1 diabetes recurrence after pancreas transplantation



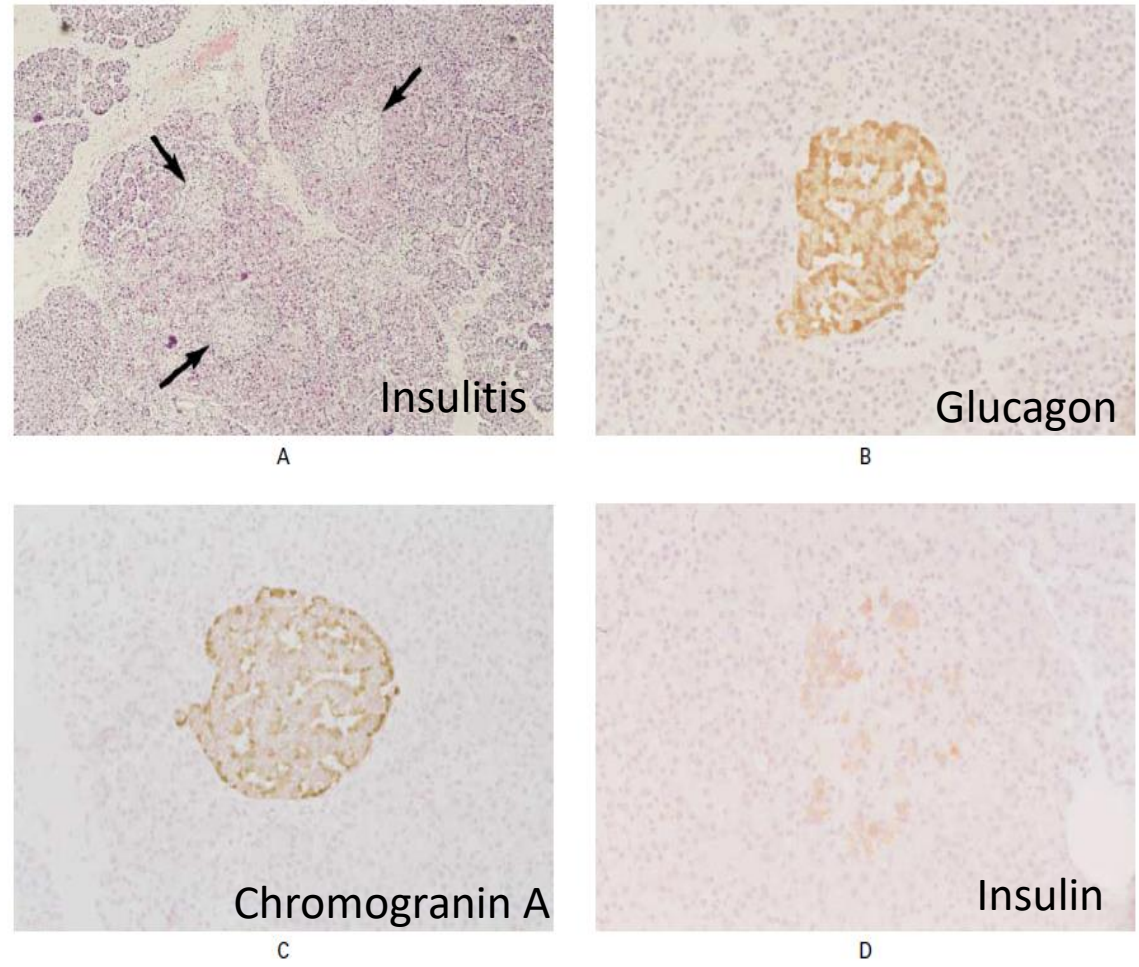


Figure 2. Photomicrographs of Sections of the Pancreatic Graft Removed from Patient 1.

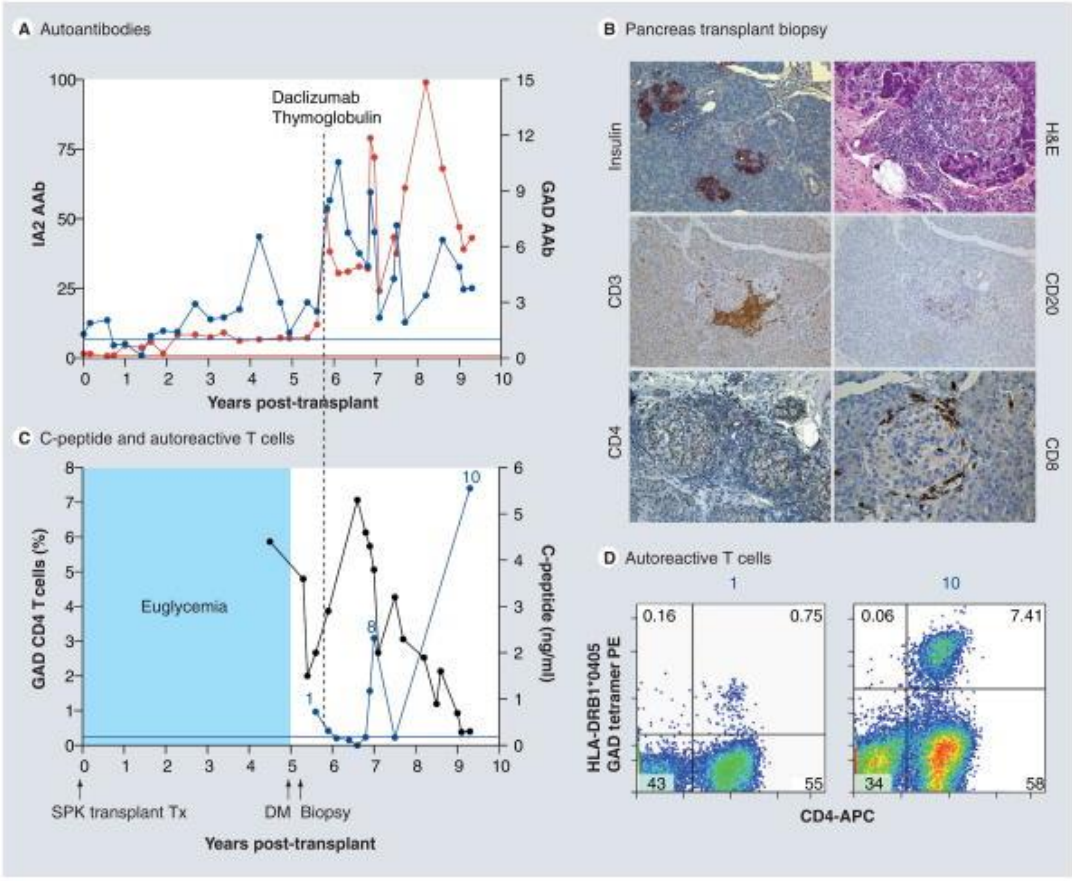
Tyden et al, NEJM 1996

Diabetes Manag (Lond). 2011 March ; 1(2): 229–238. doi:10.2217/dmt.10.21.

Recurrence of autoimmunity in pancreas transplant patients: research update

Alberto Pugliese[1,2,3,†], **Helena K Reijonen**[4], **Jerry Nepom**[4], and **George W Burke III**[1,5]

[1]Diabetes Research Institute, University of Miami Miller School of Medicine, 1450 NW 10th Avenue, Miami, FL 33136, USA



Hyperglycemia without rejection and no functional changes of the exocrine pancreas (urine amylase) or kidney (serum creatinine) grafts, with selective loss of insulin secretion;

Biopsies demonstrating insulitis and/or β-cell loss;
The persistence or reappearance of autoantibodies, prior to diabetes recurrence;

The presence of circulating autoreactive T cells around the time of diabetes recurrence and on further follow-up, which *in vitro* predominantly produced proinflammatory cytokines (e.g., IFN-γ);

In vivo evidence that the SPK transplant patients' autoreactive CD4 T cells specific for the islet autoantigen GAD65 can specifically mediate β-cell destruction in HLA-mismatched islet grafts when T cells and islets are co-transplanted under the kidney capsule of immunodeficient mice;

The presence of autoreactive T cells in the circulation of several patients, both CD4 and CD8 T cells, correlated with disease activity and progression. In patients who received additional immunosuppression in an attempt to salvage the residual β-cell mass demonstrated at biopsy, autoreactive T cells were no longer detected after treatment but reappeared on later follow-up. Their return was followed by a further and complete loss of C-peptide.

Type 1 Diabetes recurrence

- **Epidemiology**
  - Probably under-estimated (no systematic monitoring of auto-antibodies)
  - 7% of patients with SPK

Vendrame et al, AJT 2016

- **Cardinal Features**
  - Selective loss of insulin secretion (low c peptide)
  - Insulitis and/or β-cell loss at biopsy
  - Auto-antibodies
  - Circulating auto-reactive CD4 or CD8 T cells

Vendrame et al, Diabetes 2010

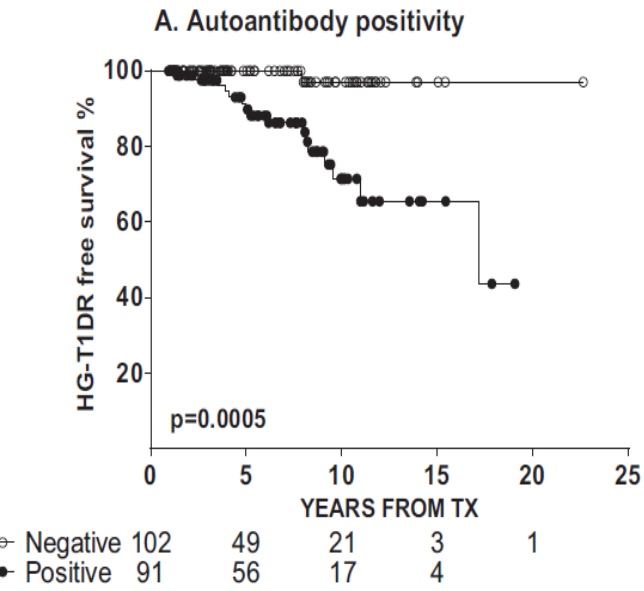
Risk factors

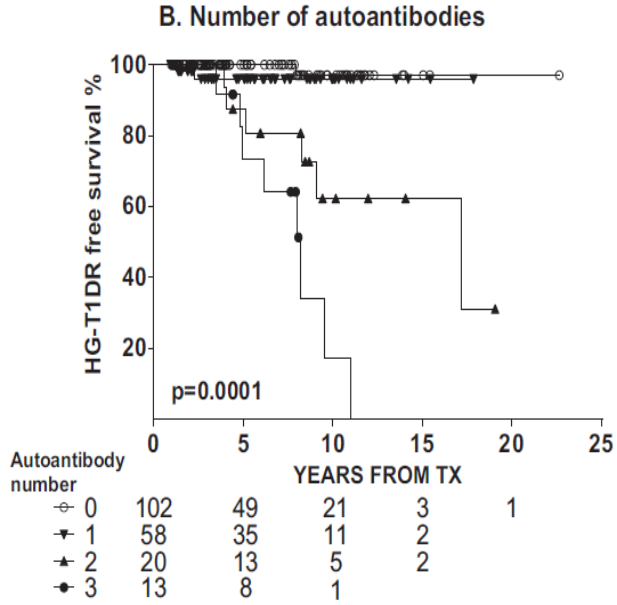
- 223 recipients of SPK in Miami between 1990 and 2012
- hyperglycemia requiring insulin, severe loss of c-peptide,
- no rejection (clinical)

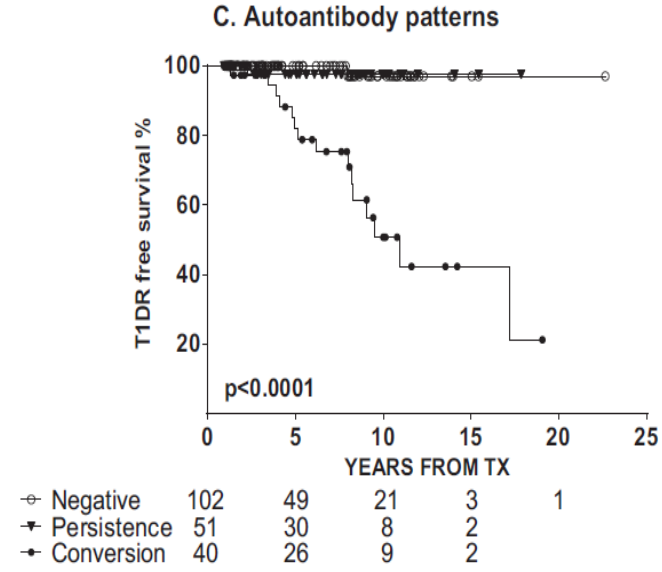
- No induction therapy
- Auto-antibodies positivity, number of auto-antibodies and **auto-antibodies conversion after Tx**
- Anti-ZnT8 auto-antibodies conversion
- T1D predisposing recipient's HLA DR3/DR4 alleles
- Donor-recipient sharing of HLA-DR alleles (DR3 mostly)

Vendrame et al, AJT 2016

Graft Survival







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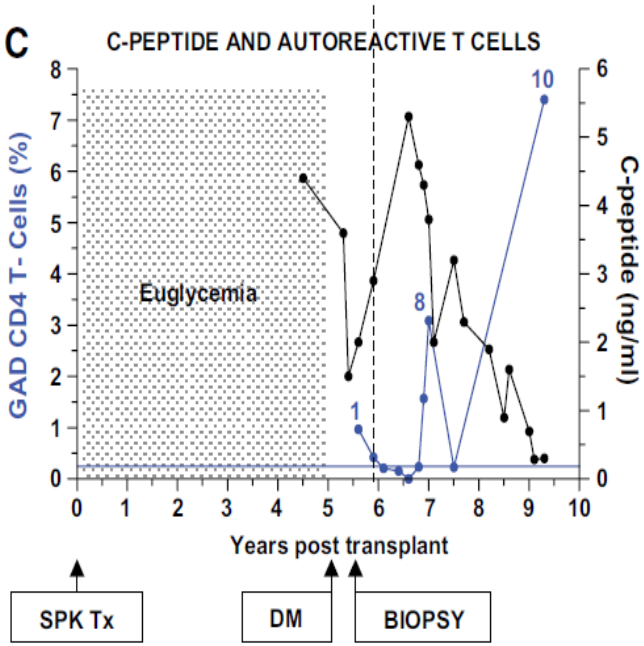
Pathogenesis

- Role of the auto-antibodies ?
- Auto-reactive CD4 T cells ?



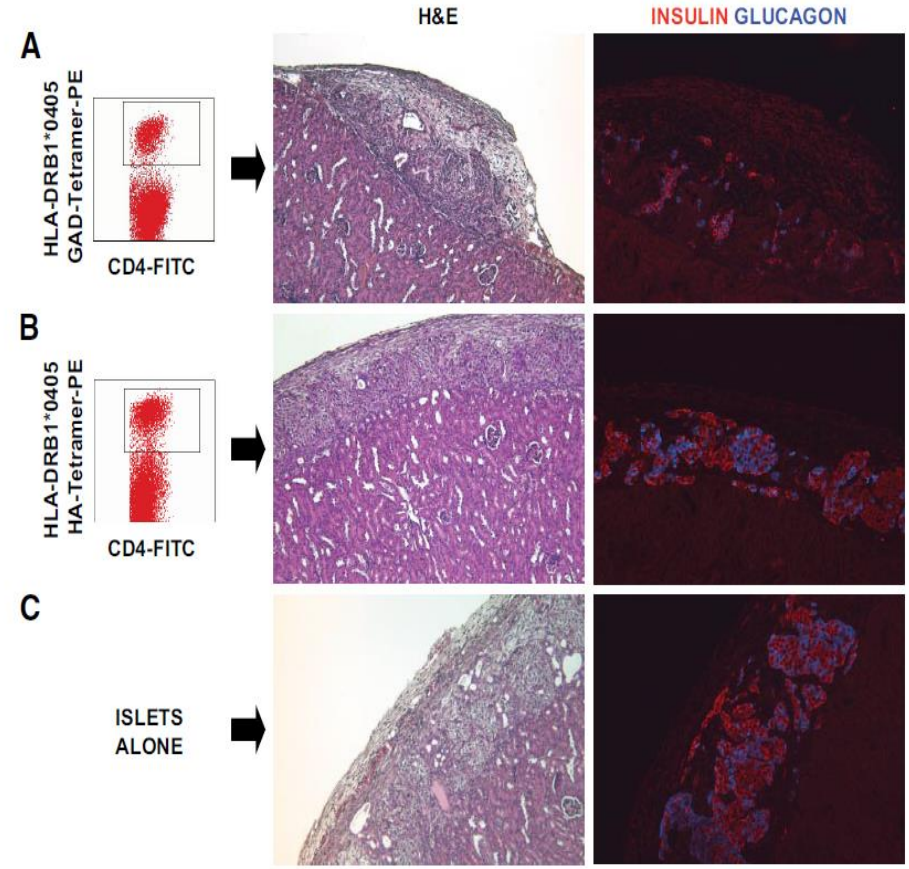


FIG. 4. In vivo assessment of the autoreactive potential of GAD-autoreactive CD4 T-cells from patient 1. A peripheral blood sample (sample no.

Vendrame et al, Diabetes 2010

Treatment

- **T-cell targeted**
  - Role of T cells predominant in T1 Diabetes: Auto-reactive CD4/CD8 T cells (memory cells)
  - Anti-T treatment in type 1 diabetes

    Gitelman S, Haller M, Lancet 2013, JCI 2015

- **B-cell targeted**
  - Role of B cells in T1D: Antigen-presenting cells
  - Rituximab in Type 1 diabetes

    Pescovitz M, NEJM 2009

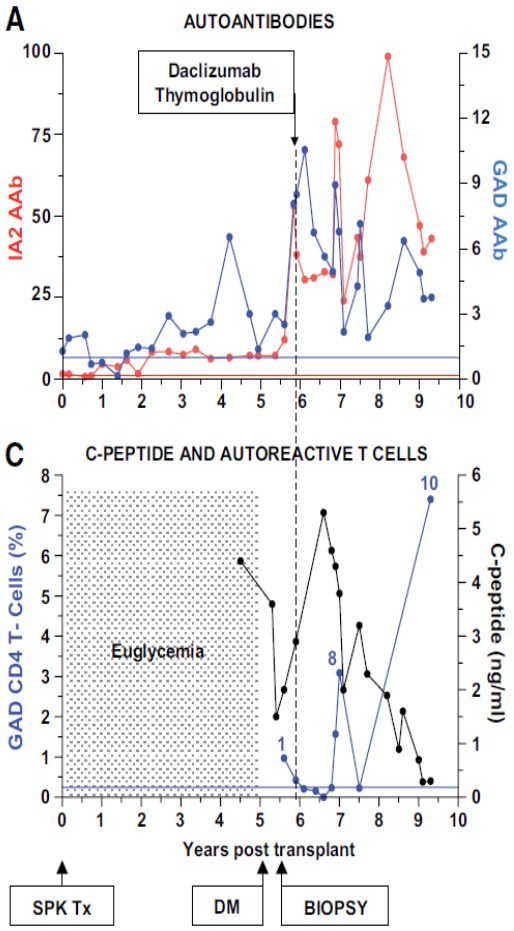
- **Plasmapheresis**
  - Role of auto-antibodies
  - Enhance the presentation of immunodominant T-cell epitope from GAD65

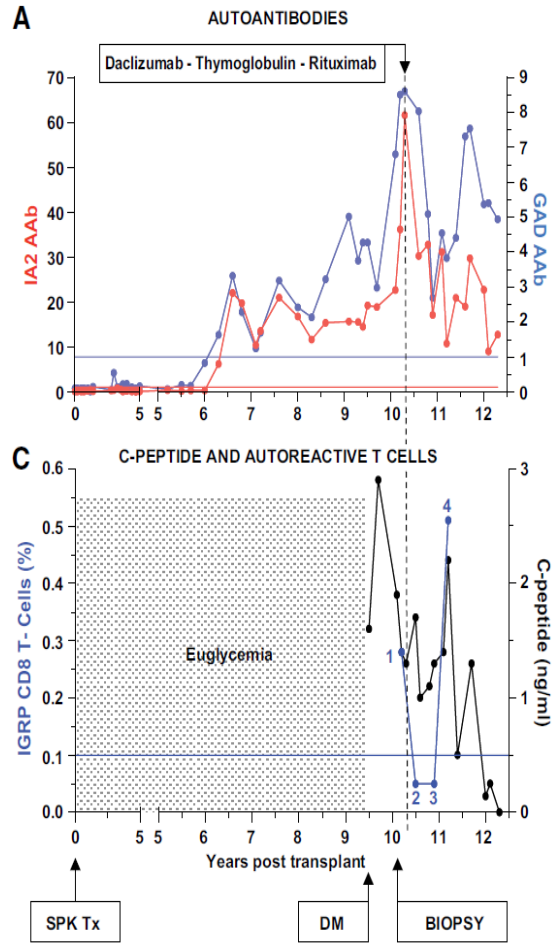
    Reijonen H, Diabetes 2000

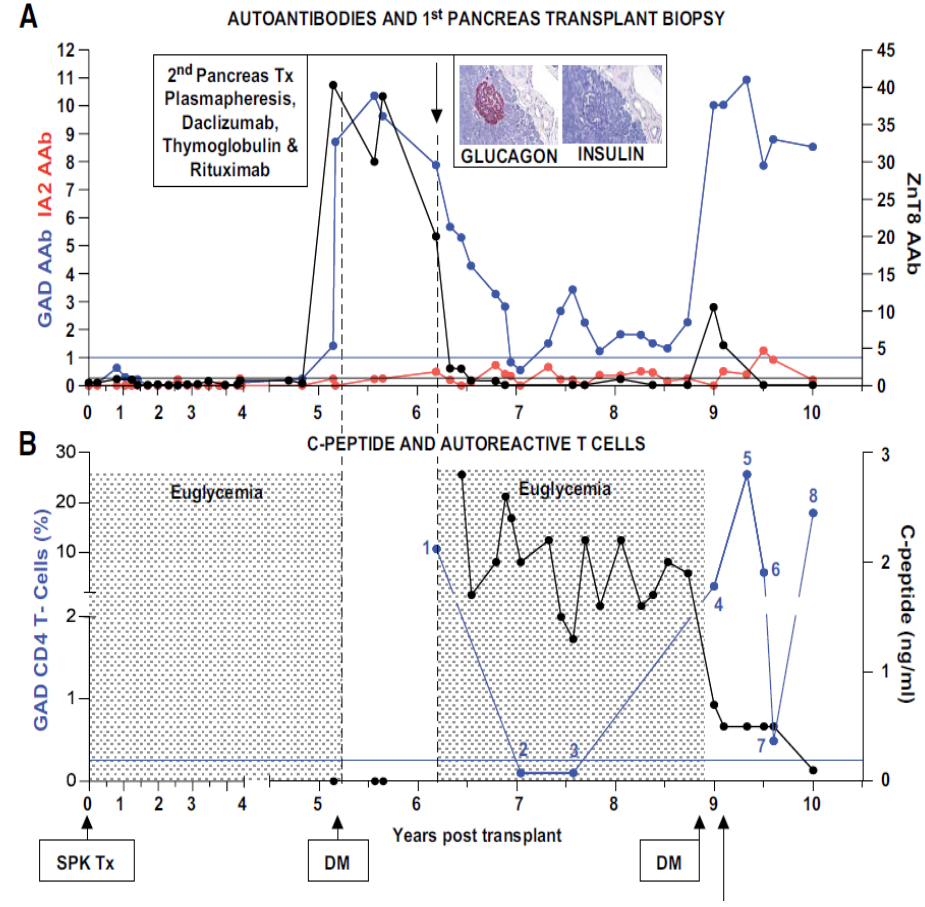
  - Effect of plasmapheresis on ICA but not GAD-Ab in T1D

    Sundkvist G, JCEM 1994

Treatment: No Success







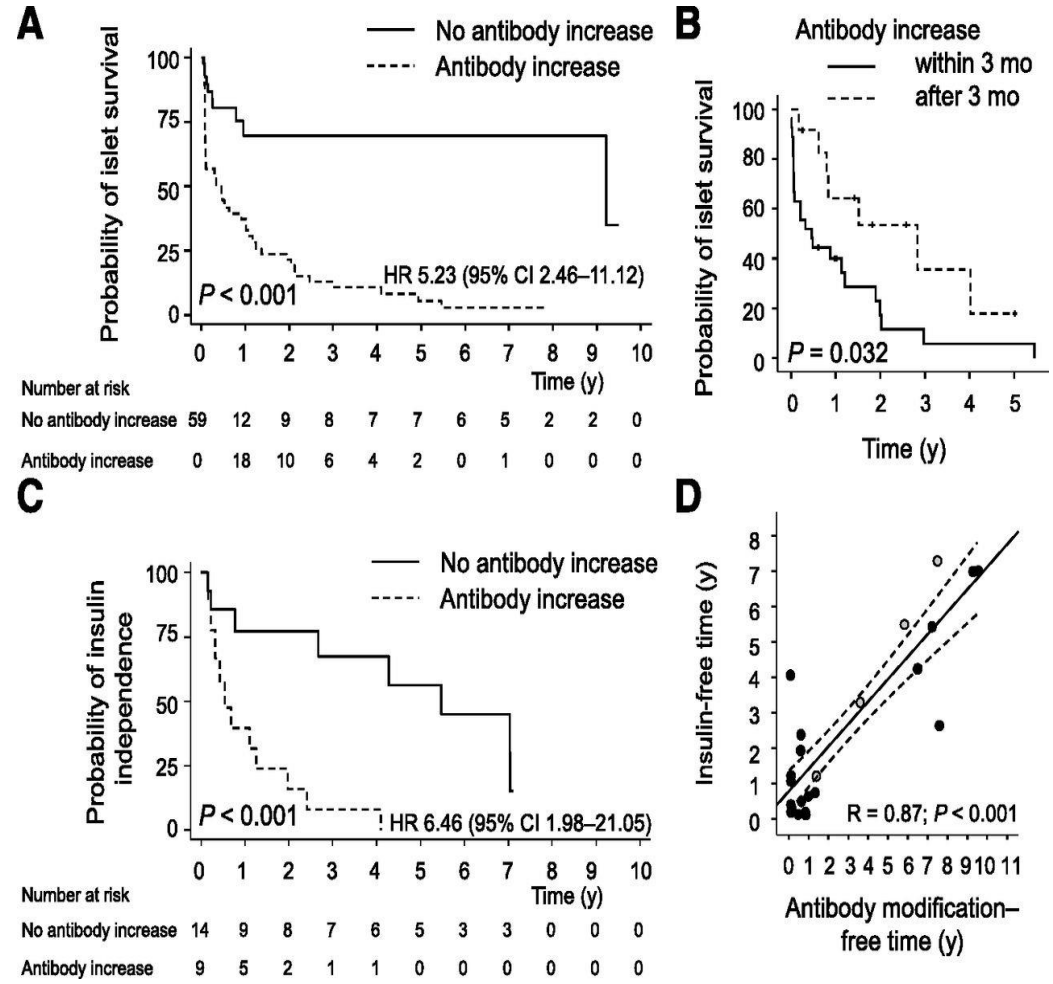
Vendrame et al, Diabetes 2010

Treatment: Success Yes…

- **Islet cells transplantation**
  - Auto-antibodies associated with T1DR

    Piemonti P et al, Diabetes 2013
  - Lymphopenia induced by IS induces homeostatic cytokines that expand auto-reactive memory T cells

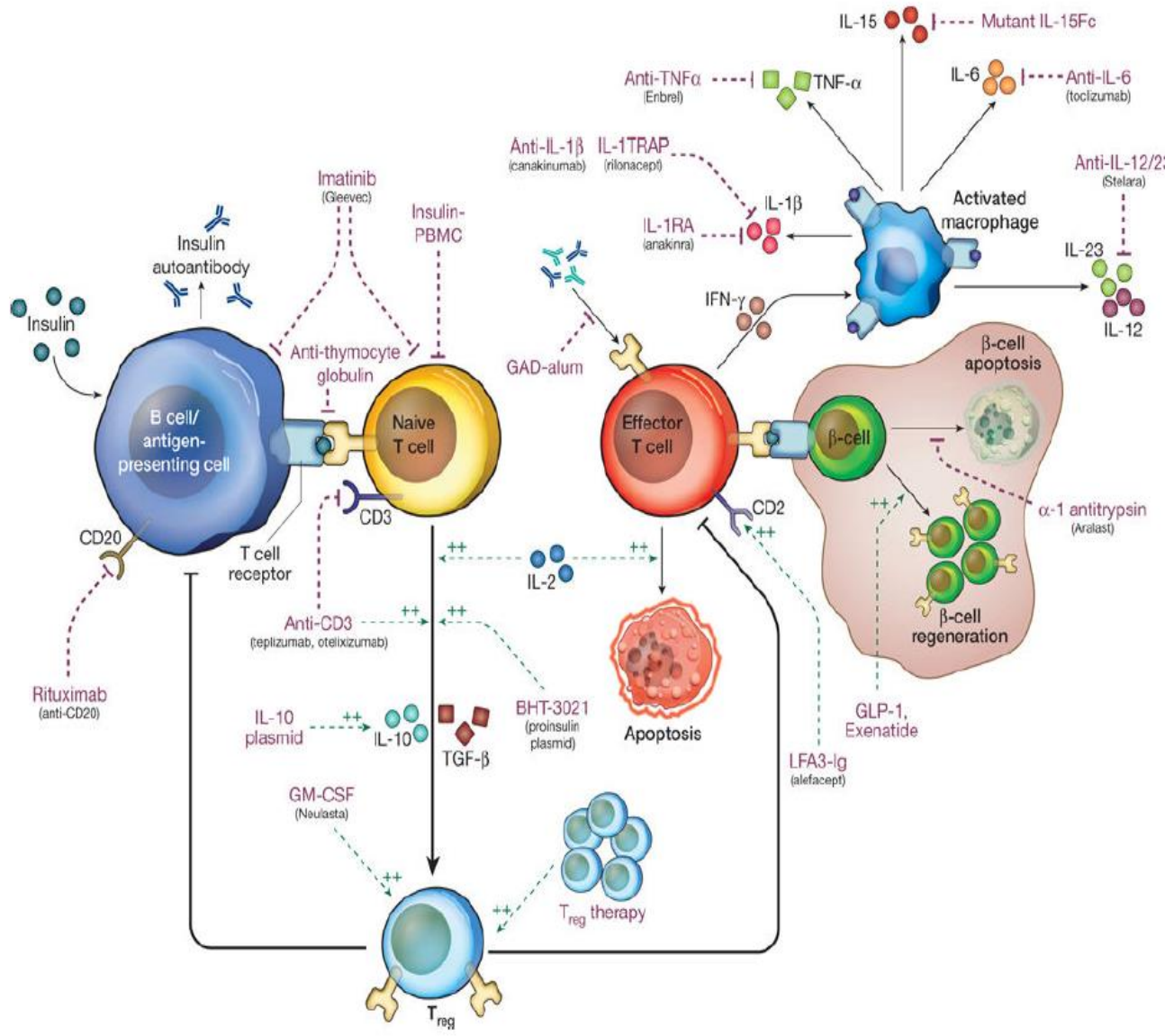
    Monti L et al, JCI 2013
  - Treatment ? Prevention?



Questions raised

- Diagnosis
  - Is biopsy mandatory?
  - Auto-Ab sufficient?
  - Auto-reactive T cells?
- Treatment
  - No effective treatment for now
  - Which target: T cells? B cells? Ab? All of them?
- Prevention
  - Immunosuppressive regimen
  - Systematic monitoring of auto-antibodies?
  - Auto-reactive T cells?
  - Intervention?

Therapeutic options in T1D (no remission!!)



- T1DR : **Risk factors**

Martins et al, Clin Transplant 201

105 SPK recipients, IS anti-thymocyte globulin, tacrolimus, mycophenolate and steroids

Prospective monitoring of auto-antibodies 1/year after Tx, no assessment of anti-ZnT8

Positive antibody (n= 46) = persistance or appearance of a new auto-antibody after tranplantation

Association of positive antibodies with HbA1c and C-peptide

Importance of new post –transplant auto-antibodies

Table 3. Multivariate analysis of factors associated with pancreatic autoimmunity

				95% CI for Exp (B)	
Variables	B	Sig.	Exp (B)	Lower	Upper
HbA1c > 5.6% (>38 mmol/mol)	1.656	0.030	5.240	1.176	23.351
HLA-match DR	−0.166	0.675	0.847	0.389	1.843
HLA-match A/B	0.610	0.061	1.841	0.972	3.487
C-peptide	−0.426	0.039	0.653	0.436	0.979
Glycemia	0.034	0.110	1.035	0.992	1.079
Pancreas acute rejection	0.930	0.341	2.535	0.373	17.230
Constant	−2.455	0.191	0.086		

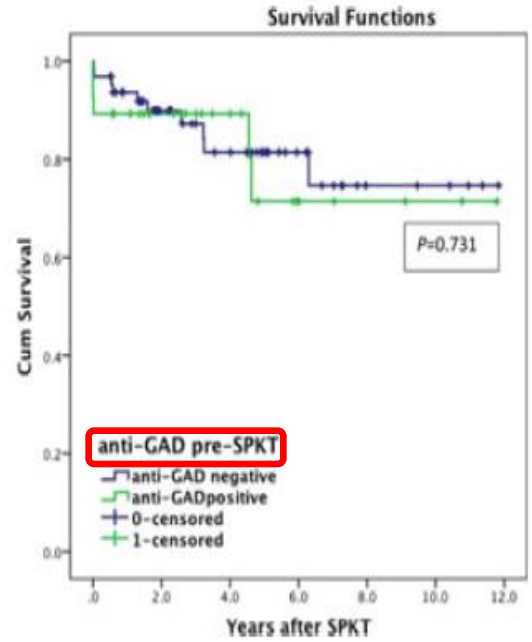


Fig. 2. Log-rank test comparing pancreas graft survival curves (Kaplan–Meier method) for the presence of absence of anti-GAD before transplantation).

- Pancreas transplantation:
  - Type 1 diabetes mellitus with complications (micro-angiopathy) or hypoglycemia unawareness
  - SPK / PAK / PTA
  - Outcomes

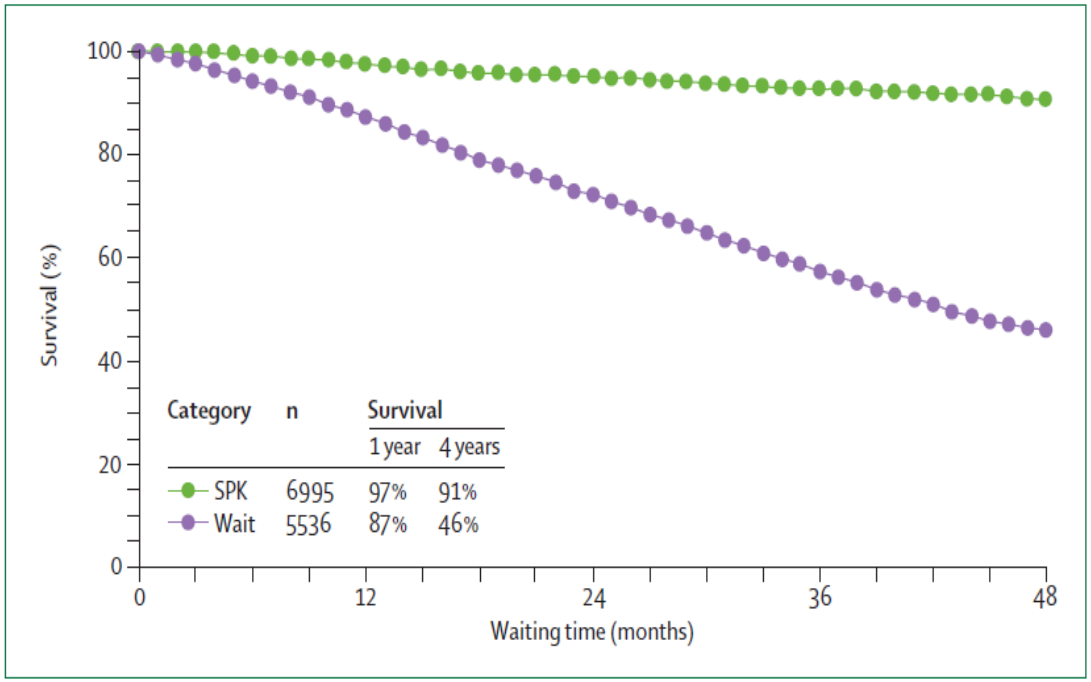


Figure 3: Survival of patients after simultaneous pancreas and kidney transplantation (SPK) versus those waiting for a pancreas transplant
Month 0 is time of SPK and entry to waiting list for those waiting for a transplant.

White et al, Lancet 2009

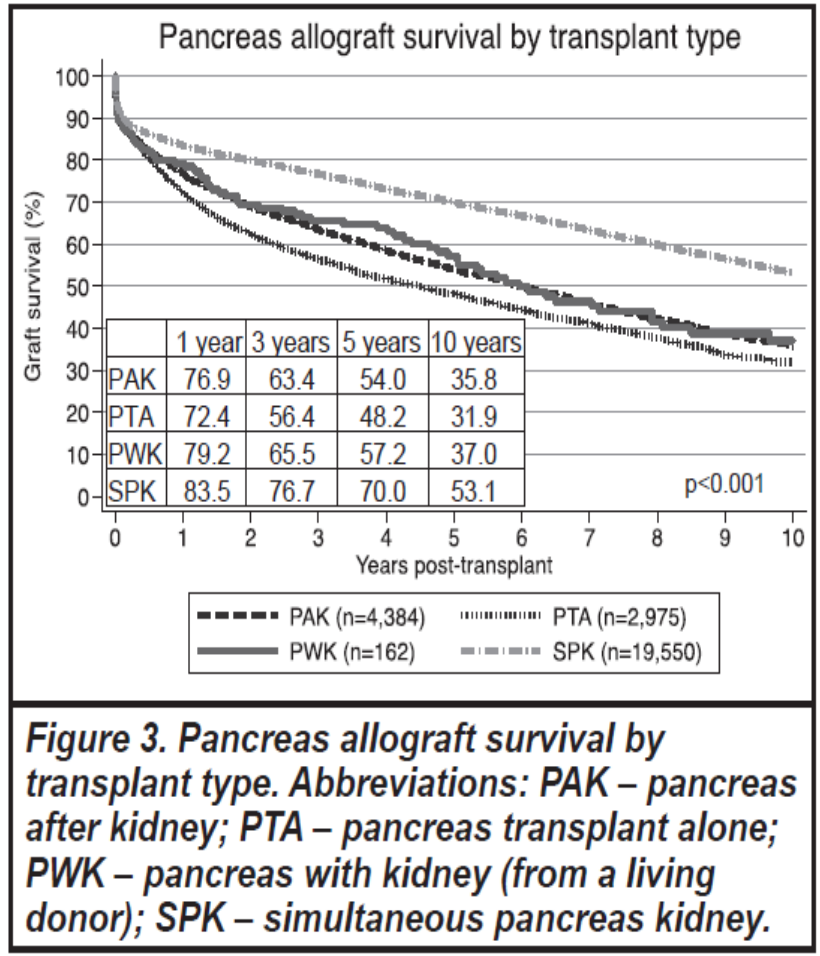


Figure 3. Pancreas allograft survival by transplant type. Abbreviations: PAK – pancreas after kidney; PTA – pancreas transplant alone; PWK – pancreas with kidney (from a living donor); SPK – simultaneous pancreas kidney.

Waki et al, Clin Transpl 2013