Invited Speakers and Oral Abstracts of the 2009 Joint Meeting of the International Pancreas and Islet Transplant Association (IPITA) and the International Xenotransplantation Association (IXA)

Monday, October 12, 2009

Invited Speakers
IPITA Main Plenary Session 1
Pancreas Organ and Beta Cell Availability

Donor preconditioning and pancreas recovery: Do's and don'ts
H. Arbogast
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Pancreas preservation: Standard and novel methods
S. Nadalin
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Organ allocation: Which pancreas for vascularized pancreas or isolated islet transplant
P. Johnson
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Islet preservation and preconditioning prior to transplantation
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Islet transplantation is emerging as a promising treatment option for qualified patients with Type 1 Diabetes. Inconsistent isolation, purification, and recovery of large numbers of high quality islets remain substantial impediments to progress in the field. Donor brain death, pancreas procurement and preservation, islet isolation, purification and culture can have a synergistic and detrimental effect on islet isolation and engraftment. Current cold preservation protocols, including the two layer method, do not prevent exposure of the majority of the human pancreas to hypoxia. Existing islet isolation and purification protocols amplify the impact of cold ischemia, with exposure of the islets to proteolytic enzymes, mechanical stress, warm ischemia and reactive oxygen intermediates. Islet culture may further expose islets to hypoxia. The absence of reliable tools for the objective assessment of pancreas and islet quality has further hindered progress. The recent development of tools for the real-time, reliable assessment of pancreas and islet quality (such as assays based on NMR and oxygen consumption rate) is expected to accelerate progress by enabling the objective evaluation of emerging approaches aimed at maximizing viable islet yield per pancreas. These approaches include: (1) pancreas preservation protocols based on liquid or gas perfusion that may minimize exposure to hypoxia, prevent autolysis, and mechanically facilitate islet liberation from the pancreatic mass; (2) use of anti-apoptotic agents (such as JNK inhibitors and antioxidants) either during organ preservation but also during islet isolation and culture to inhibit the activation of proapoptotic pathways; (3) improved oxygenation, temperature and pH control during pancreas digestion and islet processing to minimize islet stress; (4) removing islets as soon as they are liberated from the pancreatic tissue mass during isolation and circumventing the need for density gradient purification by magnetic retraction using continuous flow sorters to minimize exposure to proteolytic enzymes and other isolation and purification stresses; (5) culture islets in specially designed gas permeable vessels using enhanced media formulations and extend islet culture (7 days), to maintain the amount of viable islet tissue in a preparation, reduce the amount of dead tissue, reduce immunogenicity and provide a "calming" environment for the islet product prior to transplantation. The approaches outlined above and their implications in islet preservation and preconditioning prior to transplantation will be discussed.

Parallel Plenary Session 1
Clinical Islet Transplantation

Collaborative islet transplant registry report 2009
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Background and Purpose: The Collaborative Islet Transplant Registry (CITR) is funded by the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK) and the Juvenile Diabetes Research Foundation (JDRF) to compile data and report results on human-to-human islet transplantation since 1999.
Patients and Methods: For each islet/beta cell infusion, data is collected on the pancreas donor(s), islet processing, and recipient status from screening through long-term after transplant, including severe hypoglycemia, hemoglobin A1C, fasting blood glucose and C-peptide, and insulin use, as well as secondary outcomes, reportable adverse events, complete graft failure and loss to follow-up. The database was closed for analysis on April 1, 2009 for data on recipients that were registered in CITR as of December 31, 2008.
Results: Islet allograft transplantation activity 1999–2008. Thirty-two North American and six European/Australian programs performed at least one islet allograft transplant in 1999–2008, reporting 412 recipients of 828 infusions from 905 donors or about 81% of the total transplant performed at these sites. One hundred seven of the recipients (26%) received a single islet infusion, 202 (49%) received two, 95 (23%) received three, and eight (2%) received a total of four islet infusions. On average, recipients received a total of 842×10^3 (SD 376×10^3) total islet equivalents (IEQs), or 13×10^3 (SD 6.0×10^3) IEQs/kilogram body weight. Of the total 412 recipients, 347 (84%) were islet-alone recipients (IA), while 65 recipients (16%) had previously received a kidney transplant (IAK).
Recipient characteristics. Recipients were 19–67 (mean 44) years old, with 28 (2–54) years of diabetes, weighed 66 kg (range 35–98) with mean body mass index (BMI) of 24 kg/m^2 (16–32). Of which 63% were female. Ninety-seven percent of the participants were on the pump or were taking three or more insulin injections per day, with mean daily insulin of 37 units (SD 13.5). The mean fasting blood was 173 mg/dl (SD 88), mean HbA_{1C} was 7.7% (SD 1.3), and the mean basal C-peptide was 0.1 ng/ml (SD 0.26).
Donor information. There were no living donors. The mean age of donors was 44 years (1–75) and the mean body mass index was 29 kg/m^2 (SD 6.5). Most died of trauma or cardiovascular causes.
Pancreas procurement. UW and Two Layer were the most common (85%) methods used for pancreas preservation. The median duration of cold

ischemia was 7 hours (range 1–27). Liberase HI was the collagenase type used during most islet processing (77%) followed by NB1 (18%).
Immunosuppression therapy. The majority (52%) of the islet transplant alone recipients at the time of first infusion were on a Daclizumab, Sirolimus, and Tacrolimus only immunosuppression regimen. Daclizumab was the sole T-cell antibody used in 59% of first infusions. Anti-thymocyte globulin was given alone or in combination in 14% of first infusions.
Graft function. After the first infusion, increasing proportions of islet-alone recipients are re-infused: 11% by Day 30, 33% by Day 75, 54% by Month 6, and 65% by Year 1 (Exhibit C-1). The proportion that is insulin independent without re-infusion remains fairly constant at 11–15% throughout the first year. An additional 8–12% of all IA recipients retain detectable C-peptide over the first year with insulin dependence but without re-infusion. Of all 347 IA recipients, 74% have at least 3 years follow up post first infusion, at which time, regardless of the total number of infusions received, about 27% are insulin independent, 30% are insulin dependent with detectable C-peptide, 27% have no detectable C-peptide, and 16% have missing data (required but not yet reported). The prevalence of insulin independence from last infusion declines from 59% at Month 4 to about 31% at Year 3 post last infusion (Figure). As cumulative rates of ever achieving insulin independence after islet transplantation, 62% of the IA and IAK recipients achieve insulin independence in the first year post first infusion and by Year 2, after all infusions per recipient, this increases to 68%. Over time there is a decrease in the sustainability of insulin independence: of those who ever achieving insulin independence, 70% retained this status 1 year after achieving it and this decreases to 45% at 3 years. Recent islet recipients (2005–2008) show better results in achieving and maintaining insulin independence than early recipients (1999–2004). Similarly, C-peptide > 0.3 ng/mL is lost over time: 17% at year 1 and 40 at year 4 post last infusion. These trends are also improving in the recent cohort.
Severe hypoglycemia and HbA_{1C}. The prevalence of severe hypoglycemic events decreases dramatically following islet transplantation. Islet transplants also substantially improve HbA_{1C} levels. Taken as a composite outcome, the percent of IA recipients with $HbA_{1c} < 6.5\%$ and absence of severe hypoglycemic episodes increases from 2% pre-infusion to 51–60% at Year 1 post last infusion Hypoglycemia awareness is also markedly improved and is eroded only by loss of graft function or loss to follow-up.
Concomitant medications. Prior to the first infusion, 43% of the recipients were on at least one anti-hypertensive medication and 34% were on a lipid lowering medication. By Year 1 post last infusion, these rates increased to 53% and 61%, respectively.
Adverse events. Sixty-two percent of the islet alone recipients experienced at least one adverse event in Year 1, while 44% experienced one or more serious adverse events in this same period. Of the 594 adverse events reported in Year 1 post first infusion for islet alone recipients, 32% were related to the immunosuppression therapy and 27% were related to the infusion procedure. Of the 312 serious adverse events reported in Year 1 post first infusion for islet alone recipients, 29% were related to the immunosuppression therapy and 33% were related to the islet infusion procedure. Approximately 82% of the serious adverse events resolved with no residual effects. Neoplasms have been diagnosed in 21 of the 412 islet recipients. None were related to the islet infusion procedure while nine may have been related to the immunosuppression therapy (basal cell carcinoma (x2), squamous cell carcinoma (x3), breast cancer, ovarian cysts, and papillary thyroid cancer (x2)). The most frequent type of neoplasm was squamous cell carcinoma (nine recipients). Seventeen recipients continued their islet transplant immunosuppression regimen; two withdrew voluntarily; and two have missing follow-up.
Reported deaths. There have been nine reports of death to the Registry for islet allograft recipients; one was a viral meningitis attributed death possibly related to the immunosuppressant therapy occurring 3 years following the person's last islet infusion.
Conclusions: Compared to 2005, fewer North American centers performed an islet transplant and there were half as many islet transplant recipients in 2008. However, more centers transplanted and more people received an islet transplant in 2008 compared to 2007. With the continuation of Clinical Islet Transplantation (CIT) Consortium protocols that began in 2008, the number of new islet cell recipients is expected to rise. Islet transplantation continues to show short-term benefits of insulin independence, normal or near normal HbA_{1C} levels, sustained marked decrease in severe hypoglycemic episodes and a return of hypoglycemia awareness. Long-term primary efficacy and safety of immunosuppression as well as effects on secondary complications are less well understood. The accumulated experience in islet transplantation indicates that the best candidates for islet transplantation are older recipients in better glycemic control; close relationships between procurement, processing, and transplant teams as well as use of Daclizumab, Etanercept, and Calcineurin inhibitors are associated with favorable outcomes.

Current and future clinical trials
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Metabolic studies to assess islet mass and function
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Functional β-cell mass can be estimated from the β-cell secretory capacity, a measure that correlates with calculated β-cell mass in animal models of β-cell reduction and with transplanted islet mass in human autoislet transplantation. The β-cell secretory capacity is derived from glucose-potentiation of insulin (or C-peptide) release in response to a non-glucose secretagogue such as arginine. Human studies have shown that even insulin-independent islet recipients may have a β-cell secretory capacity of only ˜25% of normal, indicating a markedly reduced engrafted β-cell mass that helps to explain the eventual return to insulin therapy experienced by the majority of recipients. Further studies indicate that the reduced secretory capacity in islet recipients cannot be attributed to calcineurin-inhibitor (CNI) toxicity since the β-cell secretory capacity is ˜100% of normal in whole pancreas and non-diabetic kidney recipients receiving comparable doses of the CNI tacrolimus. Also, infusion of the incretin hormone glucagon-like peptide-1 does not increase the β-cell secretory capacity in islet (or whole pancreas) recipients, suggesting that the impaired secretory capacity is not due to a functional β-cell defect but rather a low β-cell mass. Recent improved results in clinical islet transplantation have been associated with the incorporation of anti-inflammatory, anti-thrombotic, and β-cell "rest" approaches during the peri-transplant period that hold promise for enhancing engrafted β-cell mass and long-term graft function for islet recipients. Improved islet engraftment can be determined through measurement of the β-cell secretory capacity, and can also be expected to restore glucagon release in response to hypoglycemia.

Islet transplantation for children: Are we ready?
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Parallel Plenary Session 2
Pancreas Transplantation: the next wave of creativity

Clinical research: Results of pancreas transplantation and effects of secondary complications
A. Secchi
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Risk/benefit ratio of PAK: Is there a case for LD kidney or SPK as the only choice?
J. Odorico
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The gold standard for T1D and ESRD-SPK

H. Sollinger
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What does the field need for the next wave of creativity?

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The Achilles heel of pancreas (Px) transplantation (Tx), done to engraft beta cells only, remains the propensity for technical complications from the extraneous exocrine tissue, and is a driving force for Islet Tx. But until high islet yields, sufficient to induce insulin-independence from a single donor, are consistently obtained from every Px, the intact organ will continue to be transplanted as is justified by the current 1 year patient and graft survival (insulin-independence) rates of >95% and >80% in all categories: simultaneous with (SPK) or sequential (PAK) to a kidney, or alone (PTA).

A factor limiting the candidate pool is the need for immunosuppression (IS), and thus most Px Txs are done in renal allograft recipients obligated to anti-rejection drugs in whom only the surgical risks need be considered. Non-uremic diabetics must have other complications more onerous than that of the currently employed nephrotoxic IS, the main one to date being hypoglycemic unawareness. To creatively expand the non-uremic candidate pool would require development of non-nephrotoxic IS, and for all diabetics to be considered would require the holy grail, tolerance, a requirement no different than for expansion of Islet Txs, an expansion that is also limited by donor shortage, the latter the main impetus to pursue stem-cell- or xeno-transplants, both still in development but the latter further along.

Of the future advances cited, the one likely to be first is non-nephrotoxic generalized IS, and when consistently effective will certainly allow more non-uremic candidates to be considered; but unless the donor pool can also be expanded, wait list time will be extended. Currently in the US at least one organ is recovered for transplantation from about 8000 deceased donors (DD) annually, but from only ~16% is a pancreas transplanted versus a kidney(s) from ~70%. The difference reflects the more restrictive criteria (such as age) for pancreas donors and the smaller waiting list, the relatively short wait times for at least a solitary pancreas driving conservatism. Thus, the question, can the criteria be liberalized if the candidate list expands and waiting time increases? As more candidates are listed and the wait increases, there will be an impetus for increased use of living donor (LD) segmental Px Txs as well as for DD split Px Txs for two recipients, approaches developed >20 years ago but utilized sparsely to date.

In the interim until Islet Tx is perfected to the point where it replaces Px Tx, how can we creatively decrease surgical and IS complications while increasing Px graft survival rates?

The surgical complications are basically the same for all recipient categories. SPK has had higher Px graft survival rates than solitary Px Txs primarily because of the ability to use elevation in serum creatinine as a surrogate early (pre-hyperglycemia) marker for rejection affecting both organs (when from the same donor), while in the PAK and PTA categories creatinine is irrelevant, and only Px exocrine dysfunction has been used. Thus, at 5 years only 50% of PAK and PTA recipients are insulin-independent. During rejection, a rise in serum amylase or lipase is only transient; as rejection proceeds it returns to normal as exocrine function is lost, so infrequent monitoring (>weekly) can easily miss rejection in enteric drained (ED) grafts until hyperglycemia ensues. At least in bladder drained (BD) grafts a decline in urine amylase is not transient and is detected with less frequent monitoring, but to avoid bladder complications ED is desirable. What is needed to make ED the gold standard for solitary Px Txs is, for example, a serum marker for rejection that is as sensitive and specific as creatinine while not transient. Proteoenomic assays also show promise for immune monitoring (and also for matching to counter T-cell memory and for tailoring immunosuppressoin), and advances in imaging technology to detect early rejection would be welcome. Such markers would allow the graft survival rate of solitary Px to be as good as for SPK Txs, and further enhance the advantage that PAK has over SPK in terms of "intention to treat" patient survival, since most PAKs are done in patients who have LD kidney transplants to preempt dialysis and obviate the high mortality risk of waiting for a DD kidney or SPK.

The need for IS, nephrotoxic or not, limits Px or Islet Txs, but even if solved there will never be enough donors to treat all who could benefit. Hence the need for research on stem cells, islet xenografts, and endogenous beta cell regeneration (with thwartation of autoimmunity for type 1 DM) to ultimately supersede beta cell (pancreas or islet) allografts in type 1 and lean type 2 diabetes mellitus (DM).

Lunchtime Workshop 1
Optimized Islet Isolation and Islet Graft Release Criteria

Protective substances during islet isolation and culture: Myths and facts

A. Pileggi
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Dissecting and predicting islet function after isolation

L. Fernandez
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Islet "graft shaping" and product release criteria: current status and future options

R. Lehmann
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Lunchtime Workshop 2
Novel immunosuppressive protocols for pancreas transplantation

Long term results of steroid free immunosuppression in pancreas transplantation

A. Humar
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CNI free immunosuppression in pancreas transplantation

R. Kandaswamy
Department of Surgery, University of Minnesota, Minneapolis, Minnesota 65455, USA

Campath induction – Long term follow-up

P. Friend
Nuffield Department of Surgery of the University of Oxford, Oxford, UK

IPITA Main Plenary Session 2
Alternative insulin-producing tissues

Embryonic stem cell-derived beta cells
H. Semb
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Endogenous beta cell regeneration
H. Heimberg
Diabetes Research Center, Vrije Universiteit Brussel, Brussels

Expansion of adult human beta cells
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Expansion of adult beta cells from the limited number of islet donors is an attractive prospect. However, while evidence supports the replicative capacity of both rodent and human beta cells *in vivo*, attempts at expanding these cells in tissue culture result in loss of beta-cell phenotype. Our recent lineage-tracing studies support the ability of human beta cells to survive and significantly replicate *in vitro*. Beta-cell delamination out of the normal epithelial structure, a process that results in dedifferentiation, seems to be required for significant *in-vitro* proliferation. Thus, ways must be found for inducing redifferentiation of the expanded cells *ex vivo*, or restoring their function upon transplantation. Elucidation of the signaling pathways altered during beta-cell adaptation to growth in culture may provide clues for cell redifferentiation. We found that human beta-cell dedifferentiation and entrance into the cell cycle *in vitro* correlated with activation of the NOTCH pathway and downregulation of the cell cycle inhibitor p57. Inhibition of the NOTCH downstream target HES1 using short hairpin RNA reduced beta-cell dedifferentiation and replication, suggesting a potential target for inducing cell redifferentiation following expansion in culture for use in cell replacement therapy of diabetes.

Mesenchymal stem cells as a source of insulin producing cells
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Background and Aims: Currently there is an inherent shortage of cadavaric donors necessary to meet the transplantation requirements of type-1 diabetics. Identification of an islet progenitor that could generate an unlimited supply of transplantable islets could potentially alleviate the shortage of donor islets. Previously we have demonstrated that mesenchymal stem cells (MSCs) can be expanded from the non-endocrine pancreas (ductal epithelium) that is normally discarded following islet isolation. Although pancreatic MSCs can be partially differentiated into islet-like cells that express endocrine hormone mRNA and transcription factors critical to islet development they do not express insulin protein. It has been proposed that these MSC progenitors are actually derived from human ß-cells undergoing reversible epithelial-mesenchymal transition (EMT) suggesting that these cells could be a potential source of new ß-cells. In this study we sought to identify a population of epithelial cells in non-endocrine pancreas that give rise to multipotent MSCs.
Materials and Methods: Double immunofluorescent (IF) staining and flow cytometry were used to assess the cell phenotype of non-endocrine pancreas tissue following islet procurement and changes to cell phenotype during *in vitro* expansion of MSCs (24 hours to 21 days) and differentiation. IF staining of paraffin embedded pancreatic biopsy sections was used to assess cell phenotype *in vivo*. Several antibodies against MSC and epithelial cell antigens along with antibodies to pancreatic endocrine and exocrine hormones were used in both methods. Flow cytometry analysis data are expressed as mean percent ± SEM.
Results: In this study we demonstrated that: (1) pancreatic epithelial cells did not express MSC antigens within the native pancreas; (2) following islet isolation fractions of the human NEPT were shown to express EpCAM and CK19 positive epithelial cells that also co-expressed the MSC antigens CD44 (32 ± 8% and 38 ± 10%) and CD29 (85 ± 4% and 64 ± 4%); (3) during *in vitro* expansion of the NEPT the number of single positive epithelial and double positive epithelial/MSCs decreased while the number of single positive MSCs increased; and (4) differentiated MSCs, although demonstrating an altered phenotype, did not revert to a true epithelial cell phenotype in our culture conditions, as epithelial cell surface markers (EpCAM, CK19 and E-Cadherin) were not re-expressed.
Conclusions: This study demonstrates that MSCs may be derived *in vitro* via a pancreatic epithelial cell undergoing EMT, however it is likely that a small percentage of MSCs that reside in the adult pancreas are expanding while the epithelial cells are negatively selected by the experimental culture conditions.

Oral Presentations
IPITA Parallel Session 1
Clinical pancreas transplantation 1

IPITA-O-1.1

Re-transplantation in 1,000 consecutive simultaneous pancreas–kidney transplants
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From December 1985 to December 2007, 1,000 consecutive simultaneous pancreas_kidney (SPK) transplants were performed at our center. Kidney re-transplantation was performed in 113 patients.

Indications for re-transplantation included acute kidney rejection (n = 19); chronic kidney graft failure (n = 87); and technical failure of the kidney (including primary non-function) (n = 7).

The following types of re-transplants were performed: living related donor kidney (LRD) after SPK (n = 42), living unrelated donor kidney (LURD) after SPK (n = 22), deceased donor kidney after SPK (n = 42), and SPK after SPK (n = 7). Kidney re-transplantation was by far the most common type of re-transplant. Technical graft failures occurred in three re-transplanted kidneys.
Results: Overall kidney survival after re-transplantation is 84.8% at 1 year, 67.9% at 5 years and 51.9% at 10 years. Survival for LRD/LURD kidneys is 87.4% at 1 year, 68.6% at 5 years and 60.9% at 10 years. Deceased donor kidney graft survival is 80.8% at 1 year, 68.1% at 5 years and 45.9% at 10 years. Overall patient survival for all re-transplanted patients is 100% at 1 year, 94.6% at 5 years and 88.6% at 10 years from the time of the SPK.
Conclusions: Re-transplantation after SPK is becoming increasingly frequent in major centers. The most frequent type of re-transplantation is kidney transplantation alone. The results in terms of patient and graft survival are good considering the increased technical difficulties and sensitization state of the recipient. Transplant nephrectomy is often required, and certain technical considerations must be taken into account to make re-transplantation successful. These will be discussed.

IPITA-O-1.2

Renal retransplantation in simultaneous pancreas–kidney (SPK) recipients with functioning pancreatic allografts
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Renal allograft loss in the SPK recipient creates the need for renal retransplantation in a patient with a functioning pancreatic allograft.

Between 2/88 and 5/09, our center performed 75 such retransplantations. The etiology for renal allograft failure was chronic rejection (n = 57), acute rejection (n = 5), primary non-function (n = 2), technical causes (n = 3), infection (n = 3) and other (n = 5). All patients were insulin-independent, and four required oral anti-hyperglycemics. Seven (10%) patients developed permanent insulin dependence (ID) immediately post-op. Twenty-four additional patients (40% total) required insulin by the end of follow-up (mean, 4.3 years). There was no relationship between the cause of renal allograft failure, the HgbA1C at retransplant, or the type of allograft (living vs deceased donor) and the development of ID. Median time to retransplant was similar between those developing ID and those who did not (7.7 vs 8.5 years, p = NS). The median time to development of ID was 1.28 years, but was quite variable (0–12.4 years) following renal retransplantation. C-peptide levels were available in 21 of these 31 patients at the time of retransplantation and in 22 of 44 of those patients who did not develop ID. Median fasting C-peptide levels were lower in those who developed ID (5.2 vs 6.95 ng/ml, p = NS). Twenty-seven of 31 (87%) patients developing ID underwent treatment for either synchronous rejection (n = 12) or isolated renal allograft rejection (n = 15).

Eighteen of these patients experienced at least one additional episode of rejection. In patients who remained insulin-free at follow-up, 34 of 44 (77%) experienced at least one episode of rejection; 10 had never been treated for rejection, and 24 experienced two or more episodes of rejection. One year patient survival was 100%. One and 3-year renal retransplant graft survival was 92% and 82%. In summary, renal retransplantation in SPK recipients can be done with excellent outcomes. Nonetheless, a significant proportion of patients will develop ID. The subset of patients which are at greatest risk of losing pancreatic graft function are those with small fibrotic pancreata visualized at retransplant. Neither fasting c-peptide levels, nor the number of prior rejection episodes, nor the original cause of kidney graft loss is predictive of ultimate return to insulin use.

IPITA-O-1.3

Evaluation of pancreas allograft quality and utilization: The pancreas donor risk index

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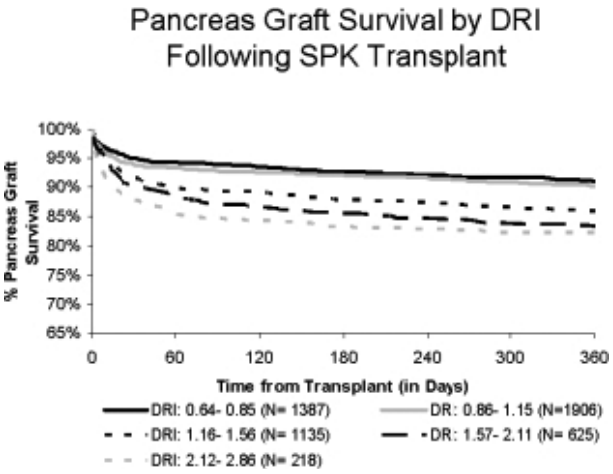
Background: Pancreas allograft acceptance is generally restricted to younger, low risk donors due to fear of early failure. However, no objective measure of allograft quality has been identified to quantify this risk for patients or clinicians. The pancreas Donor Risk Index (PDRI) predicts allograft survival based on donor characteristics available prior to transplantation.
Methods: Recipients SPK, PAK, and PTA from the Organ Procurement and Transplantation Network (OPTN) were examined from 2000–2006. A Cox regression model was constructed to predict the 1 year risk of allograft failure adjusted for donor and recipient and type of transplant (SPK, PTA, or PAK). The coefficients for donor characteristics were used to construct the PDRI.
Results: 9401 pancreas transplants were performed of which 1639 failed within the first year post transplant. The one year graft survival rates varied significantly between transplant types: SPK: 85%, PAK: 78% PTA: 78% $p \leq 0.001$. Among the donor characteristics examined, eight factors were included in the DRI (table).

The DRI was associated with graft survival in all types of transplants after adjusting for recipient characteristics. Among recipients of SPK transplants, one year graft survival varied from 80% to 58% at one year as a function of the DRI (figure). Among isolated pancreas recipients, the variation across DRI categories is even greater with patient survival varying from 73–45% for PTA and 71–44% for PAK.
Conclusion: The P-DRI can be used to predict graft survival following simultaneous and isolated pancreas transplantation (Index of Concordance = 0.67). Increasing PRDI resulting from changes in the donor pool (higher BMI, older age, and DCD), predicts poor outcome particularly among patients receiving PAK or PTA.

Donor Characteristics	Reference Donor (DRI = 1.00)	Change Factor Value to:	DRI
Gender	Male	Female	0.87
Age	28	45	1.56
Black Race	No	Yes	1.27
Asian Race	No	Yes	1.17
BMI	24	30	1.17
Height (cm)	173	190	0.9
Cause of Death CVA/Stroke	No	Yes	1.23
Cause of Death CVA/Stroke in PAK	No	Yes	0.93
Pancreas Preservation Time (hrs)	12	20	1.13
DCD	No	Yes	1.39
SCr >2.5	No	Yes	1.22



IPITA-O-1.4

Outcomes in >2,000 pancreas (PX) transplants (TX) at a single institution

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Background: From 1978 to 2008 >2000 PxTxs were done at one institution (UM). We assessed factors influencing outcomes over 30 years.
Methods: Of 2020 Px Txs, 377 were re-Txs; 482 enteric-, 1490 bladder-drained; 720 simultaneous Px-kidney (SPK), 753 Px-after-kidney (PAK) &547 Px alone (PTA). Analyses were by the 1st vs 2nd 1000, decades, or by Eras: (I) 1978–1994 (n = 640), (II) 1995–2000 (n = 657), (III) 2000–2008 (n = 710) reflecting immunosuppressive protocols, the last steroid-free. Pt (P) &graft (G) survival (insulin-independence, death-censored) rates (R) were done by the Kaplan–Meier method, risk factors (duct-management, age, gender, re-Txs and era) by Cox regression. Coronary artery disease risk factors increased from 30% to 70% between Eras II and III.
Results: PSRs differed little by category or era &was 94% at 1 &83% at 5 yrs for all recipients of 1st Txs. Px GSR (primary and re-Txs) at 5-years in Eras I, II &III for SPK (n = 252, 268, 193) were 68, 74 &72%; for PAK (n = 179, 273, 294), 30%, 60% &59%; &for PTA (n = 207, 112, 223),

26%, 57% &42% ($p < 0.01$ all comparisons). The incidences of technical-failure (graft-thrombosis, removal for infection) were similar for the three categories but overall declined from Era I to II and III: 19, 10 &11% ($p < 0.001$). By Cox regression, for SPK, only re-Txs and Era I were associated with increased risk for graft-failure; for PAK and PTA, Era I, enteric-drainage, and re-Tx were independent risk factors ($p < 0.05$). Of 245 pts transplanted >20 years ago, 96 (40%) are still alive; of these 12 (13%) have functioning grafts. Long-term-function-rates are increasing ($p < 0.05$): 5 year rates for the first 1000 cases by category (423 SPK, 323 PAK, 254 PTA) were 73%, 46% &32%; for the second (297 SPK, 430 PAK, 295 PTA), 73%, 62% &47%. Ten year-function for 1988–1998 vs 1978–1988 was 62 (n = 370) vs 58% (n = 36) for SPK; 39 (n = 218) vs 16% (n = 87) for PAK; &30 (n = 124) vs 12% (122) for PTA cases ($p = 0.01$ all comparisons).

Conclusions: Px GSRs improved during the first 15-years of application (the learning curve), particularly in solitary (PAK, PTA) Txs (2/3 of our cases). For the last 15-years risk-adjusted early outcomes have been static but with no decline on steroid-free-immunosuppression. Long-term-results continue to improve.

IPITA-O-1.5

Pancreas transplantation in selected type 2 diabetes mellitus recipients

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Background: Pancreas transplants (PTx) are rarely performed in patients with high C-peptide levels. These patients have high daily requirements of insulin and show different degrees peripheral insulin resistance. They may be classified as type 2 diabetics but there are concerns that peripheral insulin resistance may overstimulate the newly transplanted beta cells leading to rapid cellular ''exhaustion'' and accelerated graft failure for non-immunologic reasons.

Methods: Between May 1996 and March 2009 289 PTx were performed including 160 SPKTx and 129 solitary PTx. C-peptide levels were measured before and after PTx in all recipients. For the purposes of this study, based on a cut-off level 1 ng/ml, patients were classified as having low (< 1 ng/ml) (Low C-P) or high (≥1 ng/ml) (High C-P) levels. High C-P patients were 20 (13 SPK, 2 SPLK, 3 PTA and 2 PAK) and Low C-P patients 269 (147 SPK, 25 SPLK, 76 PTA and 21 PAK). Two of 15 uremic High C-P patients (13.3%) were transplanted preemptively as compared with 70 Low C-P patients (40.9%) ($p = 0.03$). Mean BMI, mean duration of diabetes from onset to PTx and mean daily insulin requirements were similar in the two groups. Mean age at onset of diabetes mellitus was 14.1 ± 0.5 years in Low C-P vs 22.6 ± 2.6 years in High C-P ($p < 0.0001$). Mean age at PTx was 38.7 ± 0.5 years in Low C-P vs 43.8 ± 1.2 years in High C-P ($p = 0.003$).

Results: Delayed graft function occurred in 3.4% pancreas and in 8.2% kidney grafts in Low C-P vs 0 and 6.7% in High C-P. Relaparotomy rate was 16.0% in Low C-P vs 10.0% in High C-P. Ten pancreas grafts were lost due to thrombosis (3.7%) in Low C-P as compared with none in High C-P; 18 (6.7%) additional pancreas grafts in Low C-P and 2 (10.0%) in High C-P developed non-occlusive thrombosis. There was no significant difference in the incidence of infection and early rejection between the two groups (14.6% in Low C-P vs 10.0% in High C-P). One-year patient, kidney and pancreas survival rates were: 95%, 90% and 85% and in Low C-P vs 95%, 100% and 90% in High C-P; 5-year figures were: 93%, 84% and 77% in Low C-P vs 95%, 92% and 79% in High C-P.

Conclusion: Good results can be achieved with PTx in patients with high levels of C-peptide. Pre-transplant metabolic work-up should be really exhaustive. Patients with low BMI, mild insulin resistance, and high daily insulin requirements may eventually be deemed suitable candidates for PTx.

IPITA-O-1.6

Panel reactive antibodies (PRA) and antibody mediated rejection (AMR) is associated with poor outcomes following pancreas transplantation

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PRA, AMR, and graft dysfunction has been demonstrated in kidney transplantation, but has not following pancreatic transplantation.

From 2000 and to 2007, 253 pancreas transplants and 144 biopsies were available for review. Fifty-seven patients had C4d stained slides. The mean follow up was 34 months. The study excluded grafts with early thrombosis due to technical failure. A ROC analysis determined the optimal peak PRA cutoff of 20% and the optimal cold ischemia time (CIT) cutoff of 16 hours. Of the 144 patients that were biopsied, 70 had no rejection and 74 had biopsy proven rejection; AMR in 5, CMR in 67 and both AMR in 2. Patients with a peak PRA >20% had similar demographics including C4d positivity, types of pancreas transplant, HLA mismatches, donor age, cold ischemia, induction agents and maintenance immunosuppression compared to recipients with low peak PRAs. The high PRA group had more retransplants, 24% vs 9%, $p = 0.03$. High PRA patients had a lower 7 year graft survival compared to low PRA patients, 76.8% vs 29.5%, $p = 0.001$. Seven year graft survival for high PRA recipients that had positive C4d+ staining on biopsies was 0 compared to 72.9% for C4d (-) patients. In comparison low PRA recipients had a 7 year graft survival of 25% and 56.2% for C4d+ and C4d (-) patients respectively, $p = 0.002$.

A multivariate analysis with recipient and donor features showed C4d positivity as the only factor associated with a poor outcome, having a hazard ratio of 6.69.

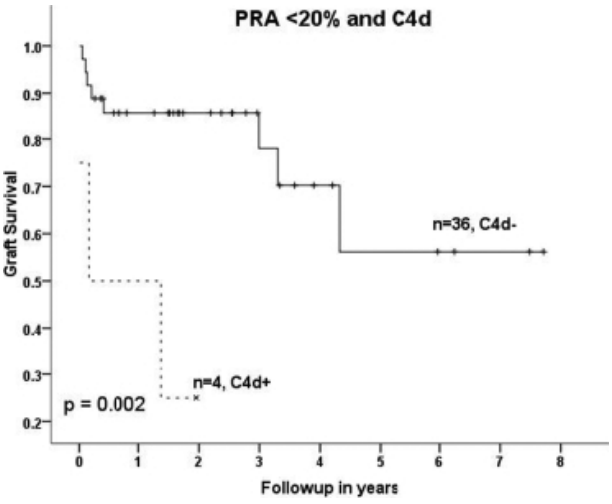

Table 1 Multivariate analysis for graft outcome

Parameter	p	HR (95% CI)
C4d+	0.04	6.69 (1.06 – 42.24)
HLA Mismatch =>5	0.08	0.27 (0.06–1.18)
Donor Age	0.33	1.03 (0.97 – 1.09)
Cold Ischemia Time	0.35	0.95 (0.85 – 1.06)
Induction	0.38	0.55 (0.14 – 2.08)
Recipient Age	0.42	1.04 (0.95 – 1.14)
Rejection	0.69	0.87 (0.45 – 1.69)
PRA > 20%	0.75	0.70 (0.08 – 6.38)
Re–Transplant	0.80	1.32 (0.15 – 11.33)

High PRA and C4d positivity is associated with worse outcomes and no long term survivors for the combined group. C4d should be routinely performed on all pancreas biopsies.

IPITA-O-1.7

Simultaneous pancreas and kidney transplantation in recipients older than 60 years of age

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Background: Older type 1 diabetic patients with end-stage kidney disease suffer from increased rate of co-morbidities, which obviously limits the use of transplantation therapy. The aim of the project was to retrospectively analyze the efficacy of transplant therapy in recipients older than 60 years. Graft function and patient survival rates were compared to recipients younger than 60 years.
Methods: Only patients with type 1 diabetes mellitus and renal failure who underwent a first simultaneous pancreas and kidney transplantation (SPKTx) were included into the study. Before transplantation, all candidates were extensively screened for cardiovascular desease and in most of them coronarography was performed. Prophylactic immunosuppressant regimen consisted of polyclonal antibody induction, calcineurin inhibitors, MMF or sirolimus and short-term course of steroids. A comparison of patient and graft survival rates was performed. Graft failure was defined as death, return to dialysis (for the kidney graft) and return to insulin therapy for the pancreas graft. Kaplan–Meier test was used to evaluate survival data. Differences in survival rates among groups were tested with the log rank test.
Results: From 1993 to 2009, 314 pancreas and kidney recipients were divided into three groups according to their age (Group A ≥60 years, n = 13; Group B 40–59 years, n = 167; and Group C younger than 40 years, n = 134). One and 5-year cumulative patient survival rates were 92.7 and 90.4% in Group A, 94.8 and 88.5% in Group B, 94.8 and 81.2%. The differences between the groups were not statistically significant. However, 5-year pancreas graft survival in older recipients was significantly better than in the other groups (A vs B 90.4% and 70.1%, p = 0.001; A vs C 90.4% and 65.8%, p = 0.008). Five-year cumulative kidney survival rates in groups A, B and C were 90.4, 88.8 and 80,5%, respectively (p > 0.05).
Conclusion: Since 1993, 4% of type 1 diabetic kidney and pancreas recipients were 60 years and older. Their 5-year patient, pancreas and kidney survival rates were not inferior than those in younger categories. Our results suggest that in carefully selected older recipients SPK transplantation may represent the optimal treatment option with satisfactory results.

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IPITA-O-1.8

Pancreas transplantation in elderly recipients

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Background: Pancreas transplantation (PTx) remains the only treatment modality that consistently restores insulin independence in insulin-dependent diabetics. In combination with a kidney, PTx may even become a life-saving Tx in uremic diabetics. Traditionally, because of the magnitude of surgery, the frequency and the severity of post-Tx complications, PTx has been reserved to relatively young individuals. However, ageing of western population has increased the number of patients aged 50 years or more seeking for PTx. We herein report our experience with PTx in recipients aged 50 years or more.
Methods: Between May 1996 and March 2009 289 PTx were performed including 160 SPKTx and 129 solitary PTx. Twenty-seven PTx were performed in recipients aged 50 years or more (≥50): 17 SPK, 9 PTA and 1 PAK; 262 PTx were in recipients aged less than 50 (< 50): 143 SPK, 27 SPLK, 70 PTA and 22 PAK. Most pancreas grafts were implanted according to portal-enteric drainage technique (< 50: 145 vs ≥50: 16). A significant proportion of grafts was transplanted with systemic-enteric drainage (< 50: 82 vs ≥50: 7), while systemic-bladder drainage was used in a minority (< 50: 35 vs ≥50: 4). Excluding recipient age (p = 0.0001), there were no differences in baseline donor and recipient characteristics. Similar immunosuppressive regimens were used in all the recipients.
Results: Delayed graft function occurred in 2.7% pancreas and 8.3% kidney grafts in < 50 as compared with 7.4% and 5.9% in ≥50 (p = NS). Relaparotomy rate was 16.9% in < 50 < span > vs 11.1% in ≥50 (p = NS). The rate of pancreas graft failure due to thrombosis was 3.4% (n = 9) in < 50 and 3.7% (n = 1) in ≥50; non-occlusive thrombosis developed in 16 (6.1%) additional pancreata in < 50 and in 4 (14.8%) in ≥50 (p = NS). None of these grafts was eventually lost due to thrombosis. The incidence of other surgical complications were similar in < 50 and ≥50. There was no significant difference in the incidence of urinary or respiratory tract infection and early rejection (17.2% in < 50 < span > vs 7.4% in ≥50) in either groups. One-year patient, kidney and pancreas survival rates were: 95%, 91% and 84% and in < 50 vs 96%, 93% and 92% in ≥50 (p = NS); five-year patient, kidney and pancreas survival rates were: 92%, 84% and 76% in < 50 < span > vs 96%, 93% and 87% in ≥50.
Conclusion: Our experience shows that PTx can safely and effectively be performed in properly selected patients aged 50 years or more.

IPITA-O-1.9

Expanded criteria donors in pancreas transplantation: The Oxford experience

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Background: Increasing pancreas transplant activity in the last few years has exposed the shortage of "standard donors" for transplantation. In an effort to increase the potential donor pool, we liberalized our criteria for acceptance of pancreases. The graft and patient outcomes from such pancreas transplants are compared with the standard cohort.
Methods: From April 2004 to April 2009, 265 pancreas grafts were procured either from 176 Standard Criteria donors (SCD) (donors after brainstem death (DBD) age 13–45) or 89 expanded criteria donors (ECD) including DBD (< 12 or > 45 years) &grafts from donors after cardiac death (DCD) (n = 28). All grafts were implanted intraperitoneally with enteric exocrine and systemic venous drainage. Outcome measures were incidence of delayed graft function (DGF) of pancreas &kidney, graft &patient survival.
Results: There were 265 transplants (261 recipients) with majority (n = 249) receiving Campath induction with Tacrolimus/MMF maintenance. There were 137 SPK, 24 PAK &15 PTA from SCD; 58 SPK, 16 PAK &15 PTA from ECD. SC donors had longer median follow-up (23 months vs 16, p = -0.004). EC donors had higher incidence of vascular cause of death (66% vs 3%, p = 0.0006) and lower incidence of head trauma (16% vs 30%, p = 0.01). SCD recipients were younger (42 ± 7 vs 46 ± 8, p = 0.0001) and had a lower BMI (24 ± 4 vs 26 ± 4, p = 0.03). ECD recipients had more readmissions (33% vs 20%, p = 0.03). Median cold ischemia, HLA mismatch, hospital stay, rejection incidence and reoperation rate for both groups were similar. ECD recipients more frequently had DGF of the kidney (19% vs 11%, p = 0.13) and of the pancreas (1.7% vs 6.7%, p = 0.06). Both groups had similar primary non-function rates (< 1%). Overall patient survival (95 vs 95.5%) and pancreas survival (SCD 92% vs ECD 90%) was similar.
Conclusions: Expanded criteria donors (defined by extremes of age and donors after cardiac death) provide comparable outcomes following pancreas transplantation. This data suggests re-visiting the definition of the 'expanded criteria' to make more pancreases available for transplantation.

IPITA Parallel Session 2
Clinical islet transplantation

IPITA-O-2.1

Islet autotransplant (IAT) outcomes after total pancreatectomy (TP) in the modern era

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Background: IAT can preserve beta cell mass after TP for painful chronic pancreatitis (CP).We previously reported on 173 IATs for 1977–2007, with ˜2/3 achieving function (fxn) and ˜1/3 insulin-independence (I-I), more durable than for allografts with more islet equivalents (IE)/kg (*Transpl 2008;86:1799*). IATs are done with brain death, organ preservation, allo- & auto-immunity and diabetogenic immunosuppression absent. Here we analyze our results for recent IATs by yield.
Methods: Islet yield &fxn was correlated in 113 pts after TP-IAT for CP in 2006–2008.Islets were prepared by collagenase digestion in a Ricordi chamber, purified on gradients when tissue volume was high (>20 cc) &intraportally embolized to the liver. Pts were assessed quarterly for islet fxn: full if I-I, partial (PF) if euglycemic on once-daily long-acting insulin (all tested were C-pep+),&failed if on standard basal-bolus insulin.
Results: Islet yield ranged from 276 to 8655 IE/kg &correlated negatively with pathology. The mean ± SD islet infusion was 3393 ± 1835 IE/kg. There were no severe adverse events from IAT but 3/113 pts (3%) died of surgery complications. 100 (88%) achieved islet fxn (II+PF), 41 (36%) II; 9 of the latter (22%) reverted to insulin but retain PF; 5/68 (7% initially with PF have failed. Currently (f/u 18 ± 11 mos), 32 (28%) are II (HbA1C 5.7 ± 0.5%); 63 (56%) have PF (HbA1C 6.4 ± 1.5%); &15 (13%) failed (HbA1C 7.4 ± 1.2%), with respective yields of 4530 ± 1933, 3253 ± 1446 &1443 ± 1002 IE/kg ($p<0.0001$). However, pts with low (<2500) can sustain II or PF but with a lower incidence than with high (>5000) or intermediate yield (IE/kg)($p<0.0003$): Clinically significant fxn occured in 69/75 (92%) of IAT recipients of 2500–8600 IE/kg &in >2/3 receiving less. I-I was sustained in >half of recipients of >5000,in ˜1/3 after 2501–5000 IE/kg, &in ˜1/8 with less.
Conclusion: IAT prevents or minimizes the otherwise inevitable and often brittle diabetes after TP for CP in a high proportion of pts. Relatively low islet yields provide good fxn in the absence of the additional factors to be overcome to optimize allograft outcomes.

Yield IE/kg (N)	Full(I–I)	Partial	Failed
<2500(38)a	5(13%)	21(55%)	11(29%)
2501–5000(54)b	16(30%)	33(61%)	4(7%)
>5000(21)c	11(52%)	9(43%)	0(0%)
a1, b5 and c3 PFs were initially I–I; a3 and b2 of Failed initially had PF.			

IPITA-O-2.2

Predictiveness of positive C-peptide for outcomes of islet transplantation

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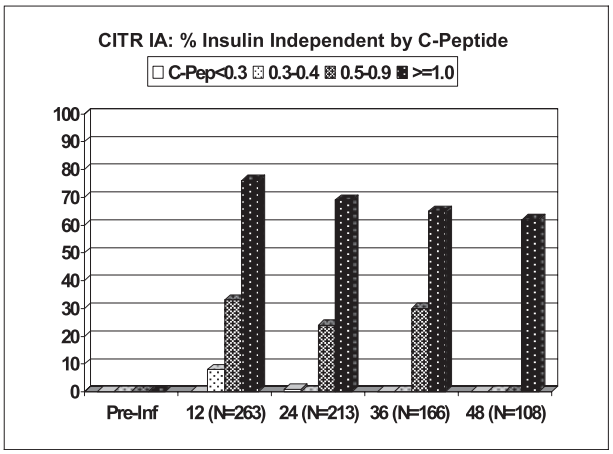
The Collaborative Islet Transplant Registry has sufficient data to define "positive" C-peptide. The Figure shows the specificity of various ranges of basal C-peptide (<0.3, 0.3–0.5, 0.5–0.9 and >1.0) for each of the main outcomes of islet transplantation: insulin independence, HbA1c<6.5%, fasting blood glucose 60–140 and absence of severe hypoglycemia at 1, 2, 3 and 4-years follow-up post last infusion (pre-infusion relationships are meaningless). C-peptide >1.0 predicts 60–75% insulin independence, though this declines over 4 years post last infusion. C-peptide of 0.5–0.9 predicts 25–30% insulin independence. C-peptide >0.3 predicts HbA1c<6.5% equally as well as C-peptide>0.5 or >1.0.

C-peptide>0.3 predicts good glycemic control, though all recipients received some benefit in glycemic control even with negative C-peptide (<0.3). C-peptide>0.3 predicts greater freedom from severe hypoglycemia episodes (SHE), although even C-Peptide < 0.3 achieves 50–60% freedom from SHE. C-Peptide > 0.3 achieves 80–100% freedom from severe hypoglyecmia episodes.



A preliminary interpretation is that C-peptide of 0.30–0.49 is almost as predictive as C-Peptide > 0.50 for HbA1c < 6.5%, fasting BG 40–160 and absence of severe hypoglycemia, but for insulin independence, even C-peptide of 0.5–0.9 is not nearly as predictive as C-peptide > 1.0. Taken together, this data may argue to use ≥0.3 as the cut-off for positive C-peptide with the caveat that C-peptide > 1.0 is needed for insulin independence, but >0.3 is good for other protection.

IPITA-O-2.3

Improvement in outcomes of islet transplantation: CITR 1999–2008

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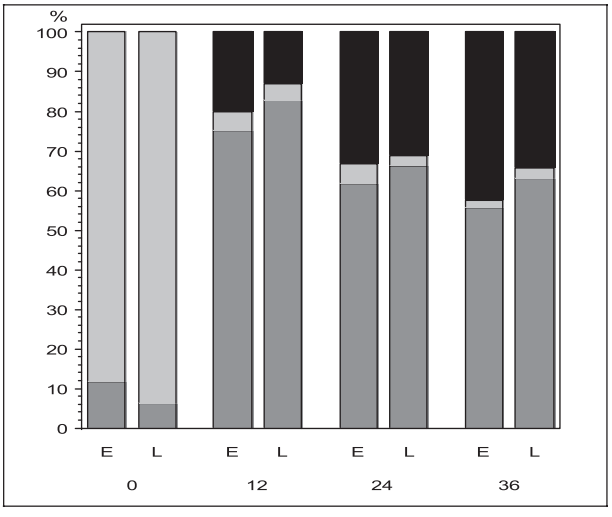
As of April 1, 2009, the Collaborative Islet Transplant Registry had information on 347 islet-alone and 65 islet-after-kidney recipients of allogeneic human islets from 28 North American, 3 European and 2 Australian centers. Improvements by 10% points are now seen in primary outcomes between those transplanted early in the decade (E: before 2005) vs those transplanted Late (in or ageter 2005), along with a general decline of about 10% points per year from last infusion. Complete graft loss (see Figure: CGL, black bars) is reported or defined as undetectable C-peptide for ≥2 annual visits without re-infusion. In this analysis, CGL trumps any other available results. Overall, the percentage of each favorable outcome declines over 1-, 2- and 3-years from last transplant ($p=0.01$), but improves from Early to Late periods: C-peptide≥0.3:



p = 0.06; insulin independence: p = 0.02; severe hypoglycemia: p = 0.06. Much of the decline over 1-, 2- and 3-years post last infusion parallels and is attributable to graft loss (Figure, black bars), which is itself attributable to identifiable factors. Multivariate models identify factors that explain the observed late vs early differences, and are related to the outcomes (Table). All the outcomes other than +C-peptide ≥0.3 ng/dl) are conditional on a concurrent +C-peptide, hence the factors that drive +C-peptide necessarily drive the other outcomes, shown in the table by the effect of a concurrent positive C-peptide. The apparent negative effect of MMF/MPA on +C-peptide may be a surrogate for switching to MMF/MPA from other agents.

Overall, islet transplantation is improving steadily and we are beginning to identify factors that can predict improved outcomes, including recently available immunosuppression strategies. The effects of multiple immunosuppression strategies, often over several infusions in the same patient, and myriad islet prepration processes, cannot be entirely factored from the Registry data.

IPITA-O-2.4

Risks and benefits of transplantation in the cure of type 1 diabetes: Whole pancreas versus islet

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Whole pancreas and islet transplantations are considered standard therapy for patients with type 1 diabetes (T1D) characterized by metabolic lability and progressive chronic complications. The balance of risks and benefits of each procedures and the comparison between them are still a matter of debate, being difficult to recruit patients appropriately. The aim of the study was to report the rate of success in the cure of T1D and the complications developed after the 2 different procedures. Sixty patients with T1D, metabolic lability and chronic complications were considered: 27 received pancreas alone (PTA), 33 received islet alone (ITA). Mean age and duration of diabetes were comparable. The success of transplant was measured on the basis of insulin independence at 1 and 12 months, on partial function (C-peptide > 0.3 ng/ml; insulin therapy); on early failure (C-peptide < 0.3 ng/ml within 3 weeks). The complications considered were: surgical interventions in addition to transplant; CMV infections; hospital admissions during the first year. Insulin independence was achieved within the 1st month in 20/27 (74%) PTA and in 19/33 (57%) ITA; among patients followed for almost 12 months 9/16 PTA and 10/18 ITA were still insulin free. Partial function was observed in 1/27 PTA and in 9/33 ITA; early failure was observed in 6/27 (22%) PTA (pancreas removal because of thrombosis) and 5/33 (15%) ITA. The complications in PTA were: 12/27 relaparotomy; 7/27 CMV activations; 7/27 episodes of hospital admission out of surgical intervention (3 urinary tract infections; 1 thrombotic thrombocytopenic purpura; 1 aortic-enteric fistula; 1 worsening kidney function; 1 necrotizing fascitis). The complications in ITA were: 2/33 CMV activation; 7/33 episodes of hospital admission (4 worsening kidney function, 2 requiring kidney biopsy; 2 viral myocarditits; 1 toxic hepatitis). In conclusion: both procedure are capable to establish insulin independence in T1D, with similar rate of insulin independence. The surgical, infective and immunosuppression therapy complications clearly affect more seriously PTA than ITA, conditioning higher episodes of intervention and hospital admissions within the first year. This study allows a better profiling of clinical indications for islet transplant, which is not burdened with severe adverse events.

IPITA-O-2.5

Influence of donor age on islet isolation and transplantation outcome

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Purpose: It has been suggested that the age of human organ donors might influence islet isolation and transplantation outcome in a negative way due to a decrease of *in vivo* function in islets isolated from older donors.

Methods: We retrospectively analyzed 332 islet isolations performed between January 2002 and September 2008 and divided them into two groups depending on donor age (n = 187 and n = 145 for < 50 and > 50 years, respectively). Pancreata were procured and processed according to established protocols. Isolation outcome was determined by islet yield, success rate (> 250'000 IEQ) and transplantation rate. Beta cell function was assessed *in vitro* by stimulation indices in static incubation assays. Transplanted patients were divided into two groups depending on donor age of islet preparations (n = 35 and n = 25 patients that received just islets from < 50 and > 50 year-old donors, respectively). Because islet recipients received more than one graft, analysis of insulin independence is impossible to perform for an individual islet preparation. Therefore, *in vivo* function was assessed by the newly developed secretory units of islets in transplantation (SUIT) index and the C-peptide/glucose ratio 1 and 6 months after transplantation.

Results: There was no difference in islet yields between the two groups (249'200 ± 11'400 and 245,900 ± 9'800 IEQ for < 50 and > 50 year-old donors, respectively). Success rates were 45% for both groups, respectively. Overall, 85 (45%) islet preparations were transplanted from < 50 year-old donors and 56 (39%) islet preparations were transplanted from > 50 year-old donors. Stimulation indices were similar for both groups. SUIT indices and C-peptide/glucose ratios 1 month after transplantation were significantly higher in patients that received islets from the younger donor population (40.8 ± 3.6 vs 25.6 ± 3.7, p = 0.006 and 1.19 ± 0.1 vs 0.76 ± 0.08, p = 0.003, respectively). At 6 months after transplantation, SUIT indices were slightly but not significantly higher and C-peptide/glucose ratios were significantly higher in patients that received islets from the younger donor population (44.5 ± 3.8 vs 35.2 ± 5.3, p = 0.162 and 1.4 ± 0.1 vs 0.94 ± 0.1, p = 0.009).

Conclusions: Our study shows that, in our donor population, donor age does not influence islet isolation outcome, in contrast to islet graft function.

IPITA-O-2.6

Islet allograft survival with ATG induction tacrolimus and mycophenolate mofetil switching to sirolimus and mycophenolate at 6–9 months

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Aim: Islet cell transplantation has proven effective at reducing recurrent severe hypoglycemia but immunosuppression impedes islet engraftment and causes significant morbidity. The aim of this study was to evaluate a novel immunosuppressive regimen where islet recipients were given tacrolimus and mycophenolate mofetil (MMF) and a 5 day course of ATG without corticosteroids. Between 6 to 9 months patients were switched to sirolimus and MMF. The aim was to promote engraftment and prevent rejection in the first 6 months whilst limiting islet and renal toxic effects of CNI.

Methods: Between 2006 and 2009 ten consecutive patient with hypoglycemia unawareness and normal renal function were enrolled in the study. All islet infusions were prepared using GMP collagenase and neutral protease.

Results: The study is ongoing. Of the 10 patients receiving at least one islet transplant the mean number of IEQ/pt was 649992 ± 388000. One recipient has had three islet infusions, four have had two islet infusions and five one infusion. Two of the 10 patients achieved insulin independence after two islet infusions and remain insulin independent 18 and 9 months respectively. Of the remaining eight, one lost their graft and withdrew, two have evidence of declining function after one infusion. The remaining five are c-peptide positve with stable function 2 to 24 months post transplant. C-peptide positive patients had a marked improvement in hypoglycemia score (HGS). Median HGS pretransplant was 2253 (1118–5369), 89 at 3 months (0–234) and 0 at 6 months (0–372). HbA1c improved in all patients. At 1 month all patients had a HbA1c < 7%. 6/7 and 4/7 had HbA1c < 7% at 3 and 6 months respectively. Transient lymphopenia and diarrhoea were the most common side effects. Of the five recipients converted to sirolimus, two failed conversion because of intolerable side effects. Two developed donor specific antibodies, one in conjunction with a failed graft.

Conclusion: ATG induction with tacrolimus and MMF, with a switch to sirolimus and MMF provides good protection against rejection in the short term. The success in switching to a CNI free regimen is limited by tolerability to sirolimus. Islet transplantation reduced the frequency and severity of hypoglycemia whilst improving glycemic control in c-peptide positive patients regardless of ongoing requirements for insulin.

IPITA-O-2.7

Immune mechanisms of teplizumab induction immunotherapy in type 1 diabetic islet allograft recipients

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Long-term survival of islet allografts in type 1 diabetes (T1D) may require establishing immune regulation to control auto- and alloimmune effector mechanisms. Studies in murine models suggest that the tolerogenic potential of non-activating anti-CD3 antibody therapy is attributed to its ability to clear pathogenic cells, polarize immune response to Th2 in short phase and induce regulatory T cells that mediate active tolerance. We have previously reported that induction immunotherapy with teplizumab (non-activating hOKT3γ1Ala-Ala) facilitated restoration of insulin independence for over 1 year in four of six recipients of single-donor islet allografts. An increase in Treg was observed and maintained posttransplant. In this study, we investigated mechanism of teplizumab induction immunotherapy and its role in continued survival of islet allografts. Single-donor islet allografts were transplanted into five additional T1D recipients. Induction immunosuppression included low dose teplizumab (15–18 mg over 12 days peritransplant) in three and high dose teplizumab (43 mg over 12 days peritransplant) in two recipients. Maintenance immunusuppression was with sirolimus and tacrolimus. One out of two recipients in high-dose and one out of three in low-dose teplizumab group has remained insulin independent for >1 year; one recipient in low-dose group has experienced stable and good partial graft function. We examined frequencies and functions of Treg, FoxP3 expression, and serum cytokine levels posttransplant. Low-dose teplizumab enhanced frequencies of Treg (CD4+CD25hiFoxP3+) early posttransplant (21 days) whereas an increase with high-dose teplizumab was only evident after 300 days posttransplant. Interestingly, low dose antibody increased expression of FoxP3 within 14 days posttransplant and remained high for rest of study period. An increase in serum IL-10 and reduction in inflammatory cytokines was observed in all recipients irrespective of dose of teplizumab.

Our preliminary findings suggest that low dose teplizumab can be effective in prolonging islet allograft survival by enhancing FoxP3 expression and increasing frequencies of Treg while concurrently limiting inflammatory cytokines and thereby activation of T cells capable of mediating rejection. Further analyses of regulatory and effector immune mechanisms are warranted to optimize teplizumab administration in T1D islet allograft recipients.

IPITA-O-2.8

Histological graft assessment after clinical islet transplantation

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This work assessed the feasibility of needle biopsy monitoring after intraportal islet transplant (n=16), and islet graft morphology was studied with the addition of autopsy samples (n=2). Pancreas autopsy samples from two non-diabetic individuals were used as control. Islet tissue was found in five needle samples (31%). Mild liver abnormalities included localized steatosis (n=8), mild nodular regenerative hyperplasia and mild portal venopathy (n=3) and hepatocyte swelling (n=2). Endocrine cell composition and distribution were similar between islet grafts and normal islets within the native pancreas. There was no or minimal immune cell infiltrate in patients on and off exogenous insulin, including two patients with ongoing negative metabolic events (increasing HbA1c or insulin requirement). The infiltrate was mainly composed of CD4 and CD8 positive cells. Overall, this study demonstrates that needle biopsy is feasible after clinical islet transplant, but of limited practical value (31% of islet sampling). Islet endocrine composition is preserved after transplantation. Islet grafts demonstrate no or minimal immune cell infiltration, even in the case of ongoing islet loss, requiring the definition of new pathological marker of immune events. On the basis of the findings, we would speculate that the islets are failing due to a combination of metabolic/microenvironmental stress and a very low-grade immunologic reaction and that the former is predominant.

IPITA-O-2.9

Pet in clinical islet transplantation

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Limited tools are available to study the fate of islets transplanted via the portal vein. To expand our knowledge concerning the peritransplant phase of clinical islet transplantation we have introduced the use of dynamic PET/CT examination. Six transplantations in five patients were performed. A fraction of the isolated islets were allowed to internalize [18F]FDG for an hour prior to transplantation. These islets were carefully mixed with the unlabled islets just before the islet infusion. From the start of the transplantation a 60-minute dynamic scanning sequence over the liver was acquired in 2D mode in the PET/CT scanner. A static 5-minute 3D examination of the whole liver was then performed, and finally the torso was examined 90 minute post-transplantation using a static whole-body sequence to visualize additional sites of uptake. Islets were readily seen. The peak of the radioactivity in the liver was found at the end of each infusion; thereafter, a mono-exponential decrease was seen. The radioactivity in the liver represented 62.9 (53.4–88.4) % of the total administered dose. A marked increase in p-C-peptide was found in parallel with the release of radioactivity from the transplanted islets. The distribution of the radioactivity in the liver was heterogeneous.

Large variations in the concentration of islets were found in the liver. The percentage of islets concentrated in areas with a higher islet density than 400 IEQ/cc varied between 1% and 32%. No accumulation of radioactivity was found in the lungs. All transplanted patients had positive c peptides, lowered their need for exogenous insulin and HbA1c at follow up 4 weeks after transplantation. No side effects associated with the PET procedure were found. It is possible to monitor the islet graft for hours after transplantation in the liver with the described technique. The uneven distribution in the liver with the islets concentrated to small areas shows that the perception that islets transplanted to the liver are evenly distributed is wrong. The good short-term outcomes indicate that the peritransplant findings with a loss of islets are not attributed to poor isolation but rather are general in islet transplantation. It also shows that the presented technique is safe and doesn't harm the islets.

In upcoming clinical trials techniques to improve both an even spread of islets in the liver and engraftment will be evaluated.

IPITA Parallel Session 3
Islet isolation: Enzymes

IPITA-O-3.1

A new method to valued efficiency of enzyme blends for pancreas tissue digestion

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One of the best successful examples of cell therapy is represented by islet transplantation since 1990. However islet isolation methods are not

completely standardized yet. More than half of isolation procedures failed to isolate adequate islets for transplantation, due to variable pancreas conditions and to unpredictable enzymatic blend efficiency. Enzymes used for pancreas digestion include collagenases and neutral proteases: their composition and activity are largely variable between different batches.

We set up a new *in vitro* method to better *in vitro* characterize enzymatic blend before its use in human pancreas. In our experimental approach human immortalized cells (ECV-304) or human islets were coltured within a 3-D type-I collagen gel in 96 wells plate. After one culture day, cells and/or islets were treated with different commercial enzymes (Liberase, Serva NB1 premium grade, Collagenase type P, Thermolysin, Neutral proteases) from different batches at different concentrations and for different times. Digestion of 3-D type-I collagen fibril gels were monitored by optical dense absorption to a fix l; morphology of released cells and/or islets were valued by confocal microscopy analyses. Cells were immunostained about expression of some adhesion molecules, like: integrins, cadherins and associated molecules, catenins, to appreciate cells morphology and islets aggregation modifications. Cell viability was assessed by SYTO 13 and ethidium bromide.

We found that Neutral proteases is less pure and more toxic than Thermolysin: it has collagenase activity and it significantly decreases cell viability. Even viable cells after neutral proteases showed an alterated morphology with an impairment of cell to cell communications. We, also, observed an higher efficiency in extraction by Serva NB1 compared to Liberase and Collagenase type P, used in the same experimental conditions.

By this *in vitro* method we were able to compare the minimal active enzyme concentration of different enzyme blends: we found that Liberase has its best value as number of extracted cell/alive cells in 130 mg/ml; while, in actual protocols for human pancreas is used to 1.3 mg/ml. Preliminary results showed a correlation between data achieved in cell lines with those of human islets, thus confirming the potential predictive role of this method for the selection of enzymes for human pancreas digestion purpose.

IPITA-O-3.2

Influence of neutral protease concentration on the digestion of collagen VI within the human pancreas

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Background: The two component system of Serva Collagenase NB1 with Neutral Protease (NP) is widely used for clinical islet isolation although the amount of NP added varies markedly between centres. The effect of increasing NP activity on the individual components of the extra-cellular matrix (ECM) of the islet-exocrine interface has not been determined. In this study, we used an *in vitro* assay to compare the effect of different amounts of neutral protease on the digestion of Collagen VI, the principal collagen subtype in the islet-exocrine interface of the human pancreas.

Methods: With appropriate consent and ethical approval, human pancreata were retrieved from six donors (ages 19–60 years). Cold ischaemia time was <10 hours. Tissue blocks ($\sim 0.5\ cm^3$) were taken and snap-frozen in liquid N2. Specimens were cryo-sectioned onto slides at 10–15-μm thickness and stored at −25°C. Specimens were thawed and incubated in HBSS (control), or with NB1 alone (5.5 U/ml) ± NP at 0.14 U/ml (NP) or at 0.28 U/ml (2NP) for 5 minutes at 37°C. Digestion of the ECM within the islet-exocrine interface was analysed by double immuno-labelling for insulin and Collagen VI. The collagen was semi-quantified by morphometry using a Zeiss KS-400 image analysis system. Fifteen to 27 islets were assessed in the specimens. Data were expressed as area of collagen/islet area. Statistical analysis was by T-test.

Results: Examination of the tissue after 5 minutes incubation showed digestion of the specimens with substantial loss of tissue from the slide in the presence of 2NP. The mean islet area increased with 2NP (16716 ± 1978 vs 13593 ± 2141 μm^2 in controls) due in part to loss of smaller islets from the specimen; +2NP only 7% islets were <5000 μ2 vs 22% in control, $p<0.05$). The peri-islet Collagen VI content was significantly reduced by 28% from 0.301 ± 0.047 to 0.218 ± 0.043/islet area + NB1 alone ($p<0.01$) and was not further reduced by addition of NP. Increasing the activity of neutral protease (2NP) had minimal effect (0.188 ± 0.019 vs 0.202 ± 0.035/islet area).

Conclusions: NB1 alone substantially degraded Collagen VI in the islet-exocrine interface whereas increasing NP lead to the loss of islet integrity and structure with minimal effect on ECM components.

IPITA-O-3.3

Vitacyte collagenase HA: A novel enzyme blend for efficient human islet isolation background

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Background: Islet isolation outcome depends critically on the efficiency of the enzyme blend used for pancreas dissociation. Mandatory regulations and safety issues have made Serva collagenase NB1 the current standard enzyme utilized for clinical purposes. Since 2008 a new enzyme blend has been available for clinical human islet isolation. In this study we investigated if Vitacyte CIzyme collagenase HA can be considered as an alternative to the Serva NB1 enzyme blend regarding isolation outcome and islet quality.

Methods: The outcome of 18 human islet isolations performed with Vitacyte HA was compared to 18 pancreases processed with Serva NB1 using identical procedures for isolation and quality assessment.

Results: Compared to Serva NB1, Vitacyte HA provided similar islet yield and purity without affecting islet integrity as expressed by survival during culture, stimulation index and cytokine expression (table 1). Although a significantly lower collagenase amount was used in isolations performed with Vitacyte HA, it resulted in significantly reduced digestion time which correlated with a reduced ADP/ATP ratio.

Conclusions: We conclude that Vitacyte HA is a highly efficient enzyme blend that does not affect morphological and functional integrity of human islets. The rapid islet release provided by this enzyme blend may be of advantage for islet quality and function.

Enzyme blend	Vitacyte HA	Serva NB1
Collagenase (PZ–U/g)	22.7 ± 1.3	27.9 ± 1.4[a]
Recirculation time (min)	22.0 ± 1.6	27.2 ± 1.3
Undigested tissue (%)	14.3 ± 1.9	16.6 ± 2.6
Yield isolation (IE/g)	2110 ± 242	2047 ± 212
Purity isolation (%)	47.1 ± 3.1	39.9 ± 3.8
IE recovery culture (%)	93.1 ± 3.9	90.4 ± 3.8
Purity culture (%)	48.1 ± 4.8	42.7 ± 4.3
Stimulation index	7.4 ± 1.8	9.8 ± 2.3
ADP/ATP ratio	0.052 ± 0.002	0.080 ± 0.011[a]

a P<0.01 by Mann–Whitney test

IPITA-O-3.4

The effect of truncated collagenase class I isomeres on human islet isolation outcome

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Background: The low standardization of enzymatic human islet isolation is mainly related to the insufficient characterization of the enzymes used for pancreas dissociation. Previous retrospective studies indicated that the efficiency of an enzyme blend is mainly determined by the integrity of collagenase class I. The objective of the present investigation was to characterize the importance of intact (115 kDa) and truncated (100 kDa) collagenase class I isomeres for successful human islet isolation.

Methods: Crude collagenase was chromatographically separated into intact (CI-115) and degraded (CI-100) collagenase class I that was recombined with purified collagenase class II at a class II-to-I ratio of 0.7. The blends were

supplemented with 1.5 DMC-U/g of neutral protease and 2.6 BAEE-U/g of tryptic-like activity (TLA), dissolved in 1.5 ml/g HBSS and intraductally injected into research grade pancreata utilizing a collagenase activity of 25 PZ-U/g. Pancreata were digested at 37°C and purified on a Ficoll gradient utilizing a Cobe 2991. Purified islets were cultured for 2–3 days at 37°C and assessed for insulin release during static glucose incubation and islet viability utilizing Syto 13 and ethidium bromide for staining.
Results: No significant differences were observed between CI-115 (n = 8) and CI-100 (n = 8) with regard to digestion time (28.0 ± 2.2 vs 24.0 ± 2.3 minutes), percentage of undigested tissue (8.1 ± 1.1 vs 12.5 ± 2.0%) and embedded islets (15.0 ± 2.2 vs 15.3 ± 5.2%), purity (52.8 ± 4.9 vs 48.6 ± 8.9%), islet equivalent number (IE) per gram (2340 ± 320 vs 3010 ± 640 IE/g), survival post culture (67.1 ± 7.5 vs 64.6 ± 5.9%), stimulation index (3.9 ± 0.7 vs 2.5 ± 0.5) and viability (76.6 ± 2.9 vs 78.2 ± 2.3). Islet morphology was almost similar in both experimental groups.
Conclusions: These preliminary findings in human pancreata indicate that truncated collagenase class I with an apparent molecular weight of 100 kDa has the same capacity to dissociate human pancreatic tissue and to release islets as intact collagenase class I. These results are supported by data obtained by different activity assays utilizing different substrates.

IPITA-O-3.5

Successful clinical islet isolation using a GMP-manufactured collagenase and neutral protease

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Pancreatic islet transplantation offers a specific, minimally-invasive approach to restore normoglycemia and insulin independence in patients with type 1 diabetes, but its clinical applicability is limited by the complexity of the islet isolation process. Pancreata (n = 14) were processed using a modified Ricordi method. Briefly, pancreata were perfused with a GMP grade Collagenase NB1 and Neutral Protease NB mixture (Serva) via the pancreatic duct followed by continuous chamber digestion. Islets were washed and then purified using a continuous density gradient. The fourteen processed pancreas had an average total islet yield of 544745 (+114862) Islet Equivalents (IEQ), suitable for transplantation. The Serva collagenase NB1 and neutral protease NB functioned within a wide range of donor ages; 17–53 years with a mean of 37 years and average BMI of 32. An average trimmed pancreas weight of 102 (+18) grams yielded 5,862 (+1,843) purified IEQ per gram of trimmed pancreas with a 95% post culture recovery. Four different lots of Serva enzyme were tested. Several contributing factors or modifications were identified as contributing to these successful isolations. These included a reduced average cold ischemia time of 5.9 (+1.5) hours. Pancreata were perfused with cold enzyme solution at a concentration of 1600U Collagenase NB1; 200U Neutral Protease NB containing 11 mmol/l CaCl2 per 100 g pancreas. Pancreata digestion time averaged 16 (+2) minutes at 37°C. The digestate was diluted into cold RPMI containing 5.0% HSA, insulin, and heparin while the chamber temperature remained at 30°C. Digest was centrifuged and pellets were pooled into flasks containing 0.625% HSA and 2% Pentastarch solution at 4°C. Islets were then purified on a continuous density isopyknic iodixanol (Optiprep) gradient after being washed in a solution containing 0.2% Pentastarch. We have determined that the biochemical activity of the Serva products and these modifications contributed to a successful clinical islet isolation and clinical islet function.

Clinical Outcomes	n
Pts with C–peptides at 1 month after Transplant	10/10*
Patients Insulin Independent >30 days	9/10*
Duration of Independence (months)	>24 mo: 2 Pts; >18 mo: 2 Pts; >12 mo: 1 Pt; >3 mo: 4 Pts

* 6 patients received single islet transplants and 4 patients received 2 islet transplants. All patients produced C–peptide after their first transplant.

IPITA-O-3.6

Comparison of liberase HI and collagenase NB1 in 199 human islet isolations

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Objective: Liberase HI (Liberase) has been the standard enzyme used for highly efficient islet isolation and transplantation prior to its mandated replacement with primarily Collagenase NB1 (NB1). The study goal was to identify relevant differences in pancreas digestion and islet isolation outcome parameters using either Liberase or NB1 in a large number of isolations.
Methods: Isolation methods and solutions were essentially unchanged during the study period. Quality parameters include insulin stimulation index (SI) via glucose perifusion and insulin content per DNA. Pancreas dissociation efficiency parameters include digestion time, percent undigested tissue, packed tissue volume, islet yield, purity, islet recovery after culture and percent of isolations fulfilling transplantation criteria (>300000 IE, >30% islet purity, a biphasic SI >2, and <5 ml packed tissue volume). Logistical problems resulted in a smaller population of Liberase isolations for quality measurements.
Results: Donor organ variables were not significantly different between the two groups. Recirculation time for the digestion phase was almost identical for both groups. Lower percent undigested tissue, higher packed tissue volume, more IE/g pancreas favored Liberase isolated organs whereas purity, SI and insulin content were superior when NB1 was used (Table 1). Post culture recoveries were similar and purities unchanged from isolation values. Transplantation criteria data was not significantly different although notable at 58% and 42% for Liberase (n = 33) and NB1 (n = 94) groups respectively.
Conclusions: This is the first study comparing large numbers of pancreata isolated using either Liberase or NB1. This study highlights important differences in isolation enzymes on pancreas digestion and certain islet quality parameters postculture. Liberase was more efficient for pancreas dissociation than NB1 although more harmful to exocrine tissue and islet functionality.

Digestion and Quality Parameters

Enzyme	Liberase (n)	NB1 (n)	p =
Recirculation time (min)	26.2 ± 0.6 (103)	26.4 ± 0.6 (96)	>0.05
Undigested tissue (%)	15.7 ± 1.3 (103)	18.0 ± 1.0 (96)	<0.05
Packed tissue (ml/g pancreas)	0.60 ± 0.02 (103)	0.47 ± 0.02 (96)	<0.001
Yield (IE/g)	4015 ± 228 (103)	2979 ± 149 (96)	<0,001
Purity (%)	43,0 ± 1,8 (103)	53,8 ± 1,5 (96)	<0,001
Stimulation Index	6,1 ± 1,0 (33)	15.0 ± 2.3 (94)	<0,001
Insulin Content (ng/ng DNA)	2,4 ± 0,2 (71)	5,0 ± 0,3 (94)	<0,001
IE = Islet equivalent			

IPITA-O-3.7

Assessing neutral protease activity in tissue dissociation enzyme mixtures

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The recent report by Brandhorst, et al. regarding the impact of trypsin like activity (TLA) on improved human islet isolation highlights the importance of neutral protease activity for successful human islet isolation. Little is known about the comparative activities of neutral protease enzymes typically used in tissue dissociation or the effect of substrate on apparent specific activities. We have used azocasein and fluorescein isothiocyanate conjugated to bovine serum albumin (FITC-BSA) as substrates to detect neutral protease activity using a spectrophotometer or fluorescent microplate instruments, respectively, to characterize several neutral proteases. Initially, we used the azocasein as substrate in an endpoint assay. The main advantages of this assay were the commercial availability of substrate and historical experience. However, after critical review of the results, we found that results were inconsistent and did not correlate with the difference in specific activities between proteases when other substrates were used to

measure activity. The development of the fluorescent microplate assay using FITC-BSA as substrate dramatically improved our analysis of neutral protease activity. In contrast to the azocasein assay, this is a homogeneous, kinetic enzyme assay that allowed determination of steady state reaction rates of multiple samples, dilutions and selected inhibitors in one microplate within 90 minutes. This assay had a restricted linear range (4–8 fold) but the intra and interassay coefficients of variation (CVs) were $<5\%$ and $<9\%$, respectively.

Further experiments using this assay were designed to assess the enzyme responsible for TLA activity found in selected lots of purified collagenase. These samples were assessed for neutral protease activity in the presence or absence of a sulfhydryl inhibitor. These experiments showed $>98\%$ inhibition of a clostripain control and $>96\%$ inhibition of TLA in collagenase, but $<30\%$ inhibition of thermolysin and *C. histolyticum* neutral protease, which strongly supports the conclusion that the TLA is due to clostripain contamination. Further work confirmed an earlier report in the patent literature of synergistic increase in neutral protease activity when purified collagenase, neutral protease and clostripain were mixed. The implications of this and other undefined synergistic activities and how this may impact islet isolation is an area of active investigation in our laboratory.

IPITA-O-3.8

Novel fluorogenic peptide for evaluating and quantifying bacterial collagenase I, II, thermolysin and neutral protease

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Islet isolation from human pancreata for clinical treatment of type I diabetes requires appropriate enzymes to digest the organ and to free the islets from exocrine tissues. The quality of collagenase in terms of variability between manufacturers and from lot to lot is a major problem that limits the use of the islet transplantation procedure. Therefore, there is a need to develop a precise and sensitive assay to evaluate and quantify the activity of collagenase and other enzyme(s) critical for the digestion of pancreata, in order to free islets without compromising their quality or quantity. Here, we describe a novel fluorogenic peptide substrate that can be used to precisely determine the activities of collagenase I, II, thermolysin and neutral protease, and the corresponding Km values were: 28.18, 24.5 and 34.6 μmol/l, respectively. The new substrate can also be used in a fluorometric assay to determine the activity of other bacterial enzymes such as dispase, which is also used for tissue dissociation. The assay is fast (1 hour), specific to collagenase and bacterial neutral proteases, and does not interfere with the presence of the pancreatic proteases trypsin, chymotrypsin and elastase. It can also be used to determine if there are any traces of enzyme activity post islet culture and is ideal for monitoring collagenase activity and to observe the effects that any enhancer or inhibitor may have on its enzymatic function, particularly during the pancreas digestion process. Thus, an appropriate ratio of lytic enzymes can be established for optimal islet yield even from suboptimal organ recoveries.

IPITA-O-3.9

Handling and short-term stability of collagenase and neutral protease components and enzyme mixtures

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Purified tissue dissociation enzymes are critical to the success of human islet transplantation as the quality of the enzymes directly impacts the number and quality of islets. There are no studies on the short-term stability of the current purified tissue dissociation products or data that addresses the stability of the product after multiple freeze thaw cycles. We have examined the impact of freeze thaw cycles on reconstituted VitaCyte collagenase and thermolysin products. Short term stability studies were performed on collagenase-neutral protease mixtures using VitaCyte, Roche, or Serva-Nordmark products. The function of the enzymes was assessed by a sensitive fluorescent microplate assay using fluorescein isothiocyantate (FITC) labeled collagen fibrils or FITC labeled bovine serum albumin as substrates to measure collagen degradation activity (CDA) or neutral protease activity (NPA), respectively. Purified collagenase or thermolysin were able to withstand multiple freeze-thaw cycles with an insignificant apparent loss of activity. Collagenase-neutral protease mixtures were subjected to short-term storage at four temperatures (−80°C, −20°C, +4°C, +37°C). Samples were removed at different intervals – up to 4 hours at 37°C, 24 hours at 4°C, and 8 weeks for samples stored frozen – and enzyme assays performed. Thermolysin in solution was stable whereas the Serva Neutral Protease lost 9.7% and 37% of activity when stored at 4°C for 4 hours and 37°C for 1 hour. Collagenase-neutral protease mixtures showed a decline in CDA when stored at +4°C or +37°C. The Serva product showed the greatest decline in activity with more than 29% of activity lost after 1 hour at 37°C and only 3.4% and 8.2% for VitaCyte and Roche respectively. Collagenase activity was more stable when mixtures were stored frozen with nearly all samples retaining almost 80% of the starting CDA. NPA for all three suppliers showed a statistically significant loss of activity after 8 weeks stored frozen. Freeze thaw data demonstrates the ability to freeze aliquots of reconstituted individual VitaCyte enzymes for short durations. Collagenase and neutral proteases should not be mixed until immediately prior to use since these enzyme mixtures are sensitive to proteolysis, especially when not frozen, potentially affecting their performance in islet isolation procedures.

IPITA Parallel Session 4
Islet engraftment

IPITA-O-4.1

Endoscopic gastric submucosa (EGSM-ITX) islets transplantation in pigs with streptozotocine induced diabetes

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Background: Islets and pancreas transplantation have become standard treatments of patients with diabetic complications. However pancreas transplantation is associated with high incidence of complications and the long-term results of islet transplantation are still unsatisfactory. Loss of pancreatic islets grafts is caused not only by immunological reactions but also due to the site of grafting and IBMIR. Gastric submucosal space could be an alternative site for transplantation. The aim of this study was to assess the possibility of endoscopic islets transplantation into the gastric submucosa. **Materials and Methods:** Twenty Landrace pigs weighing 19–24 kg were obtained for the study. Seven were controls (C-group) and 13 were Transplantation group (TX group).In both groups diabetes was induced by streptozotocine (stz) infusion at 200 mg/kg. At 7 days post stz infusion pigs underwent endoscopy. Immunosuppression consisted of tacrolimus,sirolimus. At 7 days post transplantation, control gastroscopy was performed to assess the gastric mucosa and to take biopsies for histopathology.10–30 days after eGSM-ITx Magnetic Resonance (MRI) examination was performed. Stomach and pancreas were obtained at autopsy for histopathology. For 10 days after diabetes induction (up to 3 days after eGSM-ITx) in both groups, insulin was given to reach glycemia between 150–200 mg/dl, after that period insulin was given only when glycemia exceeded 600 mg/dl. **Results:** There were no differences in insulin requirement and glycemia up to the day of eGSM-ITx between the groups. Tx-group received a mean of 6000IEQ/kg. Mean glicemia in the day of Tx was 445 in C-group and 470 in Tx group (p = NS). At 1,3,7,21 and 30 days post-transplantation glicemia in

C-group was: 452,555, 600,586,573 vs 215, 278, 220, 213, 123 in Tx-group ($p < 0.05$). Tx-group animals had a significantly lower insulin requirement and significantly lower mean glycemia since the first day post transplantation. This trend was observed till the end of study at 1 month. There were no signs of perforation, ulceration or bleeding after the eGSM-ITx on gastroscopy and histopathological examination. In MRI scans unspecific thickening of gastric wall was observed at sites of islet deposition.
Conclusion: Endoscopic islets transplantation into gastric submucosa is feasible and a safe procedure in an experimental setting. Its potential for clinical application needs further studies.

IPITA-O-4.2

Bone marrow as an alternative site for islet transplantation

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Background: The liver is the current site for pancreatic islet transplantation but has many drawbacks due to immunological and non-immunological factors as well as important technical limitations. We asked whether pancreatic islets could be engrafted in the bone marrow (BM), an easily accessible and widely distributed transplant site that may lack the limitations seen in the liver.
Methods: Pancreatic islets were implanted into the BM of STZ diabetic C57BL/6 mice. Islet survival, function and morphology were evaluated in comparison with the liver site.
Results: Pancreatic islets engrafted efficiently in BM and were able to maintain glucose metabolism up to 1 year. In the syngeneic model of marginal islet mass transplantation BM guaranteed a higher probability to reach euglycaemia than liver (2.4 fold increase, $p = 0.02$). Glucose metabolism in mice that achieved normoglycaemia by islet transplantation into the BM was similar to that of non diabetic mice for all the parameters evaluated (fasting and not fasting glycaemia, insulinemia, HOMA-B and glucose tolerance after IVGTT or OGTT). Morphologically, islets in BM showed an increased size with a compact morphology and a conserved ratio between a and β cells. Islet transplantation in BM did not affect hematopoietic activity and marginally affected bone structure.
Conclusions: The BM is an attractive alternative site for pancreatic islet transplantation. The results of our study open a research line with potentially significant clinical impact not only for the treatment of diabetes but for other diseases amenable to treatment with cellular transplantation.

IPITA-O-4.3

Prevention of non-immunologic loss of transplanted islets in monkeys

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Background: The promise of islet cell transplantation cannot be fully realized in the absence of parallel improvements in tolerizing regimens and in the engaftment of healthy and resilient islets. The loss of islets in the pre-, peri- and early post-transplant periods is profound. To determine the potential role that transplantation of only a marginal mass of functioning islets may play in triggering late non-immunological graft loss, we studied the effect of treatment with alpha-1-antitrypsin (AAT) in a non-human primate autologous islet transplant model. A marginal mass of autologous islets, i.e. islets prepared from 70–80% of the pancreas, was transplanted into subtotal pancreatectomized + streptozotocin treated insulin deficient diabetic hosts. In this model, islet function is insidiously lost over time and diabetes recurs eventually in all recipients by months post-transplantation. AAT, an acute phase reactant, serves to terminate adverse inflammation. Hence, we tested the hypothesis that over time physiologic stimulation of an initially marginal b-cell mass may evoke to non-specific inflammation that instigates self-perpetuating and progressive metabolic deterioration.
Methods: Cynomolgus monkeys were partially (75–80%) pancreatectomized and given streptozotocin. Group 1; received no treatment and group 2; received a short course of alpha-1-antitrypsin (AAT). Two days later all monkeys received autologous islet transplants infused into the portal vein. Blood glucose levels were measured twice daily, c-peptides twice a week and IVGTT monthly.
Results: Each of three control autologous islet transplant recipients slowly and progressively lost graft function and became insulin dependent on 160, 195 and 210 days (mean of 185 day). Histological analysis showed reduced number of small and degranulated islets in the absence of inflammation. In contrast five autologous islets transplanted in monkeys that have received AAT have functional islets at 2 years.
Conclusions: These results demonstrate that (1) auto-islet transplants loose function with time in cynomolgus monkeys in the absence of any immune response and (2) blocking inflammation prevents islet mass loss and increases islets cell mass in monkeys. Finally a short course of AAT prevented non-immunological islet failure and enabled functional expansion of islet mass such that graft function improves rather than deteriorates over time.

IPITA-O-4.4

Role of chemokines during IBMIR and development of new therapeutic target

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Background: The conventional technique for transplanting isolated islets is by intraportal injection, with the islets being trapped in the liver. Human islets exposed to human blood trigged an ''instant blood mediated inflammatory reaction'', IBMIR, characterised by platelet consumption, and activation of the coagulation and complement systems. The purpose of this study is to develop a model of study of these reactions and to determine the cellular mechanim at the origin of islet loss during IBMIR.
Methods: Several *in vitro* models have been developed to identify the IBMIR : syngenic model with pancreatic islets and macrophages from Lewis rat, allogenic model with pancreatic islets and macrophages from wWstar rat and xenogenic model with human pancreatic islets and mouse balbc macrophages. Inflammatory reactions are studied by the measure of macrophages migration with a modified Boyden chamber. Pancreatic islet supernatants were prepared using several islets preparation purities from 90% to 50%. Chemokines secretion was measured in the supernatant using ELISA test (CCL-5, CCL-3, CXCL-1). Finally, IBMIR was inhibited using antagonist of CCR-5, PSC-RANTES (10 pg/ml) or anti-coagulant strategy (Pentosan or heparin).
Results: Study of chemotaxis showed comparable migration of macrophages using the three *in vitro* models (1.41 ± 0.12 using syngenic model, 1.52 ± 0.18 using allogenic model and1.25 ± 0.22 using xenogenic model with 90% purity). However, macrophage migration was significantly increased when the purity of islet preparation decrease from 1.41 ± 0.12 to 1.92 ± 0.19 for syngenic models at 50% of purity ($n = 6$, $p < 0.001$). In accordance with these results, the measure of chemokines release in islet supernatant showed a dose-dependent increase of chemokine secretion with the decrease of islet purity. For example, CCL-5 secretion was 25.8 ± 2.5 pg/ml with 90% of purity and 218.7 ± 24.2 with 50%. Moreover, PSC-RANTES reduced significantly chemotaxis from 1.92 ± 0.19 to 0.67 ± 0.18 ($n = 6$, $p < 0.001$). Similar inhibition was obtained with pentosan and heparin.
Conclusions: Chemotaxis technology seems to be a good tool to study *in vitro* inflammatory reaction. This model showed that exocrine tissue contamination in islet preparation increases IBMIR. Finally, heparin-like strategy could improve islet viability during transplantation.

IPITA-O-4.5

Quantification of transplanted iron oxide-labelled islet cells by 3-dimensional 3T magnetic resonance imaging (MRI)

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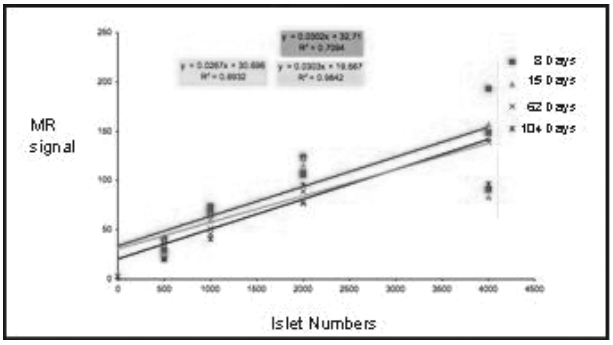
Background: Monitoring mass and function of islet grafts is vital for the improvement of results of islet transplantation in type I diabetes. MRI provides non-invasive imaging for iron-labelled islets. Quantification of the engrafted islet mass has not yet been reported.
Methods: Syngeneic Resovist-labelled islets were transplanted into the portal vein of SD rats. Increasing islet numbers were transplanted (0,500,1000,2000,4000). Imaging was carried out on a clinical 3T MRI scanner. Scanning was performed 1 day, and 1, 2 and 8 weeks after surgery. Respiratory triggering was performed with a trigger delay of 150 ms. Images obtained with a novel 3-dimensional Ultrashort-Echo-Time (UTE) technique were compared with conventional 2-dimensional acquisition sequences. Quantitative assessment included measurement of the number of iron-related pixels (over all liver slices) and correlation with number of transplanted islets.
Results: The isotropic 3-dimensional images can be viewed with the same resolution in all three orientations. When imaging at day 1, surgical disturbance makes visualization of clusters difficult. At 1, 2 and 8 weeks, cell visualisation and quantification is more defined with isolated enhanced spots within an uniform background. UTE images show a good correlation between the number of counted pixels and the number of transplanted islets, and this is reproducible over time.

A rapid decrease of the signal was observed in rats transplanted with xenogeneic human islets. The novel technique also offers an improved signal-to-noise ratio, due to lower background and better signal detection due to control of motion artifacts.



Conclusion: This novel MR imaging technique offers reproducible quantification of transplanted islet grafts. Development of the technique on a clinical MRI scanner makes its application in a human clinical study promising.

IPITA-O-4.6

A strong candidate approach to prevent the instant blood-mediated inflammatory reaction in clinical islet transplantation

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Background: The instant blood-mediated inflammatory reaction (IBMIR), in which activation of both the coagulation and complement cascades plays a key role is one of the main obstacles to successful islet engraftment. At present, however, no useful protocol is clinically available. Therefore the aim of this present study was to examine whether complementary peptides against an active region of C5a, proved to be safe due to extremely low molecular mass, in combination with a clinically available anti-coagulant could provide an effective protocol for suppressing IBMIR.
Methods: Complement receptors on the pancreatic tissues and isolated islets were analyzed by immunohistochemical staining and flow cytometry. Two point five islet equivalents/g of syngeneic rat islet grafts were transplanted intraportally into four groups (control, gabexate mesilate, C5a inhibitory peptide (C5aINH), combination: n = 8 per group) of streptozotocin-induced diabetic rats. The recipients which were injected with equivalent amounts of saline served as a control. Plasma samples were collected at 0, 0.5, 1, 3 and 6 hours after transplantation and analyzed. The curative rate, intravenous glucose tolerance test and insulin amounts in the liver of the recipients were also evaluated.
Result: Both C5a receptors and C5L2 were expressed on the isolated islets (C5a receptor: 8.01 ± 2.55%, C5L2: 3.71 ± 1.00%), but not on the pancreatic tissues prior to isolation procedures. Thrombin-antithrombin complex was significantly suppressed in the treated three groups ($p = 0.004$), and HMGB1 known to be a detrimental inflammatory mediater was lower in the C5aINH treated two groups.The curative rate was remarkably improved (between control and combination_$p = 0.005$, among four groups –Ð vs 40 vs 50 vs 100%, $p = 0.08$).The glucose tolerance was significantly improved in the treated three groups ($p = 0.0001$).Insulin amounts in the liver was considerably higher in the treated groups than that in control group. Notably, the increase of body weight in the recipients was not affected by treatment.
Conclusions: These data suggest that induced C5a receptor on the isolated islets might accelerate the IBMIR. Hence C5a inhibitory peptide combined with gabexate mesilate could be a strong candidate approach, without side effects, to control the IBMIR induced in clinical islet transplantation.

IPITA-O-4.7

Testis specific gene as novel tool for detection of islet damage in the peripheral blood of mice with a portal islet transplant

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Aim: The aim of this study was to evaluate a realtime PCR assay for quantification of testis specific gene (TSG) in peripheral blood for monitoring graft damage after portal islet transplantation in a mouse model.
Methods: Two groups of female mice received portal transplants of 300 islets from male FVB donor mice after induction of diabetes by intraperitoneal streptozotcin injection (220 mg/kg): (1) Iso Tx (FVB, n = 9) and (2) Allo Tx (C57BL/6, n = 5). Blood was sampled by tail snip for DNA extraction and glucose measurement. Real-time PCR was used to measure the DNA levels of the pYMT2/B gene. The liver was harvested at the end of the study for immunohistochemistry.
Results: Despite normoglycemia from the first postop day until the study end at day 50, the Iso Tx group differed in the histology of islet grafts. The finding of perfectly healthy islets without any cellular infiltrate in four animals (Iso Tx I) contrasted with the observation of islets with mononuclear cell infiltrates with more or less disrupted borders in five animals (Iso Tx II). Interestingly, TSG copy numbers were low or unmeasurable only in Iso Tx I with normal islet morphology, but consistently elevated throughout the study in Iso Tx II with disturbed islet morphology, thus indicating ongoing islet damage. In allografts, mice were normoglycemic on the first day post Tx, but became hyperglycemic after 4–10 days due to histologically proven rejection on day 11. Throughout the observation period TSG copy numbers were highly elevated, reflecting ongoing islet damage, which did not differ much throughout the observation period.
Conclusions: The TSG realtime PCR assay is a noninvasive method for monitoring damage of islet transplants. It was specific because it detected

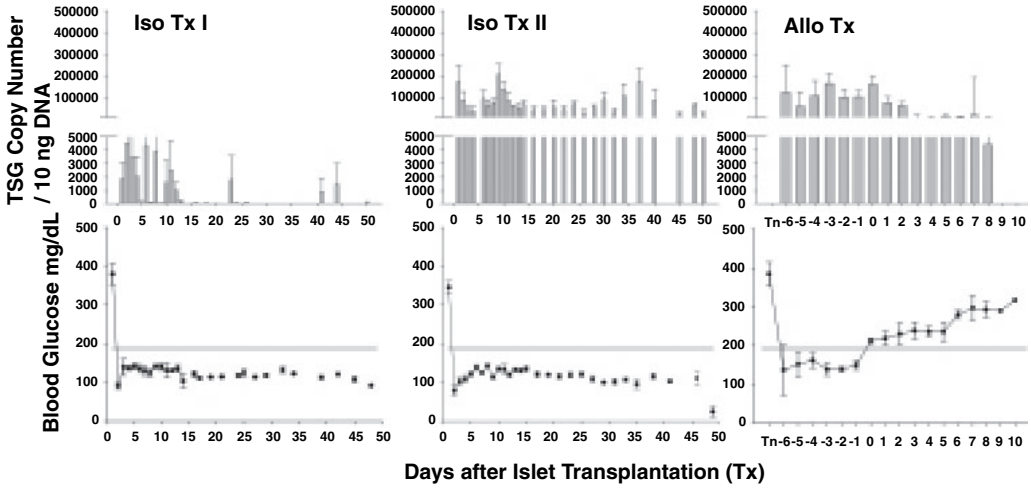

Figure for IPITA-O-4.7.

islet damage only in histologically proven disruption of islet morphology. Thereby, TSG measurement allowed, for the first time to our knowledge, to identify slow islet damage resulting from a weak immune reaction induced by H-Y incompatibility in some female recipients.

immunomodulators, e.g. CTLA4Ig and IL-1RA preserve more functioning allo-islets in an injectable, temperature-sensitive hydrogel, CPNHA, in the subcutaneous space.

IPITA-O-4.8

Prolonged survival of subcutaneous islet allografts in cpnha, an injectable temperature-sensitive hydrogel, with local CTLA4IG and IL1RA

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To explore the plausibility of using injectable temperature-sensitive hydrogels as a subcutaneous (SC) milieu for islet transplantation, pluronic F127 and hyaluronic acid (HA)/chitosan-modified PNIPAAm (CPNHA) were evaluated. The effect of CTLA4Ig and interleukin-1 receptor antagonist peptide (IL-1RA) on islet grafts function in temperature-sensitive hydrogels was examined in a rodent allo-transplantation model. In the releasing kinetic study, time required for the cumulative amount of albumin to reach 50% of the totally released albumin from 24% F127 and 10% CPNHA containing 4 mg/ml of albumin was 6 ± 2 (n = 3) and 5 ± 2 (n = 3) days, respectively. Using tetrazolium salt (MTS) cell proliferation assay, significantly more non-viable islet cells were found in 4-days culture containing 24% F127 comparing with islets cultured without F127 (OD492: 0.11 ± 0.01, n = 6 vs 0.16 ± 0.01, n = 6, $p < 0.05$). Meanwhile, the OD492 absorbance of 30 islets cultured with 10% CPNHA gel for 4 days did not differ from that of islets cultured without CPNHA in MTS assay. The stimulation index did not differ between islets cultured with and without 10% CPNHA for 12 days (6.67 ± 1.83, n = 4 vs 3.42 ± 0.74, n = 4, $p > 0.05$). Seven out of 12 mice receiving 1 ml of 24% F127 on their back SC were dead at day 5 post implantation. Although none of four mice receiving F127 containing 300 allo-islets and 2 mg of both CTLA4Ig and IL-1RA were dead at 4 weeks, no positive insulin-staining cells were found in the immunohistochemical examinations of all retrieved implants. On the contrary, none of 12 mice receiving 0.5 ml of 10% CPNHA on their back SC were dead at 4 weeks after implantation. Many positive insulin-staining islet cells were observed in the 2 weeks implants retrieved from mice receiving 300 allo-islets in CPNHA plus 2 mg of CTLA4Ig and IL-1RA. In conclusion, hydrogel containing high moiety of HA and chitosan is biocompatible, non-toxic and works as a subcutaneous artificial milieu for islets transplantation. The addition of local

IPITA-O-4.9

A small molecular GPR119 agonist stimulates beta cell replication and improves islet graft function

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G protein-coupled receptor 119 (GPR119) is expressed in β cells and enteroendocrine L cells. Activating GPR119 can directly stimulate insulin secretion and indirectly stimulate incretin hormones secretion. In this study, we investigated whether PSN632408, a small molecular GPR119 agonist, can directly stimulate β cell replication and improve islet graft function in mice. To determine β cell proliferation *in vitro*, C57BL/6 mouse islets were cultured in RPMI1640 for 4 days, with and without exendin-4 and PSN632408. Culture medium was changed daily and BrdU was added on day 3 for overnight. Insulin and BrdU double immunofluorescence staining was performed on intact islets and BrdU+ βcells were accounted using confocal microscope. To determine islet graft function, 100 C57BL/6 mouse islets were transplanted into each streptozotocin induced-diabetic C57BL/6 mouse. These recipient mice were given BrdU and with or without PSN632408 at 10 mg/kg/day. At 4 weeks, nephrectomy was performed to remove islet grafts for insulin and BrdU double immunofluorescence staining. Without treatment, BrdU+ β cells in each cultured islet were 9.6 ± 8.8 cells. With 0.1 μmol/l exendin-4, BrdU+ β cells in each islet were 21.5 ± 10.6 cells ($p < 0.01$). BrdU+ β cells in each islet were 18.1 ± 8.8 cells with 0.1 μmol/l PSN632408 treatment; 25.1 ± 13.5 cells with 1 μmol/l PSN632408 treatment; and 44.2 ± 19.7 cells with 10 μmol/l PSN632408 treatment ($p < 0.01$). Although all recipients achieved normoglycemia at 4 weeks with or without PSN632408 treatment, normoglycemia was achieved in significantly fewer days in PSN632408 treated mice (n = 8, 8 ± 5 days) than in vehicle treated mice (n = 8, 19 ± 7 days, $p < 0.05$). The nonfasting blood glucose levels were 389 ± 97 mg/dl at 1 week, 299 ± 86 mg/dl at

2 weeks, and 232 ± 70 mg/dl at 3 weeks in mice with vehicle treatment; and were 201 ± 108 mg/dl at 1 week, 161 ± 42 mg/dl at 2 weeks, and 136 ± 23 mg/dl at 3 weeks in mice with PSN632408 treatment ($p < 0.01$). The percentage of insulin+ and BrdU+ positive β cells in islet grafts were significantly higher in PSN632408 treated mice than that in vehicle treated mice (19.4 ± 7.4% vs 4.7 ± 4.3%, $p < 0.01$). Our data demonstrated that GPR119 agonist can directly stimulate β cell replication and improve islet graft function. Activating GPR119 is a new therapeutic approach to enhance β cell mass by stimulating βcell replication before and after islet transplantation.

Tuesday, October 13, 2009

Invited Speakers
IPITA Main Plenary Session 3
Cellular Immunotherapeutics and Immunoregulation

Mesenchymal stem cells/BM mediated immunomodulation
L. Piemonti
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Immunomodulation by dendritc cells
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Our NIH-funded protocol approved by the FDA is currently underway in phase I clinical trial with an adult (18 year or older) cohort documented with insulin-requiring T1D of at least 5-year duration. Leukocytes are obtained from the patient's blood by apheresis and dendritic cells (DC) are generated *in vitro* and engineered in GMP facilities to remain functionally immature. They are then injected into the patient by intradermal administration at an anatomical site proximal to the pancreas. DC will migrate to the nearest lymph nodes where they will start to anergize naive T cells, precluding their maturation into diabetogenic killers. This approach didn't show so far any unforeseen negative effect. It should be more successful when DC injections start close to the clinical onset of the disease, so to rescue still functioning insulin-producing beta cells and to possibly induce regeneration. This is the goal of the phase II clinical trial in which we'll test its efficacy in new-onset patients.

Inhibitory signals and T regulatory cells in control of islet allo and autoimmunity
C. C. Anderson
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Drug interference in immune regulation
M. Saemann
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Lunchtime workshop 3
Islet Imaging

Islet Imaging
Functional imaging of islet mass
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The noninvasive imaging and quantification of pancreatic ß-cells is considered as a high-priority field of investigation since 1999. As recently reviewed,[1] potential tools in such a perspective includes hypoglycemic sulfonylureas and glinides, β-cell specific monoclonal antibodies, ^{18}F-L-3,4-dihydroxyphenylalanine, somatostatin-receptor ligands, and ^{11}C-dihydrotetrabenazine as a marker of vesicular monoamine transporter type 2. Novel approaches, such as optical coherence tomography and diffusion MRI (as developed for the direct and fast detection of neuronal activation in the human brain), are now also under investigation. Noninvasive imaging of islet function might also be useful to evaluate a number of physiological or pathological variables in pancreatic islets, such as pancreatic microvasculature after intravenous injection of a paramagnetic contrast agent, levels of β-cells apoptosis using cyanine-5,5-labeled annexin V, migration of T-cells labeled *ex vivo* with perfluoropolyether nanoparticles, or monitoring the response of CD8+ T cells against β-cells during the progression of type 1 diabetes mellitus using as MRI probe supramagnetic iron oxide nanoparticles with a coating that specifically binds to a subpopulation of CD8+ T cells. A suitable analog of D-mannoheptulose might also be used for the functional imaging of islet mass, since the uptake of this heptose by β-cell is mediated at the intervention of GLUT-2. Further investigations on the latter approach were hampered by the ill-informed objection that GLUT-2 is expressed at very low levels and is not functionally essential in human β-cells, so that the proposed imaging technique was considered not applicable to humans. This view indeed eventually resulted in the rejection of grant applications. Yet, as early as 1966, D-mannoheptulose was reported to inhibit insulin secretion in humans. Moreover, in 2000, it was documented that this heptose is indeed taken up by isolated human pancreatic islets. Recent investigations in the same perspective has drawn attention to the possible use of other heptoses devoid of the inhibitory action otherwise exerted by D-mannoheptulose on both glucose phosphorylation and glucose-stimulated insulin release in rat pancreatic islets.

Reference

1. Malaisse WJ, Louchami K, Sener A. Noninvasive imaging of pancreatic β cells. Nat Rev Endocrinol 2009; 5: 394–400.

MRI imaging of grafted allogenic islets in transplant patients
C. Toso
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PET imaging of islet transplant recipients
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Limited tools are available to study the fate of islets transplanted via the portal vein. To expand our knowledge concerning the peritransplant phase of clinical islet transplantation we have introduced the use of dynamic PET/CT examination. Six transplantations in five patients have been performed. A fraction of the isolated islets were allowed to internalize [^{18}F]FDG for an hour prior to transplantation. These islets were carefully mixed with the unlabled islets just before the islet infusion. From the start of the transplantation a 60-min dynamic scanning sequence over the liver was acquired in 2D mode in the PET/CT scanner. A static 5-min 3D examination of the whole liver was then performed, and finally the torso was examined 90 min post-transplantation using a

static whole-body sequence to visualize additional sites of uptake. Islets were readily seen. The peak of the radioactivity in the liver was found at the end of each infusion; thereafter, a mono-exponential decrease was seen. The radioactivity in the liver represented only 62.9 (53.4–88.4) % of the total administered dose. A marked increase in p-C-peptide was found in parallel with the release of radioactivity from the transplanted islets. The distribution of the radioactivity in the liver was heterogeneous. The uptake pattern over time was similar for the four main liver segments, but large variations in the concentration of islets were found in the liver. The percentage of islets concentrated in areas with a higher islet density than 400 IEQ/cc varied between 1 and 32%. No accumulation of radioactivity was found in the lungs. All transplanted patients had positive c peptides, lowered their need for exogenous insulin and HbA1c at follow up 4 weeks after transplantation. No side effects associated with the PET procedure were found. It is possible to monitor the islet graft for hours after transplantation in the liver with the described technique. The uneven distribution in the liver with the islets concentrated to small areas shows that the perception that islets transplanted to the liver are evenly distributed is wrong. The good short-term outcomes indicate that the peritransplant findings, are not attributed to poor isolation but seem to be a general phenomenon in islet transplantation. It also shows that the presented technique is safe and doesn't harm the islets. In upcoming clinical trials techniques to improve both an even spread of islets in the liver and engraftment will be evaluated.

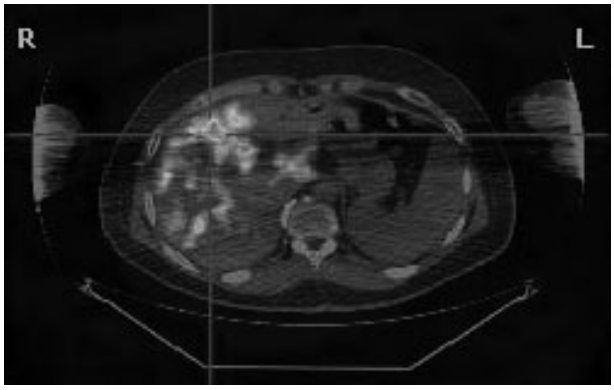

Fig. 1. [18F]FDG-labeled islets found in "hot-spots" after clinical islet transplantation.

Lunchtime workshop 4
Post-transplant Endocrine Disorders

Post-transplant new onset diabetes mellitus

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Disturbances of normal glucose tolerance (including impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM)) are common following solid organ transplant. Estimates of the frequency of these metabolic abnormalities vary depending on how abnormal glucose tolerance is defined but may be as high as 74%. NODAT is associated with a 60% increased risk of graft failure and a 90% increased risk of death. While acute rejection is associated with increased risk of death-censored graft loss, NODAT increases the risk of death with a functioning graft, which would be expected since NODAT is an independent risk factor for major cardiovascular events. In addition, NODAT is associated with risk for chronic allograft nephropathy as well as severe sepsis and microvascular complications of diabetes.

Observational and experimental studies have confirmed a number of important risk factors for NODAT, some of which are potentially modifiable. Many of the risk factors for NODAT are similar to those for Type 2 diabetes (age, obesity, family history of diabetes, IGT and non-caucasian ethnicity). Other risk factors are specific to the transplant setting (deceased donor, acute rejection, Hepatitis C infection) including, importantly, immunosuppressant drugs.

Immunosuppressant drugs (steroids, calcineurin inhibitors, and sirolimus) increase the risk for NODAT by effects either to increase insulin resistance, impair insulin secretion, or both. When, as is usual, these drugs are combined the metabolic effects may be enhanced. By minimizing exposure to these drugs it is hoped that the risk for NODAT can be reduced.

While it is possible to identify individuals at high risk for NODAT, the effectiveness of strategies to reduce NODAT have yet to be proven. Currently it would seem prudent to be vigilant to identify cases, to promote healthy lifestyle change to improve insulin sensitivity and to actively manage cardiovascular risk factors.

Post-transplantation diabetes mellitus – the Giessen Center experience, guidelines and recommendations

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De novo diabetes mellitus (posttransplant diabetes mellitus, PTDM) following solid organ transplantation is associated with a significant decrease in patient- and transplant-survival and thus resembles a serious complication. Due to non-uniform definition of PTDM the reported incidence ranges from 2 to 54%. The development of a PTDM is supported by modifiable- (weight, immunosuppressive regimen, etc.) and non-modifiable factors (age, ethnicity, etc.).

A retrospective analysis of all consecutive kidney-transplantations (NTX) from 2000 till 2006 at Giessen Transplant Center (GTC) was done to evaluate the prevalence of a PTDM, the contributing risk factors as well as other possible predictive parameters over a time period of 12 month post NTX. Additionally 21 patients were screened prior to NTX in a prospective setting.

From a total of 271 consecutively transplanted patients from 2000 till 2006, 181 patients were analyzed. A persisting PTDM was found in 36/181 (19.9%) consistent with current literature.

Risk factors contributing to the development of a PTDM were age (46 vs 63 years, $p < 0.001$, respectively No-PTDM vs PTDM), Body-Mass-Index (23.4 vs 26.0 kg/m^2, $p = 0.002$) as well as the Fasting Plasma Glucose prior to NTX (91 vs 103 mg/dl, $p < 0.001$). Patients of the No-PTDM-group had a significantly lower rate of HLA mismatches (3.2 vs 3.9, $p = 0.023$) and received more frequent a living-related-organ (37.9 vs 16.7%, $p = 0.018$). The immunosuppressive regimen (Tacrolimus vs Cyclosporine A) had no significance but showed a trend towards more PTDM cases in patients treated with Tacrolimus (23.8 vs 14.5% PTDM vs No-PTDM-group, $p = 0.367$).

Within the prospective setting no patient had diabetes mellitus prior to transplantation. Twelve of the screened patients have already been successfully transplanted at GTC. Three months post NTX 5 of 9 (55.6%) patients showed to have diabetes mellitus, which in 2 of 7 (28.6%) cases was also persistent at 6 month post NTX.

The groups No-PTDM and PTDM did not show differences in Age, BMI, and FPG prior to NTX. However the PTDM-group showed to have impaired glucose tolerance (IGT) in the 2 h OGTT at screening prior to transplantation (159.3 vs 131.5 mg/dl). Three month after successful kidney-transplantation the IGT aggravated to be a manifest PTDM (226.0 vs 124.0 mg/dl). Considering only the FPG at these screenings would not have detected a single case of PTDM.

By systematically screening patients with an OGTT impaired glucose metabolism can be detected early allowing the examiner to take therapeutic interventions, by which mortality and loss of graft function can be minimized. In addition to current guidelines the use of an OGTT in prospective screening of glucose metabolism should be attributed more importance and is therefore recommended.

Post-transplant osteoporosis
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Parallel Plenary Session 3
Late Islet Graft Failure

Monitoring islet destruction
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Autoimmunity
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Consideration of factors affecting chronic islet allograft dysfunction
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Transplantation under the Edmonton Protocol using a steroid-free, sirolimus (SRL) Tacrolimus (Tac) based maintenance immunosuppression regimen yielded the first demonstration of consistent diabetes reversal by transplantation of isolated pancreatic islet allografts. However, longer-term follow-up has revealed that Edmonton protocol islet transplants exhibit a gradual loss of function resulting in insulin-independence rates at 5-years that are $<10\%$, a result dramatically inferior to that achieved by whole organ transplantation. A variety of theories have been put forth to explain chronic islet dysfunction including immune mediated loss (rejection or autoimmune recurrence), site related dysfunction, marginal mass exhaustion, and immunosuppression related toxicity. Recent experimental data suggests that the drugs employed in the standard Edmonton regimen may exert potent anti-proliferative activity in islet beta cells. Sirolimus in particular has well described anti-proliferative activity and in fact is now routinely utilized as a chemotherapeutic agent for certain malignancies.

We will review experimental data supporting a role for drug toxicity in the impairment of beta cell regeneration that may be required to sustain the transplanted islet mass. In addition, we will review clinical data comparing is islet-alone transplants under the Edmonton protocol with islet-after-kidney (IAK) transplants treated with CNI-MMF-steroids (but no SRL) to assess whether the SRL free immunosuppression regimen allowed improved long-term success.

Recent advances in "*in-vivo*" islet imaging
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The measurement of β-cell preservation as a clinical end point is critical for an early assessment of new anti-diabetic therapies. The new FDA draft guidance highlights the need for a regulatory path regarding pre-diabetes and disease modification. However to date, it is only possible to estimate β-cell mass indirectly, namely using secretory indices such as fasting C-peptide, reduction in insulin dose and β-cell responsiveness to a glucose challenge. Since these tests lack the necessary sensitivity and specificity for human proof-of-concept studies, an imaging solution would prove invaluable, particularly for longitudinal assessments of β-cell survival. Among all available modalities, nuclear PET imaging may be particularly well suited as it offers potential for β-cell mass determination with high sensitivity and specificity. With β-cells representing only 1–2% of the whole cell population in the pancreas, the main challenge however resides in the selection of a radiolabelled ligand with a reasonably high affinity for a β-cell specific target as well as high specific activity. A handful of targets recently emerged as promising platforms for the development of such ligands, e.g. VMAT2, GLUT2, ZnT-8, mGluR5, SUR1 and possibly others. Data gathered from antibodies and small peptides that bind to cell surface receptors of target cells while not binding to undesired cells (ie β-cells and exocrine pancreas) have also been put forward. Although the value of β-cell specific imaging is clear, delineation of β-cell mass from number and function for every target identified remains one of the biggest hurdle to overcome. Absence of target modulation upon disease progression, sufficient signal-to-noise ratio, high detection window, favorable pharmaco-kinetics, lack of toxicity at tracer doses, appropriate kinetic modeling are among other key-aspects for successful tracer development which will also be addressed in this presentation. Given the inherent challenges of β-cell imaging (i.e. the path from discovery to qualification still will likely take years for a relevant marker), it is essential that the risk of failure be mitigated by using a staged investment approach with clear go/nogo criteria set up front. In this context, participation in multicenter consortia on β-cell imaging may also be considered as one of the ways forward to bring all the expertise to bear on the issues.

Parallel Plenary Session 4
Metabolic and Transplant Surgery for Type II Diabetes

Introduction: does surgery for T2D make sense?
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Metabolic surgery for obese patients with T2D
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Metabolic surgery for non-obese patients with T2D
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Pancreatic transplantation for patients with T2D and ESRD
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In the recent past, type 2 diabetes (T2D) was a contraindication to pancreatic transplantation. However, initial intentional (and unintentional) experience with simultaneous pancreas-kidney transplantation (SPKT) in patients (pts) with T2D and end stage renal disease (ESRD) suggested that augmentation of endogenous insulin production through pancreatic transplantation in pts with C-peptide positive, insulin-requiring diabetes resulted in insulin independence, improved glucose counter-regulation, and enhanced quality of life. There may be tremendous overlap in the

"definitions" of type 1 vs T2D, which are historically differentiated based on age and pattern of onset, detection of C-peptide and islet/anti-GAD antibodies, initial need for insulin and total daily insulin dose, presence or absence of diabetic ketoacidosis, obesity, ethnicity, HLA association, and other associated auto-immune phenomena. To add to the confusion, it is well established that the immunosuppressive medications requisite to transplant may cause T2D. Single center and registry reports have documented equivalent SPKT outcomes in pts with either type 1 or T2D although clearly a selection bias exists for pts in the latter category. Selection criteria for SPKT in T2D include pts <55 years of age with a BMI <30 kg/m^2, insulin-requiring for a minimum of 5 years with a total daily insulin requirement <1 u/kg/day, a fasting C-peptide level <10 ng/ml, absence of severe vascular disease or tobacco abuse, adequate cardiac function, and presence of "complicated" diabetes. Because SPKT is associated with a shorter waiting time, enhanced donor quality, increased life expectancy, higher graft survival, improved quality of life, and better preservation of renal function compared to deceased donor kidney transplantation alone, characterization of the "type" of diabetes may be irrelevant and insulin-requiring diabetic pts should be evaluated for SPKT based exclusively on their predicted ability to tolerate the surgical procedure (with higher inherent complication rate) and requisite immunosuppression as well as comply with a more stringent post-transplant follow-up regimen.

Joint IPITA-IXA Plenary Session 1
Consensus on the Infectious Risk in Xenotransplantation

Overview: risk assessment in xenotransplantation

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Advances in experimental xenotransplantation have increased the feasibility of clinical trials of xenotransplantation. As with human allotransplantation, the prevention of infection associated with xeno-transplantation is central to the success and acceptance of this technology. Particular concern exists regarding activation of latent viruses, including retroviruses, from xenograft tissues in human recipients. Despite significant advances in knowledge regarding such pathogens, the absolute risk for such infections remains unknown in the absence of human studies. For xenotransplantation, the equation of infectious risk includes unique factors: (i) Screening of "donor swine" can be designed to be *more rigorous* than screening used for deceased human organ donors; (ii) Donor samples must be archived for use in future epidemiologic studies; (iii) Many *in vitro* studies use cell lines selected for "infectability" of human cells by viruses or produce virus at high titers - with uncertain relevance to *in vivo* infection; (iv) Infectious risk may relate to the specific cells or tissues selected for transplantation; (v) The risk for infection will depend on many factors including: whether the tissues transplanted are vascularized organs or cells; whether the tissues produce pathogens infectious for normal human cells; the "virulence" and quantity of pathogens produced; the recipients' susceptibility to infection including functional receptors, cellular capacity for productive infection, and the nature and intensity of immunosuppression.

The factors controlling infectious risk are equally ill-defined in allotransplantation and for xenotransplantation. To enhance clinical safety in xenotransplantation, it is essential that further research be conducted to define the factors controlling infectious risk including blocks to retroviral infection inhuman cells, differences between donor tissues and strains, and optimal immunosuppressive regimens. A reasonable goal is to make xenotransplantation "as safe as is possible" with assurances that any infectious risk will be explored and minimized for the general population.

PERV: donor screening and post-transplant detection

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Porcine endogenous retroviruses (PERVs)-A and -B, present in the genome of all pigs and being released by normal pig cells with the ability to infect human cells, pose a risk for xenotransplantation using pig cells, tissues or organs. In contrast, PERV-C is not ubiquitous and only infects pig cells. In addition to these viruses, recombinant PERV-A/C viruses have recently been described that are able to infect human cells, that are characterised by very high titre replication and whose proviruses have been found *de novo* integrated in the DNA of somatic pig cells, but not yet in the pig germ line. The risks presented by PERV-A/C recombinant viruses could easily be eliminated by using pigs not containing PERV-C in their germ line, effectively preventing recombination with PERV-A. Screening for and selection of PERV-C-free animals will therefore reduce the risk of PERV-A/C transmission to humans. However, when the incidence of PERV-C in different pig strains and 181 non-transgenic and multi-transgenic pigs was evaluated, 97.2% were found to have PERV-C incorporated into their germ line. Donor pig screening may also be performed using an assay based on the measurement of virus expression by real time RT PCR before and after stimulation of pig PBMCs that allows discrimination between pigs with high or low PERV expression. In this way, selection of pigs free of PERV-A/C and having a low expression of PERV-A and -B may be achieved. Whether the use of transgenic pigs expressing PERV-specific siRNA to inhibit PERV expression significantly decreases the risk of PERV transmission also needs to be evaluated.

Screening xenotransplant recipients for PERV infection can be done in a number of ways. Provirus integration and PERV expression could theoretically be easily detected in the peripheral blood mononuclear cells (PBMCs) using PCR and RT PCR but as the cells in which PERV replicates is still unknown, it is unclear whether this will be reliable. As in every retrovirus infection (including HIV-1) the detection of virus-specific antibodies is clear evidence for infection and replication and the absence of specific antibodies indicates absence of such infection. Antibodies may be detected by Western blot or ELISA using purified virus or recombinant viral proteins as antigens. The detection of antibodies specific for more than one PERV antigen is necessary to exclude false positive results due to the presence of antibodies cross-reacting, for example, with the Gag protein.

Blocks to retroviral infection: how real is human risk?

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In the course of pig-to-human xenotransplantation as a strategy to overcome the human organ shortage, porcine pathogens that might not show any apparant phenotype in their natural host could exhibit a risk of fatal infections to humans. The presence of known pig infectious agents as well as zoonotic and dissimilar agents should be reduced by specific pathogen free (spf) breeding. However, porcine endogenous retroviruses (PERV) whose genetic information is integrated in the genome cannot be eradicated by this way. Therefore, it is important to investigate whether human and porcine cells display natural and effective mechanisms counteracting productive infections caused by PERV. The mammalian immune system has developed numerous strategies to limit or restrict virus replication including an intracellular defence as part of the innate immunity. This cellular front is composed by several constitutively expressed genes which prevent or at least suppress retroviral infections. For example members of the tripartite motif (TRIM) and the apolipo-protein B mRNA-editing polypeptide (APOBEC) families as well as tetherin and zinc-finger antiviral protein (ZAP) were identified to act against viral attacks at different steps of the retroviral life cycle. While the TRIM5 class (especially TRIM5α) targets incoming retroviral capsids leading to a rapid uncoating of the capsids, the APOBEC3 class hypermutates retroviral genomes during reverse transcription, whereas tetherin disturbs the release of retroviral particles at the membrane of the infected cells and ZAP directs the degradation of the viral RNAs.

Our research focusing on APOBEC and TRIM led to the conclusion that human and porcine APOBEC3 cytidine deaminases exist that inhibit PERV replication *in vitro*. The role of human and porcine TRIM5 proteins

is still under investigation. So far, a single *TRIM5* gene in the pig genome was identified, but the functional properties of the porcine TRIM5 protein are still unclear. Recently, Wood et al. (2009) published data indicating that PERV are resistant to the restrictive nature of different TRIM5 proteins including human and monkey TRIM5α. Based on this we investigate the impact of porcine TRIM5 on PERV using the human TRIM5α as a negative control, expecting that PERV will be insensitive to porcine TRIM5.

So far, there is no effective clinical treatment for viral diseases based on genetically designed and pharmacologically active host factors. Further characterization of cellular restriction factors and the viral life cycle might lead to their development.

Herpes viruses and interspecies infectious risk

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Xenotransplantation exposes the host to known and unknown pathogens of the donor pig (donor-derived xenosis). A major effort has been undertaken to minimize the risk of transmission from the donor using specialized breeding techniques. With the exception of endogenous retroviruses and some latent viruses, exclusion was successful and has eliminated a majority of donor pathogens. Enhanced replication of many host pathogens will be stimulated by the immune responses induced by transplantation and by the immune suppression used to prevent graft rejection. Infection of the graft may occur with host-derived pathogens with unpredictable consequences due to the cross-species situation. Infectivity may be decreased as entry or replication is altered by missing receptors or inability to use the cellular machinery. Replication of organisms in the xenograft and the inability of the human host to respond to human pathogens in the context of a xenograft infection due to immune suppression, or the presentation of such pathogens in the context of pig instead of human MHC (major histocompatibility complex) could impair control of such infections. Recent data suggest that some host-derived herpesviruses, such as human cytomegalovirus (HCMV), may indeed infect porcine tissue and are associated with a pro-inflammatory phenotype. Exclusion of latent viral infections may not be achieved in all donors. Both may lead to interspecies infection. This presentation focuses on host-derived as well as donor-derived herpesviral pathogens and their potential harmful role in xenograft or host infection.

Conclusions and discussion

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Joint IPITA-IXA Plenary Session 2
Progression Towards Clinical Islet Xenotransplantation

The Transplantation Society –- the expanding role: Science, clinical practice, education, ethics and regulations

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Pig-to-primate islet xenotransplantation: state of the art

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Ethics: safety vs efficacy

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A morally justifiable balance between the risks (or probable harms) and anticipated (or foreseeable) benefits of clinical research protocols is a bottom-line prerequisite for the permissibility of clinical trials. The process of clearly and completely identifying the risks and anticipated benefits of a clinical trial is called a harm/benefit analysis (HBA). An HBA that logically proves that the anticipated benefits of the trial justify its risks is the moral pre-condition for ethically-responsible subject recruitment and for carrying out the scientific and medical features of the trial. Unfortunately and perhaps surprisingly, the topic of how to conduct a logically complete, coherent, and ethically-defensible HBA for complex research initiatives – including research in xenotransplantation – has rarely, if ever, been explored. Codes of research ethics and xenotransplant regulations present researchers and members of research review committees with a variety terms and topics that are neither logically ordered nor systematically related one another and to the ethical foundations of harm-benefit assessments. What all do terms and topics such as "safety," "efficacy," "risks," "minimizing risks," and "satisfactorily managing" risks refer to? How do they logically relate to each other, and what is their ethical import? This presentation will seek to provide answers to these rarely-raised questions.

Regulatory requirements for clinical xenotransplantation

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The role of the WHO and the Changsha communiqué

L. Noel
Essential Health Technologies. World Health Organization, Geneva, Switzerland

Progression towards clinical islets xenotransplantation in China

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Background: WHO established an outline of xenotransplantation clinical trial for all the WHO member states in 2009, named Changsha Communiqué. It will maximize safety and effectiveness in xenotransplantation. Based on Changsha Communiqué, China has established a roadmap of clinical islet xenotransplantation. There are several critical issues of clinical islet xenotransplantation for the road map.
Critical Issues of Clinical Islet Xenotransplantation: (1) Chinese government has organized three consultation meetings to draft National regulation on xenotransplantation so far. This includes the procedure for approval, scientific standards, ethical Standards, biosafety standards, background of staff, facility standards, source animal standards, recipient standards, transplantation protocol, Biosafety surveillance system, database. Chinese

government will regulates xenotransplantation clinical trial according to Changsha Communiqué very strictly.

(2) Research data from the non-human primate model is the second critical issue. It should show the xenotransplantation therapeutical procedure is efficient to reverse hyperglycemia of non-human primate diabetic model. Our group has initiated a program of transplanting NPI into diabetic rhesus monkeys.

(3) DPF Medical grade pig donor is the third critical issue of clinical islet xenotransplantation. We have screened 12 isolated pig colonies in China. Outcomes show 2 pig colonies with potential as islet donors because these pigs have deficient PERV-C and high yield of NPI. Our Lab will establish a pig facility for DPF donor pigs.

Summary: Roadmap of clinical islet xenotransplantation in China will base on Changsha Communiqué. It includes national regulation, non-human primate research and setting up medical grade donor pig facility.

Oral Presentations
IPITA Parallel Session 5
Clinical Pancreas Transplantation 2

IPITA-O-5.1

Alemtuzumab induction in simulaneous kidney–pancreas transplantation in the United States: a review of a National Data Registry

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Background: Alemtuzumab (AZ) is a monoclonal anti-CD52 antibody used as an induction agent in organ transplantation. There are several single center reports regarding AZ use in simultaneous kidney–pancreas transplantation (SPK). We sought to further assess the utility of AZ as an induction agent for SPK in the US by analyzing the Scientific Registry of Transplant Recipients (SRTR) database.

Methods: We examined SPK recipients in the US from 1/2002 to 10/2008 from the SRTR database. Primary outcomes were overall pancreas allograft, renal allograft and patient survival by induction agent. Induction was classified as none, IL2 receptor blockade (IL2-RB), rabbit anti-thymocyte globulin (rATG) and AZ. Outcomes were assessed with Kaplan–Meier and Cox proportional hazard survival models adjusted for donor, transplant and recipient variables. Secondary endpoints were analyzed including: initial hospital length of stay (LOS), re-hospitalization within 6 months, and treatment with anti-viral therapy within 6 months of transplant. Multivariate logistic and generalized linear models were used for dichotomous and continuous endpoints respectively.

Results: During this time period 6,050 patients underwent SPK. Induction agents reported were: None 22%, IL2-RB 18%, rATG 49%, AZ 10%. There were no statistically significant differences in patient, pancreas or renal allograft survival. There was a trend towards improved renal allograft survival in patients receiving IL2-RB. Median LOS was 9 days. Patients receiving AZ had a significantly shorter LOS than all other regimens ($p < 0.0001$). Those receiving rATG or AZ were more likely to be re-hospitalized within 6 months after transplant ($p < 0.0001$). Patients receiving AZ were more likely to undergo anti-viral treatment by 6 months post-transplant (AZ 69%, rATG 51%, IL2-RB 46%, none 32%; $p < 0.0001$).

Conclusions: There are no differences in patient, pancreas or renal allograft survival using AZ induction. AZ may confer an advantage in the perioperative period as evidenced by a decreased hospital LOS. However, this benefit may be lost due to frequent re-hospitalizations or need for anti-viral therapy. There do not appear to be any long-term differences between induction agents. Centers may need to alter immunosuppressive or viral prophylaxis regimens when using this induction strategy.

IPITA-O-5.2

Daclizumab (DAC) maintenance therapy in pancreas (PX) transplantation (TX)

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Background: Calcineurin-inhibitors(CNI) are the basis of immunosuppression(ImmSx) >20 years, but are nephrotoxic with other side effects. Conversion to CNI-free ImmSx is desirable in patients with side effects. We describe here our experience with a DAC based regimen in Px recipients.

Methods: 25 Px recipients with progressive CNI nephrotoxicty of native or transplanted kidneys, as manifested by elevation of creatinine and biopsy evidence of no rejection with CNI lesions, or neurotoxicity or glucose toxicity, or a combination thereof, at even low CNI levels, were selected for stopping CNI and being placed on monthly IV DAC maintenance therapy(1 mg/kg) with either mycophenolate mofetil(MMF) or siroliums(SIR) as the sole oral ImmSx. They were compared with 25 controls 1:1 matched by transplant type &number, age (within 5 years), year of transplant and duct management. Of the 25 in each group, 11 were primary Tx, 11 were 2nd Tx and 3 were 3rd Tx; 13 were pancreas after kidney(PAK), 10 pancreas transplant alone(PTA) and 2 simultaneous pancreas–kidney(SPK) recipients. Mean age was 42 10 (DAC) and 42 8 (controls); and 13 females and 12 males in each group. Patient (Pt) and graft survival (GSR) and immunological loss rates were calculated by the Kaplan–Meier method. Death with a functioning graft (DWFG) was counted as a graft failure. For the immune loss rate, DWFG was censored.

Results: Daclizumab Controls p (log-rank)
1/3/5 year GSR (%) 88/ 79/ 60 67/ 44/ 44 0.06/ 0.01/ 0.05
1/3/5 year immune loss rate (%) 8/ 17/ 22 28/ 53/ 53 0.05/ 0.01/ 0.02
1/3/5 year Pt Sur (%) 96/ 96/ 80 92/ 86/ 74 0.5/ 0.2/ 0.3

Conclusion: Pt survival rates were similar in DAC patients and controls. Px graft survival rates were significantly higher in the CNI-free DAC group than in the controls kept on CNI. The immune loss rate was reduced by more than half in the DAC vs control group. The control group had similar CNI related problems as the DAC group resulting in a tendency to lower CNI dose but without a compensating drug, putting the pancreas at risk in the non-DAC group. DAC maintenance in selected patients with CNI toxicity/intolerance appears to be safe, effective and well tolerated. A randomized trial to compare this regimen against CNI based therapy is needed. Timing of CNI withdrawal, impact on kidney function and use/non-use of T cell induction are questions that will need to be answered.

IPITA-O-5.3

Simultaneous pancreas and kidney transplantation from living donors in Japan – Outcome of eleven consecutive clinical trials in a single institution

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Objectives: Based on a severe shortage of deceased donors in Japan, the first simultaneous pancreas and kidney transplantations from living donors

(LDSPK) were conducted in 2004 and eleven cases of LDSPKs have since been, performed (13 cases in Japan).

Patients and Methods: [Recipients] Eleven type 1 diabetic patients with end-stage renal diseases underwent LDSPKs from January 2004 to February 2009. The age and gender were 34.9 + 4.9 years and 4 males/7 females. All of them showed frequent hypoglycemic unawareness because of negative serum C-peptide levels (CPR; < 0.03 ng/ml). [Donors] The donors included seven mothers, three fathers and one brother. Three donors were ABO-incompatible for the recipients. All of them fulfilled the donor criteria for LDSPK. [Operations and immunosuppression] The donor operation was initiated with a right nephrectomy followed by distal pancreatectomy with an open laparotomy (8 donors) or laparoscopic procedure (3 donors). LDSPK was performed using a pancreatico-cystostomy. Immunosuppression was achieved by quadruple therapy using tacrolimus, MMF, predonisolone, and basiliximab.

Results: None of the donors showed any complications, including pancreatic fistula, diabetes or renal dysfunction, from 3 months to 5 years after the operation. One recipient, who showed an extremely high level of anti GAD antibody, developed a primary nonfunction of the pancreas graft after transplantation. The1-year and 3-year patient survivals of the recipients were both 100%. The Pancreas/Kidney graft survivals were 90.9%/100% at 1 year and 90.9%/90.9% at 3 years. Insulin independency was maintained in nine cases (82%) from 3 months to 5 years. The quality of life of the recipients as evaluated by the Short-form 36v2 showed a dramatic improvement in both the physical and mental summary scores by LDSPKs.

Conclusions: The results of consecutive clinical trials demonstrated that LDSPK may therefore be a good therapeutic alternative and a potent tool for the treatment of type 1 diabetic patients with ESRD.

IPITA-O-5.4

Simultaneous kidney pancreas transplantation in select patients with type 2 diabetes mellitus having positive C-peptide and negative GAD-65 antibody

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Background: Simultaneous pancreas kidney transplantation (SPKTx) has been predominantly performed in patients with ESRD and Type 1 Diabetes Mellitus. Few studies that have reported equivalent graft survival in select Type 2 Diabetes Mellitus (T2DM) patients have defined T2DM mainly as presence of C-peptide.

Methods: Retrospective comparison of recipient and donor characteristics in addition to allograft (kidney and pancreas) and patient survival after SPKTx between T2DM vs T1DM. T2DM defined as: C-peptide present, GAD65 antibody absent, absence of diabetic ketoacidosis and use of oral hypoglycemics during the course of disease. BMI < 30 and use of < 1 unit/kg of insulin/day are also used in selecting T2DM patients for Tx.

Results: Between 6/2003 and 9/2008, we have performed 86 SPKTx, 6 were excluded from this analyses due to technical graft loss within 30 days of Tx. (n = 80: 70 T1DM, 10 T2DM). Of the 70 with classic clinical presentation of T1DM, 10 (16.7%) had detectable C-peptide, of which 8 (13%) had C-peptide in the normal range. Table 1 describes baseline characteristics of groups.

Table 2 describes outcomes of the study cohort. Cox regression survival analyses found no significant differences in allograft (pancreas and kidney) and patient survival.

Conclusion: Select T2DM patients (defined by a composite of clinical presentation, positive C-peptide, absent GAD65 antibodies) demonstrated pancreas and kidney allograft function comparable to T1DM after SPKTx. Further follow-up is needed to determine the long-term outcome.

Table 1 Descriptive analysis of the study cohort (IPITA-O-5.4)

	T1DM (n=70)	T2DM (n=10)
Mean age*	44	51
%White, African Am, Hispanic, Native Am**	87,1.5,10.4,1.5	30,10,30,20
% male*	88	90
Mean BMI*	24.8	27
Mean age of DM onset**	15	30
Mean duration of DM pretx (years) **	29	19
% documented retinopathy**	85	50
% documented neuropathy*	60	80
% documented autonomic dysfunction*	50	50
* Pvalue: NS, **P value <0.01		

Median follow-up time was 682 days.

Table 2 Outcomes after SPKTx in study cohort (IPITA-O-5.4)

Outcomes	T1DM	T2DM
Clinical acute kidney rejection, % + ve	10	30
Clinical acute pancreas rejection, % +ve	15	10
% with mean HbA1C >6.0 at 1month	38	20
% with mean HbA1C >6.0 at 12 months*	8.2	0
CrCl 1 month (MDRD equation) Mean SD	69+/−25	60+/−12
CrCl 12 month (MDRD equation) Mean SD *	68+/−25	69+/−18
* patients with >=1 year follow–up, P value not significant		

IPITA-O-5.5

Pancreas transplantation from obese donors

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Background: Obesity (Body Mass Index (BMI) > 30 Kg/m^2) has reached epidemic proportions in the USA. Currently, 1/4 to 1/3 of the population over age 20 is obese. Consequently, there are an increasing number of potential organ donors that are obese, but would otherwise be appropriate donors for pancreas transplantation (PTx). This study is a retrospective review of all PTx performed at a single center comparing donors with BMI > 30 to donors with BMI < 30.

Methods: A retrospective review was performed of all PTx performed at IUH between 2003 and 2009 (n = 308). All allografts from obese donors were scrutinized at the time of procurement and on the backbench to confirm that despite the donors weight, the pancreas itself appeared grossly normal. Enteric exocrine and systemic venous drainage was used in all cases. Immunosuppression: rabbit antithymocyte globulin induction, steroid withdrawal, tacrolimus and sirolimus or mycophenolate mofetil maintenance. Data included recipient and donor demographics, 7 days, 90 days and 1 year graft survival, peak 30 day serum amylase and lipase, length of stay (LOS), readmissions, HbA1C and C-peptide.

Results: Of the 308 PTx donors, 268 (87%) had a BMI < 30 and 40 (13%) had a BMI > 30. Donors with BMI > 35 (n = 7) tended to be younger (mean age 21 years, range 14–33 years) and have relatively normal serum amylase, lipase and glucose. There was no significant difference in Recipient transplant type, gender, race, age and BMI, donor age, gender, serum amylase and lipase and total ischemic time. There were significantly more African American donors in the > 30 group (28.6% vs 11.6%, p = 0.02). There was no significant difference in length of stay or 90 day readmissions, 7 day, 90 day or 1 year graft or patient survival, 30 day peak serum amylase or lipase, HbA1C or C-peptide. Of the seven recipients of allografts from donors with BMI > 35, the only major technical complication was a pancreatic leak ultimately leading to renal allograft loss from a pseudoaneurysm.

Conclusion: Although technically challenging, PTx of allografts from obese donors can be accomplished with similar results compared to normal BMI donors.

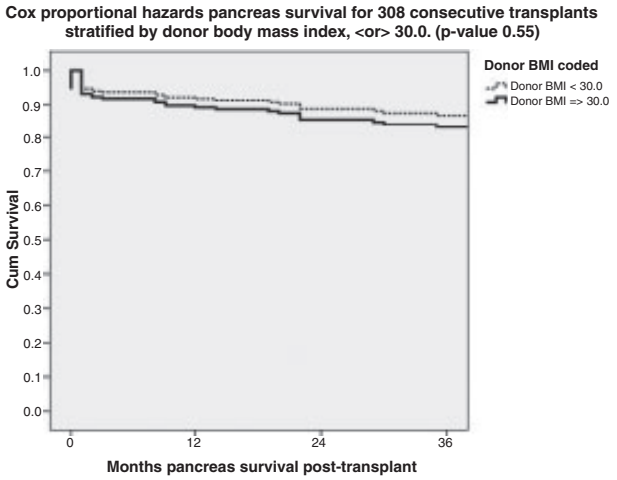

Figure for IPITA-O-5.5.

IPITA-O-5.6

Alemtuzumab with rapid steroid taper in simultaneous kidney pancreas transplantation: Comparison to induction with antiyhymocyte globulin

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We reviewed our experience with Alemtuzumab (Alem) induction with rapid steroid taper (RST) in simulataneous kidney pancreas transplantation (SKPT) and compared to a historic control group who received rabbit antithymocyte globulin (r-ATG) induction with RST. 74 of the 91 SKPTs performed at our center between Jan 2005 to Nov 2008, received immunosuppression with RST (positive crossmatch Txs with coninued steroids were excluded from this study); r-ATG induction 33 pts (1.5 mg/kg×4 for a total dose of 6 mg/kg) and Alem induction 41 pts (30 mg single dose). Maintainance immunosuppression consisted of Tacrolimus and Mycophenolate mofetil. Steroids were discontinued after post op day 4. Protocol kidney biopsies were performed at months 1, 4 and 12. All pts in the r-ATG group and 75% of the Alem group had atl east 1 year followup (minimum followup 6 months). The mean age of the pts (r-ATG 45 ± 13 years Alem 47 ± 12 years), rate of premptive Tx (r-ATG 36% Alem 22%), incidence of type 2 DM (r-ATG 9% Alem 17%), mean length of stay (r ATG 7.8 ± 3.2 days Alem 8.1 ± 5.0 days) and percent of pts steroid free at 1 year (r-ATG 82% Alem 80%) were all similar between the 2 groups. All clinical acute rejection episodes were biopsy proven. Rate of acute rejection (kidney and/or pancreas), CMV infection, BK nephropathy, graft survival and graft function in the 2 groups are shown in the following Table. Death with functioning graft was included in calculating the graft survival.

Conclusion: Induction with r-ATG or Alem with RST is safe and effective in SKPT. Both induction agents are associated with excellent short term outcomes. The incidence of acute rejection, CMV and BK nephropathy are similar between the two groups. Mean HbA1C at 1 year is lower in the Alem group. Further long-term follow-up is needed to confirm these excellent results.

	Alemtuzumab (n=41)	r-ATG (n=33)	p value
Clinical acute rejection (K or P)	15%	12%	NS
Subclinical acute rejection	5%	6%	NS
CMV infection	10%	18%	NS
BK nephropathy	2%	6%	NS
1-yr Kidney graft survival	93%	97%	NS
1-yr Pancreas graft survival	88%	94%	NS
Serum Creatinine (mg/dl)	1.4 ± 0.4	1.2 ± 0.4	NS (0.0559)
% with serum creatinine >1.5	12	23%	NS
MDRD GFR	61 ± 21	70 ± 20	NS (0.08)
Fasting blood sugar (mg/dl)	93 ±15	94 ± 12	NS
HbA1C	5.3 ± 0.4	5.6 ± 0.4	0.0021

IPITA-O-5.7

Impact of post-transplant delayed graft function on long-term graft survival in pancreas transplant recipients

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Background: Several factors related with donor or graft itself have been studied as risk factors of poor graft outcomes. In this study we evaluated the impact of post-transplant graft function in surgically successful cases on long-term pancreas graft survival.

Patients and Methods: Eighty-four pancreas transplant recipients who underwent pancreas transplantation including simultaneous pancreas–kidney transplantation, pancreas after kidney transplantation and pancreas transplantation alone between January 2000 and April 2009 in a single center, were enrolled in this study. Persistent insulin-dependence (n = 2), in-hospital mortality (n = 2), or graft loss (n = 5) caused by complications within 30 days were excluded in this study. We defined immediate post-transplant insulin-independence as no requirement of insulin for maintaining blood sugar level below 200 mg/dl. We divided all the recipients into two groups, immediate post-transplant insulin-independence group (Group A, n = 37) and delayed graft function group (Group B, n = 41). We analyze the effect of immediate post-transplant insulin-independence on long-term graft survival. We reviewed medical record including recipient, donor and procedure-related factors, retrospectively. Graft survival was analyzed with Kaplan–Meier Methods.

Results: Seventy-five of 84 pancreas recipients have been followed up at least 1 month with functioning graft. Median follow-up duration was 32.0 (1–101) and 33.0 (1–110) months. Donor age was 29.4 ± 12.1 in Group A and 34.7 ± 10.8 in Group B (p = 0.049). Otherwise, there was no significant difference in both groups including donor, recipient and procedure-related factors. Rejection episodes occurred in 5 cases (13.9%) of Group A and 13 cases (33.3%) of Group B and graft failure developed in 2 (5.6%) in Group A and 8 (20.5%) in Group B. Graft survival rates at post-transplant 5 year were 92.6% (2/36) in group A and 70.6% (8/39) in group B.

Conclusion: Although this study is based on small series, it is suggested that delayed graft function in pancreas transplantation was related with higher rejection rate and poor long-term graft survival.

IPITA-O-5.8

Risk factors for delayed graft function after simultaneous pancreas–kidney transplant and its influence on patient and graft survival

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Background: Kidney Delayed Graft Function (KDGF) is a prevalent and severe complication after Simultaneous Pancreas–Kidney Transplant (SPK). Its risk factors and influence on patient and graft survival have not been completely studied so far.

Methods: From 1996 to 2009, 122 SPK were retrospectively analyzed regarding the occurence of KDGF and its pre and post-transplant risk factors . KDGF was defined as the need of dialysis during the first week post-transplant. These patients were divided in Group 1 – with DGF and 2 – without DGF and several variables were analyzed.

Results: KDGF occurred in 25 patients (20.5%) after SPK (Group 1). There was no difference between the two groups regarding donor age, cerebrovascular event as cause of donor death, recipient gender and age and use of induction. Preemptive transplant was more prevalent in Group 2 (21.6% vs 8.3%, p = 0.038) which showed also lower cold ischemia time (11.1 vs 13.4 h, p = 0.004). There was no difference on 1-year pancreas (72% vs 70%, p = 0.653), kidney(76% vs 81.4%, p = 0.607) and patient (76% vs 84.5%, p = 0.410) survival between Group 1 and 2 respectively, although higher rate of mortality was achieved in patients with KDGF.

Conclusions: KDGF has occurred in one fifth of SPK patients in our country. No preemptive transplant and higher cold ischemia time were the strongest predictors of occurrence of KDGF. Patient and graft survival were not influenced by KDGF.

IPITA-O-5.9

Comparative assessment of immunosuppressive regimens in pancreas alone transplantation

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Background: Pancreas alone transplantation (PTA) in IDDM patients between 2004 and 2009.
Aim: To compare transplantation outcomes according to immunosuppressive therapy.
Methods: Between 2004 and 2009, 38 PTA have been performed; 8 of these patients developed graft thrombosis, 1 had a hemorrhagic infarct of the graft and 1 had a primary nonfunction. Therefore these patients were excluded from this study. Of the remaining 28 patients, 20 were pancreas-alone transplantations (PA) and 8 were pancreas-after-kidney transplantations (PAK). All of them received the whole organ with enteric diversion of exocrine secretion, 8 with portal-venous and 20 with systemic-venous graft drainage. It has been used two regimens of immunosuppressive therapy: in 2004 it was prednisone, mycophenolate mofetil, ATG (Anti-thymocyte globulin) and cyclosporine A, while from 2005 to 2009 was prednisone, mycophenolate mofetil, ATG and tacrolimus. Patients who developed acute rejection were first treated with methylprednisone (n = 5), and then with OKT3 (n = 2) or ATG (n = 2) in case of steroid-resistant rejection.
Results: Overall survival of transplanted patients was 96%; after 1 year, pancreas graft survival was 72%, after 5 years was 56%. Cyclosporine A group included nine patients (mean age 40 years, range 27–56), while tacrolimus group included nineteen patients (mean age 42 years, range 27–61). Donors demography is shown in the following table:

The 2 groups are homogeneous for donor age and weight (Mann–Whitney was respectively 0.52 and 0.1) but not for BMI (Mann–Whitney 0.04).

1 year graft survival was 55% in the cyclosporine A group while it was 80.7% in the tacrolimus group (p 0.12); after 5 years, graft survival was 33% in the cyclosporine A group and 72.7% in the tacrolimus group (p 0.062).

Episodes of acute rejection affected 55% of patients treated with cyclosporine A and 21% of the patients treated with tacrolimus (p 0.13).
Conclusion: These findings confirm the higher efficiency of tacrolimus improving PTA survival and decreasing the incidence of acute rejection.

	Tacrolimus group	Cy A group
Donor mean age (yrs)	24	26
Donor mean weight (kgs)	64	73
Donor mean BMI	22,2	24,3

IPITA-O-5.10

Long term outcomes following pancreas transplant patients in a single institution

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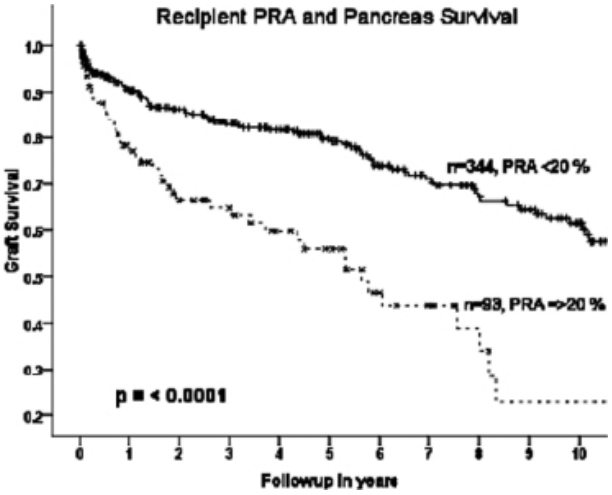
Background: Although survival following pancreas transplantation has improved over the past 15 years, long term chronic rejection continues to play a significant role. We reviewed our experience to identify specific risk factors that are associated with poor long term outcomes.
Method: From 1991 to 2005, 700 pancreas transplant patients that had at least 3 years of follow-up were reviewed. The mean follow up was 7 ± 6 years. The study excluded grafts with early thrombosis due to technical failure and the endpoint was return to insulin therapy. A ROC analysis was performed to determine the optimal cutoff for PRA and cold ischemia time, 20% and 16 h respectively. PRA data was available on 437 patients.
Results: PRA had a significant impact on graft outcome. The 10 year graft survival for high PRA (> 20%) and low PRA recipients was 23% and 60% respectively, p < 0.0001.

There was no significant difference in recipient age, HLA mismatches, PRA, hepatitis C positivity, CMV mismatch, donor age, cold ischemia time, and induction therapy between low and high PRA groups. However more pancreas transplants alone were performed in the high PRA group, p = 0.02. A multivariate analysis revealed that recipients with high PRA had a hazard



ratio of 3.50 for graft loss, p ≤ 0.0001. Hepatitis C positivity had a hazard ratio of 2.37, p = 0.03. Induction agents regardless of which were used had a protective effect with a hazard ratio of 0.10 (0.02–0.40), p = 0.001.
Conclusion: High PRA and hepatitis C positivity had negative impact on long term graft survival. Induction therapy has a protective effect on long term graft outcome.

IPITA-O-5.11

Kidney–pancreas transplantation in hiv infected individuals: the italian experience

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Background: Until a few years ago, HIV infection was considered an exclusion criteria for organ transplantation. However, more recently, because of the significant increase in life expectancy of HIV-infected persons with highly active antiretroviral therapy (HAART), solid organs transplantation has been introduced in this patients population in several centers around the world.
Purpose: To evaluate the patient and graft survival and the impact on HIV progression of kidney–pancreas transplantation in HIV-infected individuals.
Methods: The *Italian National Centre for Transplantation* has designed a protocol to be applied on a national basis. Inclusion criteria required a CD4 count ≥ 200/mm^3 and undetectable HIV viral load for at least 3 months for patients on HAART. The program was voluntarily adopted by the transplant center of the University of Insubria, Varese, Italy.
Results: From January 2006 through May 2009 a total of 4 HIV infected patients (3 male and 1 female, mean age 42 years, range 35–49) underwent cadaveric kidney–pancreas transplantation after a median waiting time of 123 days (range 84–520). Median CD4 cells count at the time of transplantation was 470 (range 431–598) and the HIV-RNA was undetectable in all recipients. HAART was started in all recipients after transplantation and HIV-RNA remain undetectable in all patients. One patients experienced an acute humoral rejection episode at 6 days after transplantation. All patients developed infectious complications at a median of 25

(range 9–128) days after transplantation. Urinary tract infections and bacteremia were the most commonly observed infections. All patients underwent re-laparotomy for post-transplant complications at a median of 9 (range 1–44) days. Drug-drug interactions between antiretrovirals and immunosuppressive agents required frequent dosage modifications. Graft and patient survival was 100% at a median follow-up of 586 days after transplantation (range 26–1234).
Conclusion: Despite the limited number of patients and the shortness of the follow-up, our study confirms excellent short term results of kidney–pancreas transplantation in HIV-infected individuals.

IPITA-O-5.12

Application of interventional radiology to portal venous thrombosis after pancreas transplantation

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Background and Objects: Portal venous thrombosis (PVT) has been reported as one of the major surgical complications after pancreas transplantation (PTx). It is usually detected too late to rescue the graft, and graft pancreatectomy remains the most common option after PVT. We present here three cases of brain death PTx in which the methodology of interventional radiology (IVR) was effective to control the PVT after PTx.
Results: (Case 1) Partial thrombus in the graft splenic vein (SV) was detected by routine ultrasonography and enhanced computed tomography on POD 6 after pancreas transplant alone. Percutaneous continuous intra-arterial infusion of urokinase for 12 days through the catheter which was inserted from the femoral artery and advanced into Y-graft completely removed the thrombus. (Case 2) Partial thrombus in the graft SV found on POD 7 after pancreas after kidney transplant (PAK) was completely dissolved by one-shot arterial infusion of urokinase via the catheter inserted in the same manner. (Case 3) Partial thrombus in the graft SV found on POD 7 after PAK was not dissolved by arterial thrombolysis. Venous infusion of urokinase through the catheter which was inserted from the femoral vein and advanced into graft portal vein and direct mechanical thrombectomy were added with placement of an inferior vena cava (IVC) filter. Even after successful thrombectomy by these strategies, arterial flow did not returned to the graft portal vein but to the recipient portal system via newly developed collateral vessels, and graft kept functioning. In all cases grafts remain functioning without extrinsic insulin under administration of oral anticoagulant. Present HbA1c levels in these cases are 5.9%?.9% and 5.7%, respectively, at 5–14 months following PTx.
Conclusions: Systemic anticoagulation therapy and surgical thrombectomy (ST) have been usually done for PVT, resulting in low success rate. Methodology of IVR is less invasive than ST and can be done safely during systemic anticoagulation therapy. Moreover, including placement of IVC filter, variety of methods such as arterial infusion, portal infusion and mechanical removal are possible. In spite of necessity of skillful techniques, IVR is quite useful option for management of PVT after PTx.

IPITA-O-5.13

Evaluation of coronary microvascular function by coronary flow reserve (cfr) measurement in simultaneous pancreas and kidney transplantation (spk) allograft recipients

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Background: In SPK recipients, 50% of deaths are due to cardiovascular diseases. We sought to evaluate coronary flow reserve (CFR) by transthoracic Doppler echocardiography, as an index of coronary microvascular function, in SPK recipients.
Methods: Twenty-three SPK recipients (11 male, aged 42 ± 8 years) without clinical evidence of ischemic heart disease, and 26 controls matched for age and sex were studied. Coronary flow velocity in the left anterior descending coronary artery was detected by transthoracic Doppler echocardiography at rest and during adenosine infusion. CFR was obtained as the ratio of hyperaemic diastolic flow velocity (DFV) to resting DFV. A CFR ≤ 2.5 was considered abnormal. Time from transplantation was 42 ± 32 months.
Results: Compared with controls, no differences were found regarding prevalence of coronary risk factors other than hypertension (80% vs 4%, $p < 0.0001$). In SPK recipients CFR was lower than in controls (2.58 ± 0.7 vs 3.55 ± 0.8, $p < 0.0001$). CFR was abnormal in 12 (52%) recipients compared with controls (1%) ($p < 0.0001$). In these patients compared with the remaining population CFR was lower (1.98 ± 0.2 vs 3.23 ± 0.6, $p < 0.0001$), total cholesterol and LDL levels were higher (206 ± 65 vs 147 ± 28 mg/dl, $p = 0.02$ and 132 ± 57 vs 70 ± 16 mg/dl, $p = 0.01$ respectively).
Conclusions: CFR is impaired in SPK recipients demonstrating the presence of coronary microvascular dysfunction. Determination of CFR in the follow up of SPK recipients could be a useful tool in the evaluation of the effects of normoglycemia in the progression of microvascular complications.

IPITA-O-5.14

Neutrophil oxidative metabolism in diabetic patients undergoing pancreas transplantation

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Background: A pancreas transplantation is the only therapy capable of returning a constant, physiological euglycemic state to diabetic patients. Considering the clinical controversies in the study of infection in diabetes and the recognized effect of insulin on the oxidative metabolism of glucose in phagocytes, the present study sought to evaluate the formation of intraphagocytic oxygen-free radicals in diabetic patients undergoing simultaneous pancreas kidney transplantation (SPK).
Methods: Twenty-five diabetic patients undergoing SPK were compared with 25 normal individuals. Evaluation of the oxidative metabolism of leukocytes was performed using the NBT test.
Results: The abnormality in the pretransplant counts (19.32–28.2%) reached normal levels at 48 h after transplantation (45.11–76.25%) and was maintained to the 5th day (46.28–76.20%).
Conclusion: An SPK in a diabetic patient normalized the formation of intraphagocytic oxygen-free radicals.

IPITA-O-5.15

Post transplant diabetes mellitus after pancreas transplantation

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Post-transplant diabetes mellitus (PTDM) has been well described after solid-organ transplant. PTDM is an increasingly recognized complication after pancreas transplant (PTX); however, the incidence, risk factors, and impact on transplant outcomes remain poorly defined. This retrospective analysis of 674 recipients from 1994 to 2005 examines the incidence of and risk factors for PTDM after PTX at a single institution. Patients who died or had graft loss within the first 6 months were excluded. PTDM was defined by fasting plasma glucose level ≥126 mg/dl, confirmed on a subsequent measurement, and/or treatment with insulin or oral hypoglycemic agent for ≥30 days while on a maintenance steroid dose. The incidence of PTDM was 14%, 17%, and 25% at 3, 5, and 10 years after PTX, respectively, and was higher after pancreas transplant alone (PTA) vs simultaneous kidney pancreas (SPK) transplant (mean follow-up 6.5 years). In multivariate analysis, three pre-transplant factors were associated with PTDM: older donor age (hazard ratio [HR] 1.04, 95% confidence interval [CI] 1.03–1.06, $p < 0.001$), higher recipient BMI (HR 1.07 [1.01–1.12] $p \leq 0.05$) and donor

positive/recipient negative CMV status (HR 1.67 [1.05–2.7] p = 0.03). Additionally, post-transplant weight gain (HR 4.5 [1.9–10.8] p < 0.01) and laboratory values at 6 months after PTX were associated with the development of PTDM, including fasting glucose (HR 1.01 [1.01–1.02] p < 0.01), hemoglobin A1c, (HR 1.12 [1.05–1.22] p = 0.002), triglyceride to high-density lipoprotein (TG/HDL) ratio (HR 0.94 [0.91–0.96] p < 0.001), and renal function in SPK recipients (mean GFR 55.4 ± 18 vs 58.9 ± 17 in PTDM vs no PTDM, respectively). Pancreas rejection conferred a higher risk of PTDM (1.94 [1.3–2.9] p 0.001). This study delineates the incidence of and identifies potentially modifiable risk factors for PTDM after PTX.

IPITA-O-5.16

Does diabetes have a role in polyomavirus nephropathy development?

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Background: Polyomavirus nephropathy (BKVN) is a cause of graft failure in kidney (KTX) and kidney and pancreas (SPK) transplant recipients. Clinical evidence shows that cumulative dose of immunosuppression (IS) can be responsible for BKVN but the pathogenesis remains unclear. A possible role for T-cell impairment and deregulation in the BKVN development has been recently reported. The relationship between diabetes (DM), autoimmunity and T and B-cells impairment have been described for type 1 and 2 DM.
Study Purpose: To establish a possible relationship between BKVN development and DM.
Methods: Retrospective analysis and comparison of 4 groups : (A) KTX affected by DM; (B) KTX without DM; (C) SPK; (D) KTX who developed post-transplant DM (PTDM). KTX and SPK with positive BK viremia underwent a renal biopsy.
Results: Between January 2006 and March 2009, 43 (KTX = 34, SKP = 9, Females = 23, Blacks = 21 mean age 49 ± 17 years) had positive BK viremia (6–20 months post-transplant). All patients received induction with Thymoglobulin (Thymo) or anti-CD 25 (Group A: Thymo n = 4; Group B: Thymo n = 11: Group C: Thymo n = 3). Maintenance IS was: tacrolimus (tac), mycophenolate and prednisone. Biopsy was performed in 41/43 patients with positive BK viremia regardless the renal function. Table 1 shows the presence or absence of BKVN, and graft outcomes (s.creatinine mg/ml). In group A and B, there was a trend (p = NS) in worsening renal function and 5 patients returned to dialysis. BKVN was often accompanied by chronic nephropathy. No SPK recipient lost the kidney or the pancreas graft and s.creatinine stabilized if not improved (p = NS). In this series, no patients developed PTDM. At BKVN diagnosis mycophenolate was discontinued and tac was reduced in 39/43 patients. LEF (dose 40–60 mg/day) was introduced. LEF levels were monthly monitored.
Conclusions: These preliminary data would suggest that DM seems not to impact or affect the incidence or outcome of BKVN. Interestingly in this series of patients with BKVN no patient had PTDM. It appears that LEF might have a role also as immunosuppressant able to minimize tac toxicities, including PTDM. These findings are meritorious of further studies.

Table 1

Groups	Creat. @BK diagnosis	Creat.@3–6 months post–BK diagnosis	BKVN + or –
A=KTX n=11(DM+)	1.9±1.3	2.2±1.5	8+ 2–
B=KTX n=23(DM–)	1.6±0.9	2.2±1.3	16+ 6–
C=SPK n=9	2.4±1.4	1.8±0.9	7+ 2–
D=PTDM	NA	NA	NA
Graft loss (dialysis) Group A= 3, Group B=2			

IPITA-O-5.17

Over 500 pancreas transplants in a single-team in São Paulo Brazil

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Background: Pancreas Transplantation (PT) is still an incipient experience in Latin America.
Methods: From 1996 to 2009, 506 PT were performed by our team and were divided in three Eras: Era 1 – 1996 to 2000; Era 2 – 2001 to 2004; Era 3 – 2005 to 2009. Whole organ PT was performed in all cases. Enteric drainage was the preferred technique for pancreas–kidney transplants and bladder drainage for solitary PT. In the most recent experience (last year) portal and enteric drainage was the technique of choice for all cases. Immunosuppressant protocol included tacrolimus, mycophenolate mophetil/sodic and steroids for all cases. Induction with anti-linfocytic drugs was used for solitary PT and for high-risk recipients of SPK.
Results: Table 1 below shows 1-year patient and graft survival according to Eras and category of PT. Improving results have been achieved in all categories but SPK.
Conclusions: This series represents the largest experience with PT in Latin America and results as good as those reported by developped countries have been achieved with the exception of SPK. It has led our programto prioritize solitary PT and SPLK category.

Table 1

Category of PT	Era 1 (1996 2000)	Era 2 (2001 2004)	Era 3 (2005 2009)
	N – %	N – %	N – %
SPK	36	109	109
1–Y Patient Survival	32 – 88.8	88 – 81	86 – 79
1–Y Kidney Survival	29 – 80.5	85 – 78	83 – 76
1–Y Pancreas Survival	27 – 75	79 – 72.4	71 – 65
SPLK	– – – –	23	37
1–Y Patient Survival		19 – 83	35 – 95
1–Y Kidney Survival		19 – 83	35 – 95
1–Y Pancreas Survival		17 – 74	32 – 86
PTA	5	62	31
1–Y Patient Survival	4 – 80	57 – 92	30 – 97
1–Y Pancreas Survival	4 – 80	46 – 74	24 – 77
PAK	2	35	57
1–Y Patient Survival	2 – 100	33 – 94	54 – 95
1–Y Pancreas Survival	1 – 50	29 – 83	49 – 86
SPK : Simultaneous Pancreas–Kidney ; SPLK : Simultaneous Pancreas and Living–Donor Kidney Transplant ; PTA : Pancreas Transplant Alone ; PAK : Pancreas After Kidney			

IPITA Parallel Session 6
Clinical and Experimental Islet Transplantation

IPITA-O-6.1

Drugs inhibit rat islet cell proliferation *in vitro*

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Aim: Beta-cell replication is thought to play a significant role in maintaining pancreatic beta-cell mass. Whether immunosuppressive drugs affect replication of beta-cells in transplanted islets is not fully understood. The aim of this study was to determine the effects of immunosuppressive drugs, used in islet transplant recipients, on islet cell replication, *in vitro*.
Methods: Rat pancreatic islet cells were incubated with BrdU (10 μM) in the presence or absence of different concentrations of cyclosporine A (CsA), tacrolimus, sirolimus or mycophenolate mofetil (MMF). Cell replication was determined by BrdU incorporation after 1–7 days of culture. Data are expressed as % of positive BrdU cells and as mean ± SEM for 3 or more independent experiments.
Results: Sirolimus (10 ng/ml) blocked almost completely islet cell proliferation from 24 h (0.17 ± 0.17 vs 2.00 ± 0.51; sirolimus vs control, p < 0.001) until 7 days of treatment (0.14 ± 0.10 vs 23.80 ± 3.63; sirolimus vs control, p < 0.01). Additionally, the inhibitory effect of sirolimus was also observed at low concentrations from 0.1 ng/ml (2.75 ± 0.97 vs 13.43 ± 0.76;

sirolimus vs control, $p < 0.001$). After 2 days of treatment, MMF also inhibited cell proliferation in a dose-dependent manner (control: 9.48 ± 2.79; MMF 1 μg/ml: 5.54 ± 1.89; MMF 5 μg/ml: 1.75 ± 0.70; MMF 25 μg/ml: 0.98 ± 0.07%). Treatment with CsA (200 ng/ml) and tacrolimus (10 ng/ml) during 5 days significantly reduced islet cell proliferation (2.52 ± 0.94; 3.68 ± 1.06; 21.13 ± 2.47 for CsA, tacrolimus and control, respectively ($p < 0.01$)). None of the immunosuppressive drugs induced a significant increase of apoptosis. Furthermore, effect of immunosuppressive drugs on cell proliferation was reversible.

Conclusion: Our results indicate that immunosuppressive drugs, at therapeutic target concentrations, inhibit pancreatic islet cell proliferation *in vitro*. It is therefore suggested that progressive graft islet dysfunction may result, in part, from an impairment of beta cell replication induced by immunosuppressive drugs.

IPITA-O-6.2

Monitoring clinical islet transplantation: Lessons learned from sequential metabolic studies

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Background: The natural history of pancreatic islet transplantation (PIT) in humans is largely undefined, in part due to difficulty assessing the graft histologically and accurately, reproducibly imaging functional graft mass. Assessing β cell function as an estimate of β cell mass is the only current method to determine the status of the islet graft. Insulin sensitivity index (Si), glucose effectiveness (Sg), and free fatty acid (FFA) dynamics were measured before and after PIT in 10 lean patients, 8 reaching insulin independence. Modified Bergman Minimal Model of Frequently Sampled Intravenous Glucose Tolerance Tests (FSIVGTT) were performed pre-transplant and 12 and 24 month post-transplant. Sequential metabolic responses to exogenous glucose (IVGTT), arginine at normoglycemic levels (Arg) (5 mM glucose) and at hyperglycaemic levels (15 mM glucose) (GPAIIS) were also obtained 3, 6, 12 and 24 months after last islet infusion. Ten non-diabetic control (NDC) subjects matched by age, gender and BMI were used as controls.

Results: Pre-transplant Si and Sg values were 3.8 ± 0.2×10–4 min^{-1} per μU/l and 4.4 ± 0.6×10–3 min^{-1} respectively, suggesting that type 1 diabetic subjects are insulin resistant. Both values were lower when compared to matched NDCs (9.7 ± 0.6 min^{-1} per μU/l and 12.7 ± 2.2×10–3 min^{-1}, $p < 0.001$ and $p = 0.004$, respectively). Islet transplantation is capable to reverse insulin resistance (IR), demonstrated by an improvement on the Si and Sg values (8.2 ± 0.3 min^{-1} per μU/l and 11.3 ± 2.6×10–3 min^{-1}, $p = 0.03$ and $p = 0.03$) respectively. Post transplant values were not significantly different from NDC. Sequential metabolic testing demonstrated (1) All PIT subjects, even at 3 month of insulin independence, had two-fold lower basal insulin and C-peptide secretion and two times lower β-cell reserve compared to NDC; (2) Differences in insulin secretion and reserve preceded changes in fasting blood glucose, and HbA1c; (3) Cut-off threshold values to predict the use of exogenous insulin therapy could be predicted several months in advance using a Receiver Operator Characteristics Analysis based on acute insulin secretion AUC for IVGTT ($p = 0.01$), Arg ($p = 0.0005$) and GPAIIS ($p = 0.0001$).

Conclusion: Despite IR reversal afforded by PIT, return to exogenous insulin dependence in some recipients may be due to impaired insulin secretory reserve related to insufficient β-cell mass leading to deterioration of islet mass.

IPITA-O-6.3

Glucagon response to hyperglycemia following islet cell transplantation in type 1 diabetes mellitus

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Background: Glucagon is inappropriately secreted in type 1 diabetes mellitus. Levels are low in response to hypoglycemia, and are high in response to hyperglycemia. Hyperglucagonemia in response to hyperglycemia has not been studied in the context of islet cell transplantation. The aim of this study is to assess whether islet transplant corrects the abnormal response of glucagon to hyperglycemia.

Methods: Thirty-five subjects who underwent islet transplant during 2005–2006 at the Diabetes Research Institute, University of Miami School of Medicine, were retrospectively studied to assess glucagon response to hyperglycemia (mixed meal testing). 6 subjects who maintain good graft function (are insulin independent) were followed over 3, 6, and 12 months and compared to a group with abnormally elevated glucagon levels comprised of both subjects who had not received islet transplant ($n = 2$) and subjects who received transplant but developed graft dysfunction and were on insulin ($n = 16$). Data were analyzed to estimate and compare mean AUCglucagon with linear mixed models regression.

Results: Mean AUCglucagon and SE in the comparison group with abnormally elevated glucagon levels and in subjects who maintain good graft function at 3, 6, and 12 months was 19093.5 3051.4, 11015 2642.6, 16509 2642.6, and 11059.2 2894.9 respectively. Comparisons of these means reveal statistically significant differences in AUCglucagon when comparing subjects with abnormally elevated glucagon levels (pre-transplant and graft dysfunction) to post transplant subjects at 3 months ($p = 0.03$) and 12 months ($p = 0.04$), but not at 6 months ($p = 0.41$).

Discussion: Results demonstrate that islet transplant restores appropriate glucagon secretion in response to hyperglycemia, an area that has not been explored. Hyperglucagonemia may be detrimental to islet function. Agents such as exenatide and pramlinitide may be useful in prolonging islet allograft function as they target glucagon. In addition, the abnormal glucagon response to hyperglycemia may be a useful marker in following graft dysfunction.

Conclusion: Islet transplant corrects the abnormal response of glucagon to hyperglycemia as demonstrated by this pilot study of type 1 diabetic subjects. This will be studied prospectively in subjects who undergo islet transplant under the CIT protocol.

IPITA-O-6.4

Lipotoxicity decreasing islet graft survival

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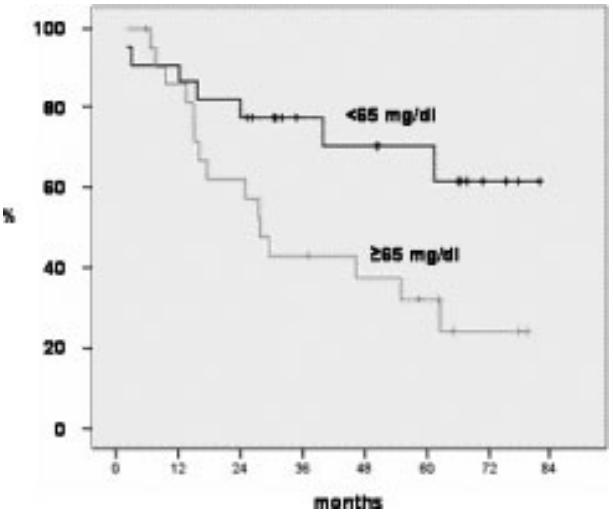
Lipotoxicity has being associated with both beta-cell apoptosis *in vitro* and with islet graft failure in animal models. The aim of this study was to evaluate if the lipid profile could modify islet graft survival in type 1 diabetes mellitus (DM) islet transplant (ITx) recipients.

Baseline demographic, anthropometrical and laboratory data, including fasting lipid profile, were collected from 44 ITx recipients. The median of each lipid fraction was determined (total-cholesterol: 177 mg/dl, LDL: 96 mg/dl, HDL: 67 mg/dl, VLDL: 13 mg/dl and triglycerides: 65 mg/dl) and comparisons were performed between subjects below and above these values.



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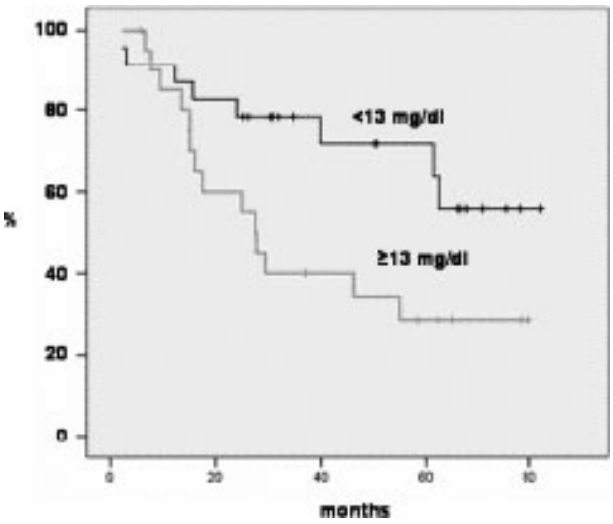

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Differences in outcomes (time to graft dysfunction and failure) were done by Kaplan–Meier curves. Cox-regression analysis was performed to control for possible confounding factors. Mean age at the time of the first ITx was 43.0 ± 8.6 years and the DM duration was 30.5 ± 11.7 years. Subjects with baseline fasting plasma triglycerides and VLDL-cholesterol above median had shorter islet graft survival (triglycerides: 39.7 ± 6.1 vs 61.3 ± 6.6 months, p = 0.029 and VLDL: 41.5 ± 5.7 vs 62.8 ± 7.3 months, p = 0.032). Total, LDL and HDL-cholesterol didn't influence islet function. Triglycerides (OR 2.9, 95% CI 1.1–7.7, p = 0.032) maintained its association with graft failure after adjustments for confounders. In conclusion, higher baseline triglycerides are associated with earlier decline in islet graft function. Prospective clinical trials should address whether it is direct caused by lypotoxicity and if strategies focusing lowering serum lipids may prolong islet graft survival.

IPITA-O-6.5

A novel calcineurin inhibitor- and sirolimus-free anti-LFA-1-based therapy enhances allogeneic islet survival and function in nonhuman primates

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There is renewed enthusiasm for pancreatic islet transplantation to treat diabetes, but inadequate long-term graft survival and toxicities associated with current immunosuppression are preventing its widespread application. New approaches targeting key T-cell costimulatory signals, like the CD28 pathway and inhibiting lymphocyte adhesion, activation and trafficking mediated by leukocyte function-associated antigen (LFA-1), have had promising results in animal models. Combining LFA-1 and CD28 blockade presents a potential novel therapeutic strategy in islet transplantation to prevent rejection while avoiding the toxicities of current therapy. To evaluate this regimen, diabetic rhesus macaques were intraportally transplanted allogeneic islets from MHC-mismatched donors and immunosuppressed with the anti-LFA-1 monoclonal antibody, TS-1/22, and LEA29Y, a high affinity CTLA4-Ig variant. Recipients treated with TS-1/22 and LEA29Y (n = 5) experienced immediate return to euglycemia and prolonged islet allograft survival (> 182, > 182, > 175, > 63, and > 63 days post-transplant), while controls treated with TS-1/22 alone (n = 1) or LEA29Y alone (n = 3) rejected 10, 58, 60 and 8 days after transplant, respectively. Daily fasting blood glucose (FBG) levels and periodic glucose tolerance tests with C-peptide measurements confirmed glycemic control and graft function. Flow cytometry verified adequate LFA-1 blockade, and all animals tolerated the regimen without clinically significant latent virus reactivation or adverse events. These results show that a calcineurin inhibitor- and sirolimus-free protocol consisting of anti-LFA-1 and CD28 blockade is a potent, yet well tolerated regimen that can effectively prevent islet rejection in a nonhuman primate preclinical model.

IPITA-O-6.6

Inhibition of pro-inflammatory cytokine induced response in human islets by Withaferin A

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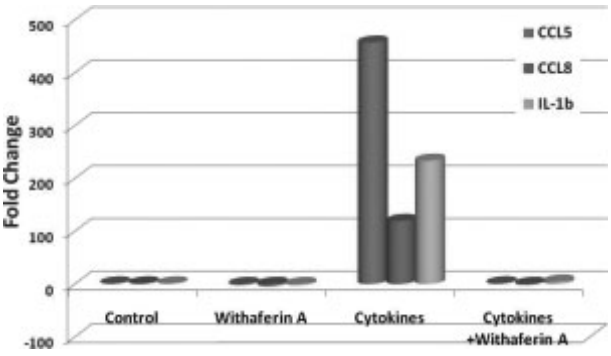
Background: Following islet cell transplantation, a significant mass of islets are lost due to non-specific inflammatory reactions. Cytokine exposure before or after transplantation can upregulate the expression of pro-inflammatory genes through NF-kB signaling pathway in islet cells that will eventually result in islet loss. We tested the effects of a naturally occurring NF-kB inhibitor called Withaferin A on the regulation of these genes in human islets.
Methods: Human islet cells were isolated by a modified Ricordi protocol. Purified islets (n = 3) were cultured for 2 days to eliminate macrophages. The effect of Withaferin A treatment on islet cell viability was examined with PDF/PI dye exclusion test and function with static glucose stimulation test.



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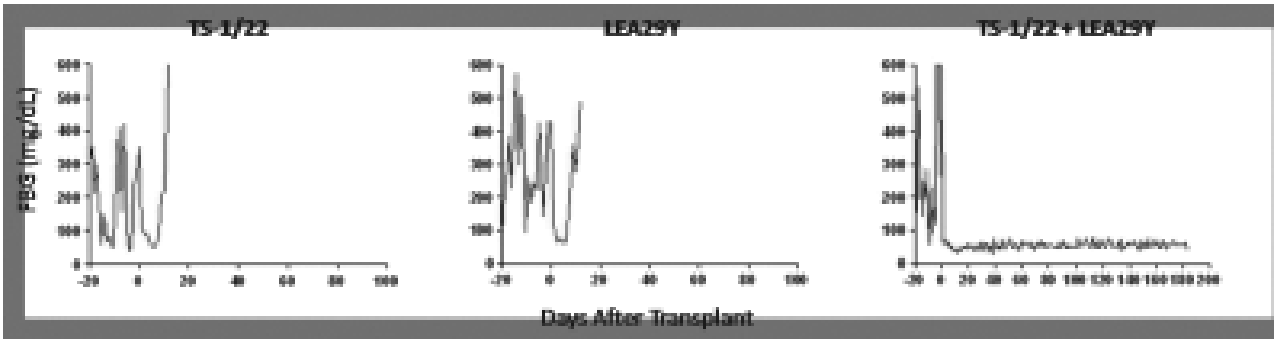

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Islet cells are treated with a cytokine mix (50 U/ml IL-1beta, 1000 U/ml TNF-alpha, 1000 U/ml IFN-gamma) for 48 h with or without Withaferin A (2 μg/ml). Treated islets are used for a real-time PCR array analysis for expression of inflammatory genes.
Results: Glucose stimulation and viability assay indicated that Withaferin A is not toxic to islet cells. Among 84 inflammation-related genes examined by real-time PCR array IL-1beta, TNF-alpha, RANTES, IP10, IP9 and MCP2 are significantly (10–300 fold) upregulated by cytokine treatment compared to control group. However, addition of Withaferin A to the culture resulted in all six genes are inhibited to baseline levels
Conclusion: Induction of chemokines and cytokines will augment innate immune response following islet transplantation. Our data demonstrated that Withaferin A can completely inhibit the inflammatory response of islet cells upon cytokine exposure. Withaferin A may be a useful addition to the immunosuppressive regimen in islet cell transplantation to minimize islet damage from inflammation.

IPITA-O-6.7

Morphological and gene expression analysis of human pancreata as a predictor for islet function

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Background: Enlarging the donor pool is severely limited, mainly because of the poor function and high incidence of primary non-function of islets from less than ideal donors. This study is aimed at identifying the morphological, histopathological and the gene expression characteristics of the donor pancreas as predictor for the outcome of human islet *in-vivo* function.
Methods: Islets were isolated from 43 human donor pancreata, and islet yield was reported as islet equivalent/gram pancreatic tissue. In-vivo function of islets from 43 isolations was tested using NOD-SCID mice. Biopsies from pancreata were evaluated after staining with HTrichome, Congo, and PAS. Correlation between *in vivo* function and BMI and positive histology (adipose tissue, islet hypertrophy or hyperplasia) was done using Somers D tests. We also examined the gene expression of the pancreata using high-density Affymetrix U133A Gene Chips® and Genespring software.
Results: Morphological characteristics such as the content of acinar and interstitial adipose cells, islet hyperplasia, and islet hypertrophy per defined area were found to have a significant reduction in in-vivo function ($p = 0.003$), whereas BMI was not significant ($p = 0.44$). Furthermore, in these isolation Long cold ischemia was the only donor characteristic that correlated with a significant reduction of in-vivo function ($p = 0.011$). Gene analysis of Pancreata that yield non-function islets showed high relative levels of expression of pro-inflammatory genes such as interleukin-1, toll-like receptor 4, T-cell activation NFKB-like protein1, as well as genes that is associated with insulin resistance such as SOCS-16and nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
Conclusion: Morphological and Gene expression study of donor pancreata can be very instrumental in predicting in-vivo function in optimal and non-optimal donor selection. The over-expression of pro-inflammatory and insulin resistance related genes may results in reduced insulin secretion and lead to islet destruction post-transplantation. Identifying and validating those genes could allow the development of a potency assay that would be very useful for screening Pancreata before clinical transplant and the possibilities of devising strategies for early intervention to reverse the adverse effect of inflammation and ischemia on the islet.

IPITA-O-6.8

***In vivo* non-viral gene delivery of human vascular endothelial growth factor improves human islet survival and function after transplantation**

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Background: Poor revascularization of graft islets is one of the most important issues for islet transplantation. Delivery of human vascular endothelial growth factor (hVEGF) gene to both the transplanted islets and the surrounding tissue may promote islet revascularization and survival. We previously showed that delivery of hVEGF to rat myocardium by ultrasound-mediated gene transfer method named Ultrasound Targeted Microbubble Destruction (UTMD) resulted in significant increases in myocardial capillary density. In this study, we examined the effect of hVEGF gene delivery on transplanted islets *in vivo*.
Methods: A marginal number of human islets were transplanted into diabetic nude mice via portal vein. Then, a non-viral plasmid vector encoding hVEGF or GFP gene were introduced into the host liver by UTMD. After treatment, their blood glucose level was checked for 30 days. After this period, serum human insulin and C-peptide levels were measured. And the extent of revascularization in graft islets was examined with immunohistochemical analysis.
Results: The expression of hVEGF after transfection to liver was detected using immunohistochemical and RT-PCR analysis. 8 out of 11 (73%) mice in hVEGF group became normoglycemia whereas 1 out of 8 (13%) in the control and 1 out of 7 (14%) in GFP group became normoglycemia at day 30. the ratio of the cured mice in hVEGF group was significantly higher than control group. During 30 days, 4 mice in GFP group and 3 mice in control group showed normal glucose level once, however, the majority of them returned to hyperglycemia. On the other hand, 9 mice in hVEGF group became normoglycemia once and only 1 mouse returned to hyperglycemia. Serum human insulin and C-peptide in hVEGF group at day 30 were significantly higher than control (Insulin: hVEGF group 109 ± 26. GFP group 37 ± 17, control group 17 ± 8 pmol/l, respectively and C-peptide: hVEGF group 791 ± 230, GFP group 115 ± 58, control group 68 ± 38 pmol/l, respectively). The density of vessels in graft islets was significantly higher than other two groups (hVEGF group 649 ± 51, GFP group 227 ± 39, control group 169 ± 36 count/mm^2, respectively).
Conclusion: These findings suggest that hVEGF gene delivery to host liver using UTMD is potential for promoting islet revascularization after islet transplantation and improving the survival and function of graft islets.

IPITA-O-6.9

Module analysis for gene expression in successful single donor islet transplantation

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Background: Successful islet transplantation from one donor is meaningful for widespread clinical application, and investigating gene expression in such case have potential to discover novel biological knowledge.
Method: Two patients with type 1 diabetes received islet transplantation from single deceased donor and obtained insulin independence. Peripheral blood mononuclear cells (PBMC) were collected before and after transplantation. RNA isolated from PBMCs was used to reverse transcribe cDNA for microarray analysis. A module analysis was performed using the list of gene with signal intensity present in at least one sample as compared with healthy control samples ($n = 10$) (Chaussabel D et al. Immunity. 2008; 29:150). Genes which significantly changed in first week after transplantation were extracted.
Results: Module analysis showed similar tendency of gene expression among two patients (Figure 1). The following modules had lower expression; B cells, T cells, plasma cells, monocytes, platelets, cytotoxic NK cells, immunosuppression, immune response, lymphocyte activation, cell cycle, proliferation, mitchondrial respiration, mitchondrial stress, and the followings had higher expression; neutrophils, erythrocytes, Interferon (IFN), inflammation, cell death, dendritic cell(DC) apoptosis. Genes which significantly changed in first week after transplantation were shown in Table 1. Inflammation genes increased in both cases but did not reach significance.
Conclusion: This study could provide basic data for clarification of mechanisms of engrafted islet loss after islet transplantation.

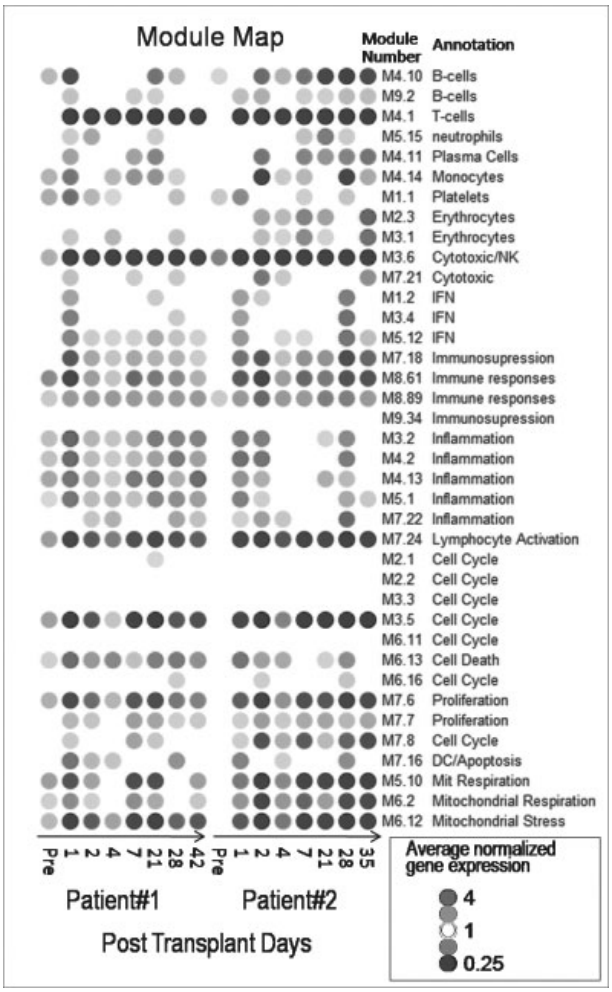

Figure for IPITA-O-6.9.

Table 1. Significantly changed genes* (IPITA-O-6.9)

Annotation	Gene
B cell	SARNP
Cell cycle	MCM4
Cytotoxic NK cell	GPR56, ABCB1, NCALD, TGFBR3, EOMES, AUTS2, PYHIN1, CD160, PRF1, SBK1, PLEKHF1, IL2RB, NKG7, SYTL2, GPR68, PYHIN1, PPP2R2B, SAMD3, TSEN54
Lymphocyte activation	ITPR3, CCDC102A, RUNX3, IL32, TMEM99, NT5C3L, TMEM118, HEG1
Mitochondrial stress	GLMN
Proliferation	CARD11, SLC12A2, UNG
T cell	IL23A, BCL2, EBI2, TNFRSF25, IL7R, FAM84B, BCL11B, CD40LG, SPOCK2
*P value (t test with Bonferroni correction) <0.05	

IPITA-O-6.10

Transcriptome analysis of isolated human pancreatic islets: expression profiles of zinc transporter genes in particular ZnT8 and ZIP14, critical islet marker genes and glucose metabolic genes

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Background: Zinc Transporter genes control the movement of zinc from the extracellular matrix to the cytoplasm, intracellular vesicles and specific metalloproteins and are thought to play an important role in insulin synthesis, secretion and storage. Glucokinase is the signal recognition enzyme in β-cells for initiation of glucose induced insulin secretion. Glucokinase and the Glucose Transporters (GLUT1-4) facilitate the movement of glucose from the circulation across cell membranes. The Zn transporter ZnT8 is expressed primarily by β-cells and a single-nucleotide polymorphism was recently identified as a critical marker for type 2 diabetes risk.

Methods: Human pancreatic islets from 10 donors were isolated by the Ricordi method. RNA was purified by Qiagen® RNA kit, prior to RT Q-PCR analysis with gene specific Taqman® primers. Polyclonal antibodies to ZnT8 and ZIP14 were raised in rabbit and mouse, respectively.

Results: Relative expression levels of 7 Zn transporter genes, 2 Glucose Transporters, Glucokinase(GcK), GLP1R and the islet marker genes Insulin(INS), Somatostatin(SST) and Glucagon(GCG) were determined. Little or no expression of ZnT4, ZnT6, GLUT2 and GLP1R was seen in any of the preparations. GcK, GCG, GLUT1, SST, ZIP14 and ZnT8 displayed highly variable expression profiles, with ZnT8 and ZIP14 the most variable of the zinc transporters. There was a high degree of correlation between the expression profiles of ZIP1,6,7 and ZnT6 ($r^2 > 0.75$); ZIP14 and both ZIP6 &GLP1R ($r^2 > 0.65$) and GcK and GLUT1 ($r^2 = 0.71$). INS was the most abundantly expressed gene followed by SST and GCG. Immunohistochemical staining of human islets showed co-localisation of ZnT8 and insulin proteins in pancreatic β-cells. ZIP14 protein did not co-localise with either insulin or somatostatin, however it did show partial co-localisation with glucagon positive cells.

Conclusion: The antibodies to ZnT8 and ZIP14 functioned with human islet tissue. Isolated human islets expressed a range of zinc and glucose related transporter genes many of which were highly variable. The observed changes in expression profiles of these genes post-isolation could potentially perturb normal glucose and insulin regulation and lead to impaired islet function and greater susceptibility to apoptosis.

IPITA-O-6.11

Preexisting donor factors and isoltion variables leading to successful islet transplantation from donors after cardiac death

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Background: Donor shortage for islet transplantation is serious problem in worldwide. However, the availability of donors after cardiac death (CD) is still controversial in islet transplantation. Here, we analyzed the preexisting donor factors and isolation variables that affected isolation of human islets from pancreas obtained from donors after CD.

Methods: Sixty-four isolations from donors after CD were analyzed using the log-rank χ^2 test for significant characteristics of donors and grafts satisfying the criteria for islet product release. Donor factors included age, gender, cause of death, final creatinine level, low blood pressure period (below 60 mmHg), and graft factors included warm /cold ischemic time, preservation solution (UW or Kyoto solution) and use of TLM. The final criteria for islet product release were including an islet mass of ≥5,000 islet equivalents/kg (recipient weight), islet purity of ≥30%, membrane-integrity viability of ≥70%, packed-tissue volume of < 10 ml, negative Gram stain, and a content of ≤ 5 endotoxin U/kg (recipient weight).

Results: The success rate of islet isolation was 53% (34/64). Mean age was 41 years. Mean warm and cold ischemia time was 7.6 min and 5.3 h, respectively. The cold ischemic time and the Kyoto solution for pancreas preservation were significant factors for successful islet isolation, and use of TLM was marginal factor by the log-rank χ^2 test. The period of the low blood pressure within 8 h showed significantly higher success rate than those over 8 h (60% vs 20%, $p < 0.05$). Islet graft survival rate of patients who had received 2 or 3 infusions (10 among 18 recipients) at 1, 2 and 3 years after the first transplant was 100, 80.0 and 57.1%, respectively, indicated comparable with reported cases using brain dead donors.

Conclusion: Use of Kyoto solution for pancreas preservation and shorter cold ischemic time as well as limited period of low blood pressure level of donors might enable successful islet isolation and transplantation from donors after CD.

IPITA-O-6.12

Analysis of clinical course of single donor islet transplantation

Morihito Takita[1]*, Shinichi Matsumoto[1], Hirofumi Noguchi[1], Masayuki Shimoda[2], Daisuke Chujo[3], Yoshiko Tamura[4], Greg S. Olsen[4], Bashoo Naziruddin[4], Nicholas Onaca[4], Marlon F. Levy[4]

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Background: Issues in clinical islet transplantation include multiple infusions for insulin independence and improvement of graft survival. Currently we implemented new pancreas procurement and islet isolation method (Matsumoto S et al. Bayl Univ Med Cent Proc. 2007;20:357) combined with new immunosuppression protocol. This modification made us possible to perform single donor islet transplantation. In this study, we analyzed clinical outcomes of our single donor islet transplantation.

Method: Two patients with type 1 diabetes received islet transplantation from single deceased donor in September and October 2008 (Table 1). Patients were administered rabbit anti-thymocyte globulin as immunosuppressive induction combined with anti-inflammatory drugs (eternacept and anakinra). Maintenance immunosuppression was obtained with mycophenolate mofetil and tacrolimus without sirolimus.

Results: Both two patients reached insulin independence on Day 51 and 65 after single islet infusion (Figure 1). Their secretory unit of islet transplant objects (SUITO) index kept more than 26 which represent adequate graft function for insulin independence (Matsumoto S. et al. Transplant Proc 2005;37:3435). Patient 1 resumed insulin injection and patient 2 remained insulin free. Diabetes Form 2.1 and the short form-36 health survey (SF-36) indicated that patient 1 deteriorated her quality of life (QOL) after resumed insulin injection even her glycemic control is excellent and patient 2 has continuously improved QOL.

Conclusion: Our new protocol made it possible to achieve insulin independence after single donor islet transplantation. However, resuming insulin injection seems negative impact on QOL even glycemic control maintain excellent.

Table 1 Patient characteristics

Characteristics	Patient 1	Patient 2
Age, year	38	53
Body weight, kg	54.9	75.9
Body mass index	20.7	27.9
Diabetes duration, year	33	44
Diabetes complications	Retinopathy	Neuropathy, Retinopathy
Autoantibodies		
GAD65	–	–
ICA512	–	–
mIAA	+	+
ZnT8	–	–
Graft		
Islet equivalents/kg body weight	9895	12216
Islet purity, %	60.0	60.0
Islet viability, %	96.8	98.8

IPITA-O-6.13

Excellence of SUITO index for assessing clinical outcome of islet transplantation

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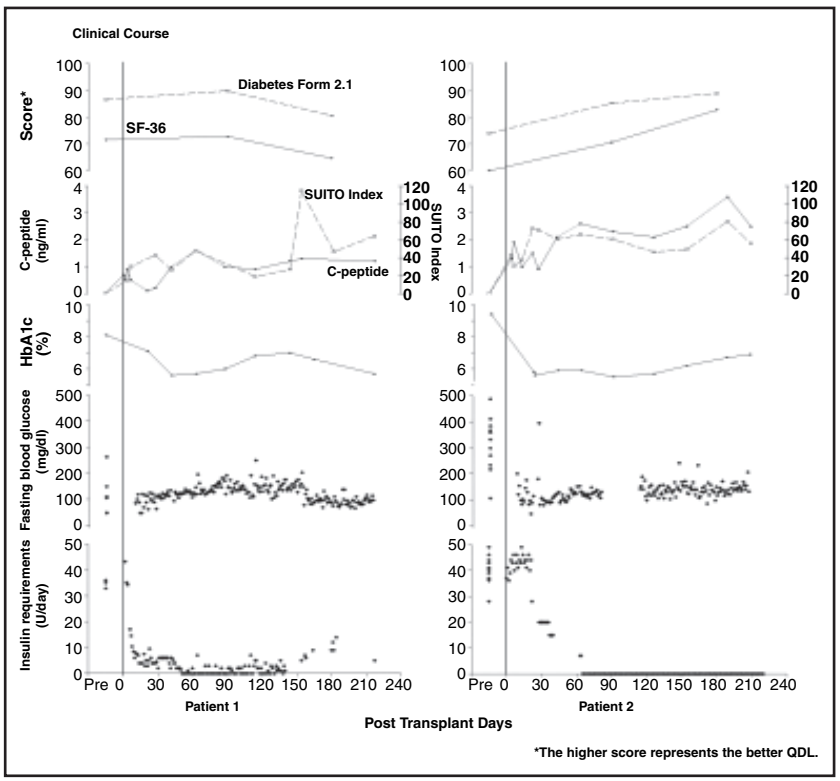
Background: Monitoring functional islet mass after islet transplantation is critical for evaluating clinical results, identifying the necessity of additional islet transplantation and predicting clinical outcome including maintenance of insulin free and/or excellent glycemic control for near future. Previously we demonstrated that average SUITO index after islet transplantation



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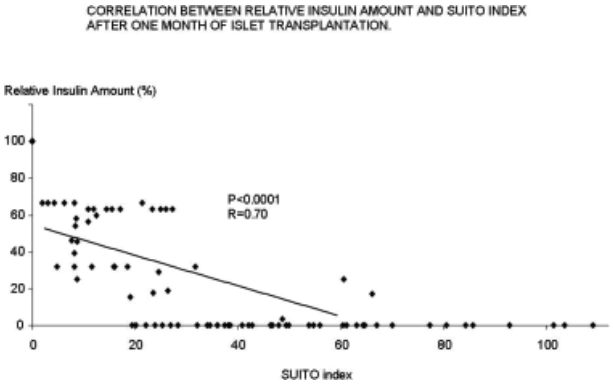
within 1 month was an excellent predictor for achieving insulin free status and reduction of insulin dose.[1,2] In this study, we have analyzed the usefulness of SUITO index for assessing clinical outcome.

Method: Five type 1 diabetic patients received islet transplantations between February 2007 and October 2008. Three patients received two transplantations and two patients received single transplantation. All five patients achieved insulin free and three of them remained insulin free at the time of evaluation. Daily relative insulin dose and SUITO index was analyzed. Relative insulin dose was calculated as; total daily insulin dose/ average pre-transplant insulin dose. SUITO index was calculated as; [fasting C-peptide (ng/ml)]/[fasting blood glucose-63 (mg/ml)]×1500.[1] The data were analyzed based on the period after islet transplantation and categorized within 1 month and after 1 month.

Results: Within 1 month after transplantation there were no correlation between daily relative insulin dose and daily SUITO index ($p = 0.068$, $r = 0.33$). No patients achieved insulin free status within 1 month after transplantation because our current protocol includes intensive insulin therapy within 1 month. After 1 month, daily relative insulin dose and daily SUITO index strongly correlated ($p < 0.0001$, $r = 0.70$) (Figure 1). When cut off value of SUITO index was decided 26 for insulin free, a positive predict value was 84.1% and a negative predict value was 89.4%.



Conclusion: SUITO index is excellent index for assessing clinical outcome after 1 month of islet transplantation.

References

1. Matsumoto S et al. Transplant Proc 2005; 37: 3435.
2. Matsumoto S et al. Transplant Proc 2008; 40, 364.

IPITA-O-6.14

Association between secretory unit of islet transplant objects (SUITO) index and glucose tolerance test after clinical islet transplantation

Morihito Takita[1]*, Shinichi Matsumoto[1], Hirofumi Noguchi[1], Masayuki Shimoda[2], Daisuke Chujo[3], Yoshiko Tamura[4], Greg S. Olsen[4], Bashoo Naziruddin[4], Nicholas Onaca[4], Marlon F. Levy[4]

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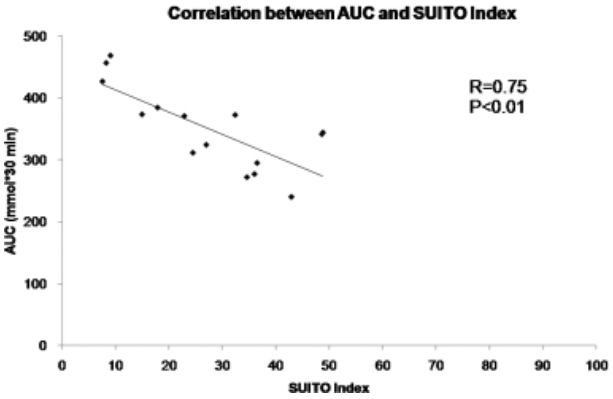
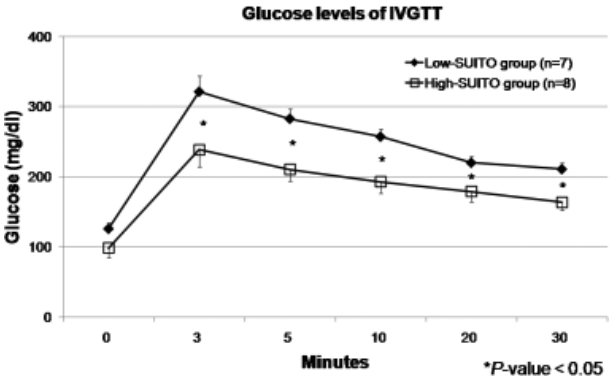
Background: We have developed the assessment tool for functional engrafted islet mass; secretory unit of islet transplant objects (SUITO) index which was easily calculated using fasting blood glucose and C-peptide (Matsumoto S. et al. Transplant Proc 37:3435,2005). On the other hand, intravenous glucose tolerance test (IVGTT) was widely used for assessing functional engrafted islet mass after islet transplantation. The present study showed the relationship between SUITO index and IVGTT.

Method: Five patients with type 1 diabetes received islet transplantations between March 2005 and March 2008. The blood samples were collected before 0.5 g/kg of dextrose 50% were infused (baseline) and at 3, 5, 10, 20, 30 min after infusion respectively. SUITO index was calculated by; [fasting C-peptide (ng/ml)]/[fasting blood glucose-63 (mg/ml)]×1500. High-SUITO group was defined as the sample when the SUITO index had more than 26 which represent that engrafted islets had adequate for insulin independence. Low-SUITO group had SUITO index which was < 26.





Results: SUITO index was significantly correlated with area under the curve (AUC) in IVGTT (Figure 1), and averages of AUC had significant difference between low and high SUITO groups (average ± SE; 398.5 ± 20.9 and 307.8 ± 15.8 mmol*min, respectively, p-value < 0.01).

Blood glucose levels in low-SUITO group were significantly higher than those in high-SUITO group during measuring period (Figure 2).

Conclusion: SUITO index had significant correlation with the results of IVGTT, and potential to be used as alternative for IVGTT that allow to avoid risk of hyperglycemia.

IPITA-O-6.15

Metabolic effects of sitagliptin in islet transplant recipients requiring exogenous insulin

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Objective: Sitagliptin is a dipeptidyl peptidase (DPP) 4 inhibitor which has demonstrated protective effects on transplanted islets in rodent models of type 1 diabetes mellitus. The aim of this study is to evaluate if the use of sitagliptin can improve islet allograft function without causing unacceptable side effects.

Methods: Four subjects with functioning islet allografts requiring exogenous insulin for maintenance of glycemic control, were started on sitagliptin 50 mg BID for a week, then 100 mg BID thereafter. Subjects were followed with basal glucose, C-peptide &HbA1c. Mixed meal

stimulation (MMTT) with &without sitagliptin was performed on 3 subjects. Acetaminophen was administered during MMTT to assess gastric emptying. Severity of gastroparesis symptoms was assessed by gastroparesis cardinal symptom index (GCSI) questionnaire (total score range from 0–5) with sitagliptin &exenatide. Adverse events were monitored. Results were compared to those who were previously on exenatide (2/4 subjects).
Results: Subjects were 1815 ± 700 (range 910–2581) days post-transplant at onset of sitagliptin and are 112 ± 29 (range 73–135) days post-treatment Prior to therapy (mean 3 months), basal C-peptide level was 0.69 ± 0.11 ng/ml, fasting plasma glucose 118 ± 8.8 mg/dl, C-peptide/glucose ratio (CPGR) 0.0.62 ± 0.1, HbA1c 7.1 ± 0.2% &insulin requirement 15 ± 2.5 U/day. At 2 months post treatment, HbA1c, plasma glucose &insulin requirements were lower (NS) (6.7 ± 0.2%; 14.25 ± 2.6 U/day; 121 ± 6.5 mg/dl respectively) and CPGR higher (0.83 ± 0.17). MMTT with sitagliptin 1 h prior to stimulation demonstrated no change in CPGR, post prandial glucose was not suppressed and there was no effect on glucagon secretion. In all 3 subjects there was delay in peak acetaminophen levels with sitagliptin (time to peak 160 ± 20 with, 90 ± 17.3 mins without sitagliptin $p < 0.05$). In all subjects the GCSI score was 0 with sitagliptin compared to 2 and 1.6 in those with exenatide. There were no adverse effects noted with sitagliptin.
Conclusion: In this small sample and short term follow up group of islet transplant recipients the effect of sitagliptin seems to be minimal. An unexpected delay in gastric emptying was observed. The metabolic effects were considerably less than those seen with exenatide but the absence of side effects warrant its continued use to look for benefits in the longer term.

IPITA-O-6.16

Post-transplant chronic anemia in islet transplant recipients is associated with reduced islet graft function and survival

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Background: In clinical islet transplantation, possible causes of progressive islet graft dysfunction and loss include: poor revascularization, chronic hypoxia, immunosuppression (IS) toxicity, gluco-lipo-toxicity, premature apoptosis and lack of regeneration. Chronic anemia negatively influences graft function and longevity and recipient survival in kidney and other transplant settings, with increased cardiovascular mortality. Contributing factors are: IS related bone marrow suppression and erythropoietin resistance; iron deficiency, from poor nutrition and frequent blood draws; and declining renal function, with lower erythropoietin synthesis.
Objectives: To evaluate the impact of chronic anemia in islet transplant recipients on islet graft function and survival.
Materials and Methods: Forty-four patients with type 1 Diabetes (19M 25F), who received an islet transplant from 2000–2007, were evaluated. Iron supplementation was given according to laboratory findings, with human recombinant erythropoietin given if Hb below 10 g/dl.
Results: Prior to transplant, Hb was 14.3 ± 1.2 mg/dl in men and 12.9 ± 0.9 mg/dl in women, (1M and 1F anemic), with iron 82.7 ± 23.7 μg/dl, ferritin 77 ± 50.8 ng/ml, MCV 87.8 ± 5.6 fl, reticulocytes 1.08 ± 0.3%, and total iron binding capacity (TIBC) 295.6 ± 44.1 μg/dl. Follow-up was 43.6 ± 27.9 months. After transplant, Hb was 12.5 ± 1.2 mg/dl in men and 11.5 ± 0.9 mg/dl in women, with iron 60.4 ± 17.3 μg/dl, ferritin 58.1 ± 41.1 ng/ml, MCV 83. ± 5.3 fl, reticulocytes 1.39 ± 0.6%, and TIBC 302.6 ± 54.4 μg/dl. Kaplan–Meier analysis of graft dysfunction vs anemia (dysfunction n = 41, insulin-free n = 3) and failure vs anemia (C-peptide neg n = 23, pos n = 21) did not show any difference between anemic (n = 31) and non-anemic recipients, classified using standard diagnostic criteria (Hb < 13 g/dl for men, < 12 g/dl for women). When the study-group median Hb values (Hb < 12 g/dl for men, < 11 g/dl for women) were used as diagnostic criteria, anemic recipients (n = 13) had significantly poorer outcomes than non-anemic ($p < 0.05$).

IPITA Parallel Session 7
Pancreas Procurement/Islet Isolation 2

IPITA-O-7.1

Pancreas persufflation for 6 h and 24 h improves viable islet yields compared with the two-layer method

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Background: Islet transplantation is emerging as a promising treatment for patients with type 1 diabetes. It is important to maximize viable islet yield for each organ due to scarcity of suitable human donor pancreata, high cost, and the high dose of islets required for insulin independence. Due to difficulties associated with organ transport following procurement, many organs arrive after 8 h of cold preservation resulting in lower islet yields. The efficacy of the two-layer method (TLM) for pancreas preservation has recently come under question. It is thought that oxygen does not reach the core of larger organs (e.g. pig, human) and that this is detrimental to islet health. Oxygen persufflation (PSF) offers an alternative way of adequately supplying this tissue with oxygen during preservation.
Methods: Pancreata were procured by *en bloc* viserectomy from non-heart beating Landrace donors. During procurement a 5 l vascular flush was performed split between the celiac trunk (CT) and superior mesenteric artery (SMA). Pancreata were divided into 3 lobes: the duodenal lobe used for immediate isolation (fresh); the connecting lobe placed on TLM for 6 or 24 h and then isolated; and the splenic lobe persufflated via the CT and SMA with 20 cc/min of 40% O_2 (g) each for 6 or 24 h and then isolated. Following isolation, islets were cultured and assessed by islet counts, morphology, DNA in culture, oxygen consumption rate normalized to DNA (OCR), and the diabetic nude mouse bioassay.
Results: PSF lobes yielded more islets per gram of digested tissue at both time points when compared to fresh or TLM. PSF islets had a consistently better morphology score than TLM. Following 2 days of culture a greater fraction of PSF islets were recovered relative to the day of isolation compared to fresh or TLM. Additionally, PSF islets had a higher viability as determined by OCR than TLM stored islets ($p < 0.05$). PSF islets were found to have a high diabetes reversal rate in diabetic nude mice when compared with historical data from fresh islets.
Conclusion: PSF shows promise for improving viable islet yields when compared with TLM. PSF also shows promise for extending the duration of pancreas preservation without significant reductions of viable islet yield. This may allow for the use of marginal donors thus expanding the donor pool suitable for transplantation.

IPITA-O-7.2

Pancreas oxygen persufflation increases ATP levels as shown by NMR

William E. Scott III[1], Joana Ferrer-Fabrega[1], Takayuki Anazawa[1], Bradley P. Weegman[1], Sam Stein[1], Shuichiro Matsumoto[1], Jay Stone[1], Appakalai N. Balamurugan[1], Bruce E. Hammer[2], Efstathios S. Avgoustiniatos[1], Kristen S. Maynard[1], Simon Stone[3], Linda Tempelman[3], David E. R. Sutherland[1], Bernhard J. Hering[1], Klearchos K. Papas[1]*
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Background: Islet transplantation is a promising treatment for type 1 diabetic patients. It is important to maximize viable islet yield due to a

shortage of suitable human pancreata, high cost and the high dose of islets required for long-term diabetes reversal. The efficacy of the two-layer method (TLM) for pancreas preservation is under scrutiny, as it is thought that diffusion limitations may prevent adequate oxygenation of larger pancreata (e.g. pig, human) and that this is detrimental to islet health. Lower islet yields have been reported for organs exposed to greater than 8 h of cold preservation by the TLM. Oxygen persufflation (PSF) offers an alternative way of supplying tissue with oxygen during preservation.
Methods: Human pancreata were procured by a combined pancreas–liver procurement from brain-dead donors. Pig pancreata were procured by *en bloc* viscerectomy from heparinized non-heart beating Landrace donors as detailed by Ferrer et al (Transplantation, v86, p1503, 2008). Pancreata were divided; the duodenal and connecting lobes were placed on TLM and the splenic lobe PSF. PSF for all organs was done by pumping 20 cc/min of 40% humidified O_2 (g) to the superior mesenteric artery and either the splenic artery (human) or celiac trunk (pig) utilizing an electrochemical oxygen concentrator (Giner Inc). Following procurement organs were transported to a 1.5T magnet for 31P-NMR spectroscopy to investigate their metabolic profile by measuring the ratio of ATP to inorganic phosphate (ATP:Pi) and by assessing PSF homogeneity by MRI (negative contrast generated by the presence of gas in the vasculature).
Results: PSF human organs exhibited prolonged elevation of ATP:Pi. When PSF was stopped ATP:Pi decreased until ATP levels were below the limit of detection. When PSF was resumed, ATP:Pi was partially restored. PSF pig organ lobes showed elevated ATP:Pi compared with paired TLM lobes. MRI showed that pancreatic tissue was homogeneously filled with gas with no gas observed in the duodenal lumen.
Conclusion: The methods developed for human and pig pancreas PSF homogenously delivers oxygen throughout the organ causing elevation of ATP. The ability to elevate ATP levels during preservation may improve current isolation outcomes and may allow the use of marginal donors expanding the donor pool. This coupled with improved islet yields seen with PSF may enable more successful islet transplantations.

IPITA-O-7.3

A new oxygen carrier improves human islet isolation outcome after prolonged cold storage

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Background: Pancreas oxygenation during cold storage has been established in islet isolation and transplantation to prevent ischemically induced tissue damage utilizing perfluorodecalin (PFD) as hyperoxygen carrier. However, studies in humans and pigs provided conflicting results about the efficiency of PFD for pancreas oxygenation. The aim of the present study was to compare PFD with a newly developed oxygen carrier composed of perfluorohexyloctane and polydimethylsiloxane 5 (F6H8S5) for long-term storage of human pancreata.
Methods: After 24 h of storage in preoxygenated PFD (n = 12) or F6H8S5 (n = 10) pancreata were processed utilizing Liberase HI for pancreas dissociation and a ficoll gradient for islet purification. Islet quality assessment was performed measuring glucose-stimulated insulin release, viability, islet ATP content and posttransplant function in diabetic nude mice.
Results: Compared to PFD F6H8S5 increased significantly the intrapancreatic pO2 (4.7 ± 1.9 vs 0.7 ± 0.4 mmHg, $p < 0.05$). This finding was associated with an increase of islet yield (3970 ± 220 vs 2440 ± 230 IE/g, $p < 0.001$), purity (60 ± 3 vs 44 ± 4%, $p < 0.01$) and recovery (76 ± 4 vs 49 ± 3%, $p < 0.001$) after culture, glucose stimulation index (3.8 ± 0.7 vs 2.3 ± 0.4, $p < 0.05$), viability (79 ± 2 vs 55 ± 4, $p < 0.001$) and islet ATP content (735 ± 232 vs 228 ± 113 pg/ng DNA, $p < 0.05$). Transplantation into diabetic nude mice resulted in a significantly enhanced cure rate after storage in F6H8S5 (67%) compared to PFD (14%, $p < 0.05$).
Conclusions: The present findings indicate clearly that F6H8S5 improves isolation outcome after prolonged ischemia compared to PFD. This observation seems to be related to the significant lipophilicity and almost pancreas-specific gravity of F6H8S5. Moreover, these characteristics facilitate pancreas shipment without using custom-made transport vessels as required for PFD.

IPITA-O-7.4

Islet isolation from juvenile pig pancreas after 24 h hypothermic machine perfusion preservation: effect of preservation solution and warm ischemia

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Objectives: Perfusion preservation technology has recently been reported to improve the recovery of islets after 24 h hypothermic machine perfusion (HMP) of porcine pancreas (Px) prior to islet isolation. This technology is now applied to juvenile pig Px, which is generally considered to be the species of choice for xenogeneic islet transplantation (Tx).
Methods: Pancreata in young (< 6 m) Domestic Yorkshire pigs (25–30 kg) were surgically removed, either before or after 30 min warm ischemia time (WIT), and the superior mesenteric artery and celiac trunk were cannulated. Px were assigned to one of 6 preservation groups: Fresh controls: cold ischemia < 1 h [G1, n = 7], Static Cold Storage: flushed &stored in UW-Viaspan at 2–4°C for 24 h with no prior WIT [G2, n = 9], HMP-perfused on a clinical LifePort machine at 4–6°C and low pressure (10 mmHg) for 24 h with either KPS1 solution (G3, n = 7) or Unisol-UHK (G4, n = 7). Additional treatment groups to evaluate the effects of prior WIT examined islet isolation after 30 min WIT *in situ* without [G5, n = 6], or with subsequent 24 h HMP with KPS1 (G6, n = 7). Islet isolation was then accomplished by ductal distension with liberase enzyme, normothermic digestion and density gradient purification. Standard assessment criteria were used including islet quantification, function using the glucose stimulated insulin secretion assay, and histology.
Results: Perfusion induced glandular edema was G3 = 138 ± 19%; G4 = 160 ± 16 and G6 = 127 ± 22%. Islet yields (IEQ/g) were: G1 = 1425 ± 610; G2 = 1002 ± 262; G3 = 2242 ± 449 ($p < 0.05$ vs G2); G4 = 1901 ± 420 ($p < 0.05$ vs G2); G5 = 1756 ± 329 and G6 = 1396 ± 243. Islet stimulation indices were equivalent between the groups and similar to controls (G1). A consistently more uniform digestion of the perfused organs was observed, with greater separation of the tissue and less entrapped islets, higher islet yield and purity.
Conclusion: HMP (24 h) using 2 alternative perfusates is well tolerated, even after 30 min WIT, leading to moderate edema but no loss of function of the harvested islets. The edema appears to aid digestion producing a greater yield and purity of islets compared with Px subjected to 24 h of static cold storage.

IPITA-O-7.5

Comparative impact on islet isolation and transplantation outcome of the new preservation solution IGL-1 vs UW and Celsior

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Purpose: Institut Georges Lopez (IGL-1) is a new preservation solution similar to University of Wisconsin (UW) with reversed Na/K contents. In this study, we assessed the impact of IGL-1, UW and Celsior (CS) solutions on islet isolation and transplantation outcome.
Methods: We retrospectively analyzed 302 islet isolations performed between January 2002 and September 2008. Pancreas were flushed and transported with IGL-1 (n = 64), UW (n = 181) or CS (n = 57). Isolation outcomes were determined by islet yields, success rates (> 250,000 IEQ) and transplantation rates. Beta cell function was assessed *in vitro* by stimulation indices in static incubation assays. Transplanted patients were divided into three groups depending on preservation solution of donor pancreas and *in vivo* function was assessed 1 month after first injection by the newly developed secretory units of islets in transplantation (SUIT) index and the C-peptide/glucose ratio.

Results: IGL-1, UW and CS groups were similar according to donor age, body mass index and pancreas weight. Final islet yields were 248,500 ± 6,000, 252,800 ± 9,300 and 249,300 ± 18,200 IEQ for IGL-1, UW and CS groups respectively. Success rates were 42, 49 and 46% in the IGL-1, UW and CS group, respectively. Altogether, 28 (44%) preparations in the IGL-1 group, 80 (44%) preparations in the UW group and 27 (47%) preparations in the CS group were suitable for transplantation. Endocrine secretory function of transplanted islet preparations, assessed by stimulation index, was slightly, but not significantly lower in the IGL-1 group compared to UW and CS groups (1.77 ± 0.81 vs 2.4 ± 1.89 and 2.23 ± 1.36). SUIT indices and C-peptide/glucose ratios at 1 month after first injection were slightly but not significantly higher in the IGL-1 compared to UW and CS groups (38 ± 5.5 vs 32.7 ± 5.2 and 29.2 ± 6.3 and 1.1 ± 0.15 vs 0.94 ± 0.13 and 0.89 ± 0.19, respectively).
Conclusion: Our study shows that IGL-1 is equivalent to UW or CS solutions for pancreas perfusion and cold storage before islet isolation and transplantation.

IPITA-O-7.6

Characterisation of collagenase class I isoforms present in commercially available collagenases used for human islet isolation

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Background: Collagenase enzyme blends and their compositions are regarded as critical impact factors during enzymatic digestion of pancreatic tissue to release islets. Enzyme structure and substrate specificity still require further elucidation to improve understanding of tissue digestion to benefit standardisation of human islet isolation procedure. Several studies identified collagenase class I and II to be essential components of commercially available collagenases. Moreover collagenase class I (CCI) consists of different isoforms including truncated isoforms which are discussed to have a negative impact on islet isolation. The objective of this study was to characterise CCI isoforms on the basis of protein sequence and proteolytic activities.
Methods: Collagenase class I isoforms of 115 kDa (CCI-115) and 100 kDa (CCI-100) were chromatographically purified from crude collagenase. CCI isoforms were characterised by High-performance liquid chromatography, Capillary Electrophoresis and SDS-PAGE. Proteins were digested and analysed by Mass Spectrometry (LC-ESI MS/MS) for sequence analysis. Protein function was determined by enzyme activity assays reflecting substrate specificities.
Results: No differences in proteolytic activity towards synthetic PZ peptide, human collagen and gelatine were observed comparing CCI-115 and CCI-100. The protein sequences of CCI-115 and CCI-100 correspond to apparent protein sizes determined by SDS–PAGE. The sequence of CCI-100 is identical to CCI-115 despite the C-terminus lacking one of two for CCI-115 previously published predicted collagen binding domains.
Conclusions: Even though collagenase isoforms CCI-115 and CCI-100 differ in protein sequence, enzymatic assay results as well as *in vivo* isolation data showed no significant difference in activity (*in vivo* data: Brandhorst et al., IPITA 2009 abstract #395: The effect of truncated collagenase class I isoforms on Human Islet Isolation outcome). Therefore we conclude that presence of truncated CCI-100 in commercial collagenase preparations has no negative impact on islet isolation outcome.

IPITA-O-7.7

Islet isolation from human pancreas with extended cold ischemia time (CIT)

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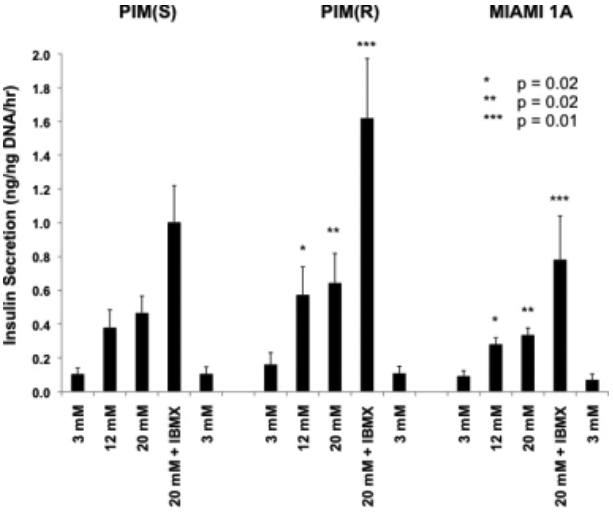
Backgound: General consensus among human islet transplant centers is that cold ischemia time (CIT) of >8 h results in reduced yields and quality. We have optimized our isolation process and enzymes used for such extended CIT pancreases.
Methods: We processed 16 extended CIT pancreases (13.2 ± 0.7 h). Donors averaged 50.8 ± 2.6 (SEM) years old with BMI of 28.6 ± 1.5. Glands were shipped in cold organ preservation solution without oxygenated perfluoro carbon. Isolations were performed under a protocol specifically optimized for digestion with the new cGMP collagenase from Roche. Purification used continuous Euroficol/UW gradients. Islets were cultured in two types of Prodo cGMP islet culture media and/or in CMRL-based media (Univ. of Miami). Glucose stimulated insulin secretion assays were performed after 8–16 days in culture.



Results: Pre-purification, post-purification and post-isolation yields are shown in Table 1. Our process liberated an average of 4278 IEQ/g of pancreas (97 ± 5 g). Purification loss averaged 13.5%. Most islets were recovered in the purest fraction (purity 79.7 ± 1.9%). Culture loss in our enhanced culture media was only 11.7%. After 2–3 days in culture viability was 92 ± 1%. Islets exhibited excellent compactness and dithizone staining. Glucose stimulated insulin secretion assays performed after 8–16 days in our enhanced islet culture media resulted in a stimulation index of 6.8 ± 1.7 (G50 to G350). This media was superior to CMRL-based media and resulted in higher glucose stimulated insulin secretion (see Figure 1). In addition, both Prodo media had better purity, compactness, dithizone staining intensity and less chaining as compared to the CMRL-based media.
Conclusions: We conclude that our human islet isolation process permits the recovery of large numbers of high quality human islets from extended CIT pancreas and that our cGMP islet culture media is superior to the current standard of CMRL-based media.

Table 1

Isolation yields from Extended CIT pancreases (n=16)

	Yield (KIEQ)	Loss
Pre–purification	415 ± 41	
Post–purification	359 ± 29	13.5%
Post–culture	317 ± 27	11.7%

IPITA-O-7.8

Deliniating the genetic architecture of human islets prepared for clinical transplantation

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Background: We hypothesize that poor islet-graft function after transplantation might be determined in part by variability in islet intrinsic factors including genes that control islet differentiation and function.
Methods: Islet preparations isolated for clinical islet transplantation were analysed for expression of a set of candidate genes (hypothesis-driven

approach) by RTqPCR; or were processed for transcript profiling utilising the HG-U133-Plus2 array (discovery approach).
Results: Expression analysis was conducted on following set of candidate genes; islet hormones insulin, somatostatin, glucagon and IAPP; genes required for GSIS GLUT2 and GCK; insulin signaling, IR, IRS1/2 and AKT; genes encoding beta cell transcription factors, PDX-1, PAX6, NKX2.2, NKX6.1, NEUROD1/BETA2, ARNT, MAFA and ISL1. All studied genes were expressed in all islet preparations. Further, the expression level (dCT) for each gene between individual islet isolates was remarkably uniform (e.g. INS, 4 ± 0.99; INSR, 7 ± 0.7; IRS1, 8.86 ± 0.7; ARNT 7.1 ± 0.66; Log2, n = 6). Notably expression of genes encoding the glucose transporter GLUT2 (6.2 ± 2 Log2) and the transcription factor NEUROD1 (6.8 ± 2.2 Log2) exhibited the most variability between isolates. For the discovery approach, microarray expression profiling with analysis of median average deviation (MAD) in a further 9 islet isolates was conducted. This identified ˜150 genes (˜0.4% of all genes) with a variable expression pattern (e.g. MAD > 1.5). Significantly, with the exceptions again of GLUT2 (MAD = 1.91) and NEUROD1 (MAD = 1.6), most of these identified variable genes have unknown roles in islet biology. Gene Set Enrichment Analysis (GSEA) revealed the variable genes exhibited a strong bias to gene expression patterns indicative of hypoxic responses; activation of NF-kB and inflammatory-responses; and tissue remodelling.
Conclusions: To our knowledge we have now generated the largest data-base profiling the expressed transcriptome of human islets. Transcript profiling revealed human islets are responding to isolation with a complex pattern of genetic remodeling. This emerging molecular architecture may impact latter islet function post-engraftment. Indeed, human islets prepared for clinical transplantation have variable expression of key genes critical for GSIS and beta cell differentiation.

IPITA-O-7.9

Collagenase does not persist in human islets following islet isolation
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Background: Optimal islet isolation requires the delivery of collagenase to the pancreatic islet-exocrine interface. However, we have previously demonstrated the presence of collagenase within human islets immediately following intraductal collagenase administration. The aims of this study were to determine if collagenase becomes internalized into islet cells during the isolation procedure, and whether collagenase remains within the islet post-isolation.
Methods: With appropriate consent and ethical approval, human pancreases (n = 6) were retrieved from multiorgan donors (age range 34–66 years) and islets isolated by standard methods. Islets were fixed in 4% paraformaldehyde at various stages throughout the isolation process: during digest collection, following UW incubation, immediately post-isolation, and then after 24 h of culture at 37°C. Islets were embedded in agar and cryo-sectioned. 8 μm sections were immunolabelled for collagenase using a polyclonal anti-collagenase antibody and immuno-fluorescence.
Results: Of the 6 islet preparations analysed, collagenase labelling was detected in only 1 sample from the first pool of islets during the collection phase. No collagenase-specific labelling was seen in islets sampled at any of the other time-points during and after the islet isolation process. Islets stained with a control, species-matched antibody showed no specific fluorescence. As a positive control, isolated islets were incubated with collagenase for 1 h at 37°C and then processed as above. Collagenase labelling was detected, indicating that islets are able to take up or bind collagenase.
Conclusion: We have previously shown that intraductally administered collagenase is able to enter human islets. This study suggests that collagenase is washed out of the islets during the collection phase of the isolation process and thus does not remain in islets after isolation. This helps to resolve some of the safety concerns that collagenase may still be present within islet grafts. However, the presence of collagenase within islets immediately following intraductal administration may cause damage to islets during the digestion process and therefore have a negative impact on islet integrity and islet graft survival.

IPITA-O-7.10

Assessment of human islet isolation with four different collagenases
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Background: The isolation of islets from the human pancreas critically depends on the efficiency of the digestive enzymes. Liberase HITM had been used as a standard until Bovine Spongiform Encephalopathy issue occurred, however, we now must use other collagenases for clinical islet transplantation. We have recently used several purified collagenases without the specific biological risk material in their process for human islet isolation. In this study, we evaluate the results of the islet isolation using four different collagenases.
Methods: Seventeen pancreata from brain-dead donors between 2006 and 2009 were used. Each pancreas was digested with the following collagenases; Liberase HI (HI) (Roche, n = 9), Liberase MTF C/T (MTF) (Roche, n = 4), Collagenase NB1 Premium Grade (NB1) (Serva, n = 7), CI*zyme*™ Collagenase HA (CI) (VitaCyte, n = 4). Islet isolations were conducted based on the Edmonton protocol for HI. Our modified islet isolation method (Matsumoto S et al. Baylor University Medical Center Proc 2007; 20, 357–362.) was used for the new three enzymes (MTF, NB1 and CI). The significance of differences among 4 groups was determined by the multiple *t*-test with Bonferroni correction following ANOVA.
Results: There were no significant differences in donor age, body mass index, pancreas size and cold ischemic time among the four groups. The isolation variables are shown in Table 1 and Table 2. The phase I time in NB1 group was significantly shorter than CI group (*p = 0.0014). The pre-purification IEQ/g in HI group was significantly lower than the others (**p = 0.0003 vs MTF, 0.0007 vs NB1 and 0.0009 vs CI, respectively). The post-purification IEQ/g in MTF group was significantly higher than HI group (***p = 0.006). And the viability in NB1 group was significantly higher than HI group (§p = 0.003). Percent embedded islet was less than half in HI group compared to the other groups.
Conclusion: These findings suggest that new three enzymes (MTF, NB1 and CI) could provide higher islet yield than HI with our modified protocol.

Table 1

	Phase I (min)	Undigested tissue (g)	% embedded islet
HI	16±2	19±3	18±7
MTF	19±2	6±1	55±7
NB1	14±1*	14±4	44±13
CI	22±1	10±5	49±14

Table 2

	Pre–purification IEQ/g	Post–purification IEQ/g	Viability (%)
HI	4443±603	2688±517	93.0±1.3
MTF	11112±1153**	6829±1235***	94.9±1.4
NB1	7962±388**	6076±989	98.8±0.4§
CI	8888±115**	5777±1272	98.3±0.5

IPITA-O-7.11

Retrospective analysis of human islet isolation using roche (Liberase HI and MTF), Serva NB1, and VitaCyte
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Successful Islet isolation depends largely on the donor characteristic and enzyme type used for pancreas digestion. Collagenase together with

thermolysin or neutral protease is a critical step to achieving this goal The purpose of this study is to retrospectively analyze islet isolation data from pancreata digested with Liberase HI (containing animal product), Liberase MTF C/T (mammalian free product), Serva NB 1, and VitaCyte.

Islets were isolated from cadaveric human pancreata (n = 145) using four different enzymes: Liberase HI (n = 70), Liberase MTF C/T (n = 22), Serva NB1 (n = 45), and VitaCyte (n = 8). Donor characteristics, IEQ recovery, islet/b cell viability, cellular composition, and insulin response (SI) were compared with the type of enzyme used.

There was no statistical difference in donor age, BMI, CIT, and pancreas weight between the four enzyme groups (p = ns). Islet counts (pre and post purification) were similar among the four enzymes tested, however, the Liberase HI was slightly better than Serva NB 1 (p = 0.0398). Higher SI was seen in islets from the Liberase MTF C/T group than Liberase HI (p = 0.000252). Liberase HI (n = 62) showed lower SI than Serva (n = 39) (p = 0.0515), or VitaCyte (p = 0.051). Lower annexin-V apoptotic cells were seen in the islets prepared with Liberase MTF C/T (n = 4) compared to Liberase HI (n = 46) (p = 0.0131). Successful islet Isolations can be achieved with the four enzymes tested. Liberase MTF C/T seems to represents a more stable enzyme and may produce consistent and reproducible islet quality.

IPITA-O-7.12

Toward standardizing islet cell processing protocol the impact of operator variables on human islet isolation

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Background: Recent trials have shown that the center's experience in islet isolation plays a critical role in successful islet transplantation. Major efforts are currently being focused toward the standardization of islet cell processing among centers. The Aim was to evaluate the effects of individual cGMP specialist's expertise could affect isolation outcomes, even within the same center.
Methods: One-hundred eight islet isolations processed for clinical trials using the same protocol were analyzed. Isolations performed during the same periods were divided into four groups (A, B, C, D, n = 31, 27, 29, 21) based on the leader of each team, who was in charge of all decisions made during the isolation.

The leaders in Group A, B and C were well experienced and the one in Group D was much less experienced.
Results: There were no significant differences among the four groups in donor age, BMI, cold ischemia time, pancreas weight, digestion time and enzyme lots. Total IEQ was significantly different between Group D and the rest (Group A/B/C/D = 321, 563/425, 761/308, 765/216, 261IEQ, $p < 0.01$). The volume of collection medium (VCM), cleaning time and isolation time were significantly different among the groups (Group A/B/C/D; 12.0/11.4/10.2/9.8 L, 47/35/51/35 min, 449/319/374/304 min, $p < 0.01$). However, only VCM is a possible factor explaining the difference in Total IEQ. Groups B and D showed the highest difference in total IEQ, pre-purification IEQ and the size of islet (IEQ/islet particle number), which were significantly lower in Group D (Group B/D = 473098/274392 IEQ, $p < 0.001$; 1.041/0.799, $p < 0.01$). Undigested tissue weight was also lower (Group B/D = 30/22 g, p = 0.069), although not significantly different.
Conclusions: The results suggested that lower total IEQ, islet size, undigested tissue weight and VCM could have been associated with a delayed switch to the dilution phase, even though digestion times were comparable among the groups, indicating that over-digestion may have played an important role in Group D with the lowest IEQ. No meaningful significant differences regarding isolation variables among Group A, B and C indicated that protocol standardization and proper training allowed creating similar isolation teams of high quality. Significant differences regarding IEQ in Group D also suggested experience as well as protocol is still important.

IPITA-O-7.13

Improved method of human islet isolation for young donors

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Background: Islet transplantation is a promising treatment for type 1 diabetes. Islet transplantation using young donors is shown more effective than older donors, however, islet isolation from young donor is notoriously difficult. This may relate to the islet ontogeny and collagen composition in the young pancreas. Therefore we examined whether the collagenase with high concentration could improve the separation of islets from exocrine tissues and result in the high islet yield.
Methods: Six pancreata from brain-dead donors of < 30 years old were used. Islet isolation was conducted based on the Edmonton protocol with our modifications. All pancreata were digested with 1 vial of Collagenase NB1 Premium Grade and 2 vials of Neutral Protease (Serva). In the standard group (n = 3), the pancreas was expanded by injecting 200 ml of cold collagenase solution (2.5 mg/ml) under a pressure controlled manner (60–80 mmHg) for 5 min, and then the pressure was raised up to 160–180 mmHg for another 5 min. In the new method (n = 3), the expansion was first conducted with approximately the same volume of the pancreas weight in grams of cold collagenase solution (about 100 ml, 5 mg/ml) using a syringe under a pressure control (60–80 mmHg) for 5 min followed by adding the perfusion solution up to 200 ml and performing the high pressure step in the same condition as the standard. The following procedure and evaluation were performed based on the Edmonton protocol.
Results: There was no significant difference in the average of used collagenase activity, donor age, pancreas weight, phase I time and undigested tissue volume. On the other hand, phase 2 time in the new group was significantly shorter than the standard (37 ± 1 vs 52 ± 4, $p < 0.05$) and the ratio of the embedded islets in the new group was significantly lower than the standard ($54 \pm 4\%$ vs $83 \pm 5\%$, $p < 0.05$). The post-purification islet equivalent per pancreas weight (IEQ/g) and the recovery rate in the new group were higher than the standard, although they didn't reach the statistical significance (IEQ/g; 6475 ± 1538 vs 3901 ± 637, recovery rate; $67 \pm 4\%$ vs $50 \pm 7\%$, respectively). There was no significantly difference in the post-purification purity, viability and final tissue volume.
Conclusion: Our simple modification with initial concentrated collagenase using a syringe may improve the separation of islets from the exocrine tissues and increase the islet yield.

IPITA-O-7.14

The standardization of human pancreatic donors for islets isolation: Relation between donor parameters and pancreas structure

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Background: The aim of this study was to establish objective criterias for a more efficient selection of human pancreatic donors.
Methods: Twenty-two human pancreases were analyzed. Donor parameters were recorded as: Age (ranged: 11–68 years old), BMI (21–38), Time of

hospitalization at ICU (1–11 days), Cardiac arrest (0–30 min)/Hypotension (0–45 min), Blood glucose (78–371 mg/dl), Amylase (8–758 IU/l)/Lipase (17–703 IU/l) levels, WIT (13–62 min), CIT (2–30 h). Pancreas were processed to study: (1) oedema, inflammation, necrosis (scored by two independant pathologists) and apoptosis (immunohistochemisty/histomorphology analysis) and (2) the pancreatic hormonal content for insulin/proinsulin/glucagon.

Results: Factor analysis demonstrated three independant factors to characterize the pancreas quality: (1) Factor 1 included Inflammatory/Necrosis/Apoptosis parameters; (2) Factor 2 included Insulin/Pro-Insulin/Glucagon pancreatic contents and (3) Factor 3 was the pancreatic Oedema. A significant correlation was found between Factor 1 and Lipase ($p = 0.0194$)/Amylase ($p = 0.0138$) sera levels. Factor 2 was significantly correlated to the donor age ($p = 0.098$) and strongly associated with Lipase level ($p = 0.0543$) and CIT ($p = 0.0528$). A donor age over 48 years demonstrated a significantly lower insulin content than donor below 30 years old (41,774 ± 25,863 ng/g vs 81,312 ± 3846 ng/g of pancreatic tissue, $p < 0.026$). The pancreatic oedema was significantly correlated to BMI ($p = 0.0294$) and a longstay at ICU ($p = 0.0040$).

Conclusions: We have selected 5 donor parameters statistically correlated to the pancreas intrinsic quality: (1) Exocrine tissue can be assessed by Lipase (< 120 IU/l)/Amylase (< 120 IU/l) sera levels (for inflammation/necrosis/apoptosis) and BMI (< 26)/ICU stay (< 4 days) (for oedema); (2) Endocrine stucture is directly influenced by the donor age (< 48 years old). All other investigated parameters as CIT, WIT, cardiac arrest, hypotension did not significantly affect the pancreas quality. Retrospectively, we have shown that among 65 human islets isolations, 38 successful islets preparations (> 350.000 IEQ, 64%/86% of purity/viability, respectively) were characterized by a minimum of 4 optimal donor parameters in 74% of cases. In contrast, 82% of failed isolations were characterized by < 3 optimal parameters.

IPITA-O-7.15

Autotransplantation of pure islets from a resected pancreatic remnant with ductal obstruction in chronic pancreatitis

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Pancreatic islet autotransplantation can prevent brittle diabetes in patients who undergo total or partial pancreatectomy for benign disease. We report a case where we obtained 100% pure islets without purification from a pancreas remnant with chronic pancreatitis after previous partial pancreatectomy. A 49-year-old woman with chronic pancreatitis underwent choledochoduodenostomy followed by a Whipple pancreaticoduodenectomy 5 years prior to referral to us. The pancreatic remnant duct became obstructed as shown by imaging, with severe abdominal pain resistant to opioid analgesics and two celiac block attempts. She was normoglycemic (hemoglobin A1C 4.8%). She underwent completion pancreatectomy. The resected pancreas had an obstructed pancreatic duct with distal dilatation. A cannula was inserted into the duct and approximately 50 ml of chilled ET-Kyoto solution was infused immediately . The pancreas was preserved using oxygenated two-layer method. The pancreatic duct was infused with collagenase; however, distention was poor, and we injected collagenase directly into the pancreatic parenchyma. During the pancreas digestion phase all tissue samples were 100% pure islets with no acinar tissue. 133,600 islet equivalents of 100% purity were isolated (4,948 IE/gram of pancreas tissue, 2,559 IE/kg body weight) and infused into the portal venous system via the inferior mesenteric vein, at the time of surgery. The hospital course was uneventful. The patient achieved good glycemic control, is insulin free more than 4 months after the procedure, with normal serum insulin levels. Histology of pancreatic biopsies showed only islet tissue and fat, with no exocrine tissue. This is the first confirmation that obstruction of the pancreatic ducts result in atrophy of the exocrine pancreatic tissue with preservation of the pancreatic islets in human subjects. Islet autotransplantation should be considered in patients who are planned for completion pancreatectomy after a previous partial pancreas resection, since insulin independence can be achieved in this clinical situation. Total pancreatic duct obstruction should not be considered a contraindication for islet autotransplantation in patients who are normoglycemic prior to pancreatectomy.

IPITA-O-7.16

One-layer method with novel compound perfluorohexyloctane increases levels of ATP relative to perfluorodecalin as shown by 31P-NMR spectroscopy

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Background: Islet transplantation is emerging as a promising treatment for patients with type 1 diabetes. It is important to maximize viable islet yield for each organ due to competition for suitable organs, high cost, and the high dose of islets required for long term diabetes reversal.
The efficacy of the two-layer method (TLM) for pancreas preservation has recently been questioned. The one-layer method (OLM) for preservation by submerging the entire pancreas in perfluorocarbon (PFC) has been suggested as an alternate method for oxygen delivery. The novel compound perfluorohexyloctane (F6H8) has recently emerged as a promising alternative to perfluorodecalin (PFD), the traditional PFC used for either OLM or TLM.

Methods: Pancreata were procured by *en bloc* viserectomy from non-heart beating Landrace donors. Pancreata were divided into 3 lobes: the duodenal lobe was placed in PFD OLM with no ductal preservation; the connecting lobe was distended with 2 ml HTK solution per gram of tissue and placed in PFD-OLM; the splenic lobe was distended with 2 ml HTK solution per gram of tissue and placed in F6H8-OLM. Following procurement organs were transported to a 1.5T magnet for 31P-NMR spectroscopy to investigate the metabolic status of the organ as represented by the ratio of ATP to inorganic phosphate (ATP:Pi).

Results: Lobes preserved in F6H8-OLM exhibited consistently higher ATP:Pi when compared with lobes preserved with PFD-OLM at similar time points. Little difference in ATP:Pi was observed for lobes exposed to PFD-OLM with or without ductal infusion of HTK solution.

Conclusion: Novel compound F6H8 better oxygenates the pancreas when used in combination with OLM when compared with PFD, the traditional compound used for either OLM or the two-layer method. Ductal infusion of HTK prior to preservation does not seem to improve the metabolic status of the pancreas during preservation but may provide other benefits which may help improve isolation outcomes.

IPITA Parallel Session 8
Stem Cells, Tolerance and Immunobiology

IPITA-O-8.1

NeuroD1 directs differentiation of cytokeratin 19-positive human pancreatic non-endocrine cells into insulin producing cells

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Background: Although the success rate of islet-cell transplantation has increased, islet shortage and graft loss remain major limitations. Therefore, promoting the formation of new beta cells is a potential therapy. It has been reported that the nonendocrine fraction, which remains after islet isolation, consists of epithelial and mesenchymal cells that can be forced to differentiate to beta cells. However, the optimal method to accomplish this goal has not been established. In this study, we introduced human NeuroD1, a transcriptional factor which plays an important role during beta cell generation, into human nonendocrine pancreatic epithelial cells (NEPEC) and promoted insulin producing cells *in vitro*.

Methods: The human unpurified islet fraction and COBE bag fraction were obtained and cultured in suspension in RPMI with FBS for 2–3 days. Then,

to deplete fibroblasts and original beta cells, they were cultured with 40 μg/ml G418 for 4 days. Then we plated these cells termed NEPEC on MatrigelTM coated dishes. NEPEC spread into a cell monolayer on the matrix within 7 days and all these cells were cytokeratin 19 (CK19) positive. Seven days after plating, a plasmid encoding human NeuroD1 gene under human CK19 promoter was transfected with lipofectamine2000 TM 3 times every other day (termed NEPEC + ND). We characterized them by morphology, immunohistochemistry, RT-PCR and ELISA for insulin and C-peptide secretion in culture media.

Results: Seven days after starting the transfection of human NeuroD1, NEPEC + ND strongly expressed NeuroD1 and insulin mRNA. At the time, the rate of NeuroD1 positive NEPEC + ND were significantly higher than NEPEC (NEPEC + ND: 85.0 ± 3.1%; NEPEC: 5.8 ± 2.2%, respectively). And insulin positive NEPEC + ND were also significantly more than NEPEC (NEPEC + ND: 7.3 ± 1.1%; NEPEC: 0.8 ± 0.2%, respectively). Insulin level in culture media at day 7 after starting transfection in NEPEC + ND group was significantly higher than NEPEC group (NEPEC + ND: 932.4 ± 17.0 μIU/ml; NEPEC: 120.6 ± 2.2 μIU/ml, respectively). C-peptide level in NEPEC + ND group at the time was significantly higher than NEPEC (NEPEC + ND: 5270.5 ± 150.9 pmol/l; NEPEC: 662.8 ± 9.6 pmol/l, respectively).

Conclusion: These findings have demonstrated that human NeuroD1 under control of a CK19 promoter can induce the differentiation of CK19 positive NEPEC's into insulin producing cells.

IPITA-O-8.2

Mesenchymal cells appearing in pancreatic tissue culture are bone marrow-derived stem cells with the capacity to improve transplanted islet function

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Adherent fibroblast-like cells have been reported to appear in cultures of human endocrine or exocrine pancreatic tissue during attempts to differentiate human b cells from pancreatic precursors. A thorough characterization of these mesenchymal cells has not yet been carried out, and there are no conclusive data about their origin. We demonstrated that the human mesenchymal cells outgrowing from cultured human pancreatic endocrine or exocrine tissue are pancreatic mesenchymal stem cells (pMSC) that propagate from contaminating pMSC. The origin of pMSC is extrapancreatic both in human and in mouse and by using GFP + bone marrow transplantation in the mouse model, we were able to demonstrate that these cells derive from the CD45 + component of bone marrow. pMSC express negligible levels of islet-specific genes both in basal conditions and after serum deprivation or exogenous growth factor exposure, and might not represent optimal candidates for generation of physiologically competent β-cells. On the other hand, when cotransplanted with a minimal pancreatic islet mass, pMSC facilitate the restoration of normoglycemia by increasing neovascularization. These results suggest that pMSCs could exert an indirect role of 'helper' cells in tissue repair processes.

IPITA-O-8.3

Glucose-responsive insulin production in hepatocytes derived from human embryonic stem cells

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Due to a shortage of donor pancreas and the limited long-term success of islet transplants, alternatives for treating Type I diabetes (T1D) are needed. Gene therapy-based glucose regulated hepatic insulin production is a promising strategy to treat T1D. We have previously reported that hepatocytes engineered with an insulin gene construct containing a liver-specific albumin promoter and glucose inducible regulatory elements corrected fasting hyperglycemia, improved glucose tolerance, and stabilized body weight in streptozotocin (STZ) -induced diabetic rats. However, due to insulin insufficiency, postprandial hyperglycemia was not fully corrected and weight loss was not reversed.

To increase the insulin output we generated a new insulin gene construct (TA1) in adenovirus which contains an additional translational enhancer and the 3′-UTR from albumin. TA1 retained glucose responsiveness and caused 25 fold higher insulin production in rat hepatocytes. In STZ-diabetic rats, TA1 corrected fasting hyperglycemia during the first month and significantly reduced postprandial hyperglycemia. Although blood glucose levels increased over the 6 month study, the reduction in blood glucose levels of TA1 treated animals compared to diabetic control animals remained significant. During the first month, the rate of weight gain in TA1 treated rats was equal to that of normal rats. Thereafter, treated rats continued to gain weight for up to 6 months but at a reduced rate.

We tested TA1 in human hepatocytes to assess its suitability for treatment of T1D. Due to the variable quality and unpredictable availability of human hepatocytes, we used hepatocyte-like cells derived from hES cells to test TA1-driven insulin production. Our results were encouraging; 266 ± 48 and 61 ± 34 ng insulin/day/sqcm was produced in medium containing 27.5 mM and 3.5 mM glucose, respectively, which compare favorably with insulin produced in primary rat hepatocytes.

In summary, TA1 greatly improved glucose-dependent hepatic insulin secretion *in vivo* and functioned well in hES derived hepatocytes, which may help overcome current shortage of insulin producing cells to treat T1D.

IPITA-O-8.4

Induction of human pancreatic progenitor cells into insulin-producing cells by protein transduction technology

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Background: Islet transplantation is a promising possibility for the optimal treatment of type 1 diabetes. However, an abundant source of tissue that satisfies the demand for b-cells has yet to be found. One attractive approach for the generation of b-cells involves the expansion and differentiation of adult human pancreatic stem/progenitor cells, which are closely related to the b-cell lineage. We previously established a mouse pancreatic stem cell line without genetic manipulation. In this study, we used the techniques to identify and isolate human pancreatic stem/progenitor cells. We also tested whether protein transduction of PDX-1 and NeuroD into human pancreatic stem/progenitor cells induced insulin and pancreas-related gene expression

Method: Forty pancreata from brain-dead donors, which were procured from either Southwest Transplant Alliance (Dallas, TX) or LifeGift (Fort Worth, TX) between 2007 and 2009, were used in this study. Human islet isolation was conducted in the standard Ricordi technique with modifications introduced in the Edmonton protocol. The cells from a duct-rich population were cultured in 23 kinds of culture media, based on media for mouse pancreatic stem cells or for human embryonic stem cells. For inducing cell differentiation, the cells were cultured with exendin-4, nicotinamide, Keratinocyte growth factor (KGF), PDX-1 protein, and BETA2/NeuroD protein for 2 weeks.

Results: The cells in serum-free media formed "cobblestone" morphologies, similar to a mouse pancreatic stem cell line. On the other hand, the cells in serum-containing medium and the medium for human embryonic stem cells formed "fibroblast-like" morphologies. The cells divided actively until day 30, and the population doubling level (PDL) was 6–10. However, the cells stopped dividing after 30 days in any culture conditions. During the cultures, the N/C ratio (nucleus/cytoplasm ratio) decreased, suggesting that the cells entered senescence. Exendin-4 treatment and transduction of PDX-1 and NeuroD proteins by protein transduction technology into the cells induced insulin and pancreas-related gene expression.

Conclusion: Although the duplications of these cells were limited, this approach could provide a potential new source of insulin-producing cells for transplantation.

IPITA-O-8.5

The mechanics of a BAFF-expanded Foxp3+ treg; IL-10 is required for islet allograft tolerance but not treg expansion

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Background: We have previously shown that mice transgenic for the B cell activation factor from the TNF family (BAFF) harbored an increased frequency of FoxP3+ T regulatory cells (Tregs) in the periphery as compared to Wild Type mice that allow for immunosupression free allograft tolerance. We have now examined the mechanism of action by which BAFF-expanded Tregs maintain allograft tolerance.
Methods: IL-10$^{-/-}$BAFF-Transgenic (BAFF-Tg) and IL-10$^{-/-}$wild type (WT) radiation chimeras were generated. Treg frequencies and allograft (BALB/c H-2d $\rightarrow$ C57BL/6 H-2b) survival were determined.
Results: BAFF promotes the expansion (≥3-fold increase) of Foxp3+ Tregs. Phenotypically BAFF-Tg Tregs were CD62Llo CD103hi and ICAM-1hi, a phenotype consistent with an ability to home to inflammatory sites and prevent effector T cell responses. Further, ˜60% of BAFF-Tg mice permanently accepted an islet allograft for >100 days (n = 10). Elimination of CD25+ T cells restored normal allograft rejection (MST ˜20 days, n = 4), demonstrating that the increased number of Tregs were responsible for their altered allo-immunity. Signaling through BR3 on B cells promotes B cell survival via activation of NFkB2, however the abilty of BAFF to promote Treg expansion was not T cell intrinsic as Tregs did not express high levels of BR3 nor trigger NFkB2 p100-p52 processing. To determine the role of B cells we examined $\mu MT^{-/-}$ $\rightarrow$ BAFF-Tg bone marrow chimeras. This revealed that in the absence of B cells BAFF could not expand Tregs and allograft acceptance was lost. BAFF-Tg mice showed increased B cells with a regulatory phenotype e.g. CD1dhi B cells. Regulatory B cells have been shown to function by producing IL-10. To test this we generated IL-10$^{-/-}$BAFF-Transgenic (BAFF-Tg) and IL-10$^{-/-}$wild type (WT) radiation chimeras. We found that IL-10 was not required for the BAFF-dependent expansion of Tregs as IL-10$^{-/-}$BAFF-Tg chimeras harboured ˜2-fold increase of CD4+ Foxp3+ T cells compared to WT (n = 5). However despite the expanded Foxp3+ T cells, IL-10$^{-/-}$BAFF-Tg chimeras rejected their BALB/c allografts (MST˜19 days, n = 5), similar to IL-10$^{-/-}$WT chimeras and WT mice.
Conclusions: IL-10 is not required for the expansion or maintenance of Tregs in BAFF-Tg mice. However, BAFF-expanded Tregs require IL-10 to achieve allograft tolerance.

IPITA-O-8.6

PD-1/PD-1l pathway is required for the induction and maintenance of tolerance to neonatal porcine islet xenografts by combined anti-LFA-1 and anti-CD154 monoclonal antibodies

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Background: We previously demonstrated that short-term administrations of a combination of anti-LFA-1 and anti-CD154 monoclonal antibodies (mAbs) induce a dominant, species and tissue specific tolerance to neonatal porcine islet (NPI) xenografts that is mediated by T regulatory cells in B6 mice. The aim of this study was to examine whether the co-inhibitory PD-1/PD-1L interaction is required for the induction and maintenance of tolerance to NPI xenografts.
Methods: Streptozotocin-induced diabetic B6 mice were transplanted with 2000 NPI under the left kidney capsule. Recipient mice were treated with short-term administrations of a combination of anti-LFA-1 and anti-CD154 mAbs (tolerogenic regimen, group 1). The other groups of mice were treated with tolerogenic regimen and short-term administrations of anti-PD-1 mAb (clone J43, group 2), or treated with tolerogenic regimen plus long-term administrations of anti-PD-1 mAb produced by hybridoma clone J43 (group 3) or by hybridoma clone 4F10 (group 4) to determine the role of PD-1/PD-1L pathway in the induction of tolerance. Blood glucose levels of recipient mice were monitored for >100 days post-transplantation. To examine the role of PD-1/PD-1L pathway in the maintenance of tolerance to NPI xenografts, tolerant B6 mice were injected with long-term administrations of anti-PD-1 mAb (clone J43, group 5). Islet grafts and spleen cells were harvested for characterization using immunohistochemistry and flow cytometry, respectively.
Results: Mice in groups 1 and 2 maintained normoglycemia for >100 days post-transplantation, however, 50% or none of the mice in groups 3 and 4 achieved normoglycemia, respectively (n = 8 in each group). Mice in group 5 became diabetic at 27 days post-treatment with anti-PD-1 mAb. Flow cytometric analysis of the spleen cells from tolerant mice showed higher expression of CD25, PD-1 and foxp3 as well as co-expression of these molecules on the majority of CD4+ T cells compared to the spleen cells from naïve B6 mice.
Conclusion: Our data indicate that the PD-1/PD-1L pathway is required for both induction and maintenance of tolerance to NPI xenografts induced by combined anti-LFA-1 and anti-CD154 mAbs.

IPITA-O-8.7

Rapid generation of tolerogenic dendritic cells (TOL-DC) for immunomodulation in clinical islet transplantation

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Background: Immunological barriers have been identified as one of the major causes of loss of islet graft function. In small animal solid organ transplantation, manipulation of the recipient with donor-derived TOL-DC has been shown to improve graft survival however currently the *in vitro* generation of monocyte-derived DC requires 7 days culture. Thus, the aim of this study was to generate and characterise TOL-DC using a 'FAST-DC' protocol that generates DC within 48 h.
Methods: Monocytes were isolated from human blood and cultured for 24 h with IL-4 (500 U/ml) and GMCSF (1000 U/ml) in the presence or absence of IFN-γ (500 U/ml). DC were matured with TNF-α (10 ng/ml) and PGE2 (1 μM) for 24 h.
Results: Both TOL-DC and control DC (CTRL-DC) expressed the DC-specific marker DC-SIGN and displayed typical DC morphology after 48 h cytokine treatment. However FACS analysis showed that IFN-γ treatment inhibited the expression of DC maturation marker CD83 by 82% in TOL-DC compared to CTRL-DC. The expression of costimulation molecules CD80 and CD86 were down-regulated by 14% and 7% respectively, whilst increasing the expression of inhibitory molecule ILT4 by 12% compared to CTRL-DC. Importantly TOL-DC showed inhibited mRNA expression of NF-κB transcription factor relB by 73% (p = 0.006), IL-12 by 90% (p = 0.005) and inhibited T-cell proliferation by 50% (p = 0.01).

CTRL-DC were typical potent allostimulatory cells. Moreover, STAT-6 phosphorylation was inhibited in TOL-DC, which we hypothesise is contributing to the 73% inhibition of IRF4 gene expression seen in TOL-DC, suggesting that IFN-γ treatment was acting through the STAT-6 IRF4 pathway.
Conclusions: IFN-γ treatment of monocytes in the presence of IL-4 and GMCSF generated TOL-DC in 48 h, which displayed reduced capacity to activate T cells. The generation of donor-specific TOL-DC have the potential to be used for immunomodulation in clinical islet transplantation.

IPITA-O-8.8

***In vivo* tracking of "color-coded" effector, natural and induced regulatory T cells in allograft response**

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Regulatory T cells play a fundamental role in maintaining immune homeostasis. We report a fluorescence "color coded" *in vivo* imaging

method, in which effector T cells (Teff) are red, naturally occurring regulatory T cells (nTreg) are green and de novo induced regulatory T cells (iTreg) are yellow.

We present novel techniques to longitudinally track islet allograft graft infiltrating cells in live animals using endoscopic confocal microscopy and *in vivo* flow cytometry to analyze the circulating T cells. We demonstrate that de novo iTreg conversion occurs *in vivo* in a classical MHC mismatched allograft model. These cells can be identified within the allograft infiltrate and in circulating blood. Teff are significantly more abundant in the allograft of untreated hosts than in hosts receiving costimulation blockade based tolerance induction treatment, while the number of nTreg or iTreg is similar between each group. This introduced method allows for serially investigating the phenotypic and functional changes of T cell subtypes during the entire course of allograft response. Non-invasive *in vivo* flow cytometry may work to predict clinical prognosis of allograft rejection or tolerance. Design principles used in this method may also be attractive in studying other perspectives of T cell immunology.

IPITA-O-8.9

CD34+ OCT4+ human pancreatic islet-derived stem cells: endocrine/endothelial features and multidifferentiation potential

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Background: Stem cells appear as an attractive source for the development of regenerative therapies for diabetes. Pancreatic islet-derived stem cells may bear advantages connected with tissue-specific functions.
Methods: We isolated, expanded and characterized stem cell populations from human pancreatic islets. Gentle isolation procedures were optimized for small pancreatic specimens. High yield expansion was obtained by culturing in Chang medium D. Adherent populations were characterized by flow cytometry and immunofluorescence. Differentiation potential was investigated after induction with specific media.
Results: Highly expandable adherent populations were isolated. The cells expressed stem/progenitor markers (CD34+ Oct-4+ Sca-1+ SSEA-4+), displayed elevated expression of endocrine (Insulin+ Glucagon+) and endothelial (vWF+ CD105+ CD146+) markers. They showed multidifferentiation potential toward pancreatic endocrine and mesenchymal commitments. The cells had an important propensity to form islet-like clusters.
Conclusions: CD34+ Oct-4+ stem cells can be isolated and expanded from human pancreatic islets: these cells display endocrine/endothelial features and remarkable multidifferentiation potential.

IPITA-O-8.10

An acidic pH and stimulation of phosphoinositide 3-kinase stimulate the differentiation of pancreatic progenitors into the insulin producing cells

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Background: The phosphoinositide 3-kinase (PI3K) pathway plays an important role in the regulation of beta cell differentiation. Here, we present a method of pancreatic non-endocrine cells differentiation based on the change of pH and stimulation of the PI3K pathway.
Methods: Non-endocrine cells were obtained from islet-depleted fractions of pancreatic tissue (n = 4). Gene expression was evaluated by RT-PCR and immunofluorescence staining at protein level. Cells were cultured for 3 days in DMEM medium containing bFGF, EGF and neonatal fibroblasts conditioned medium. Afterwards, cells were cultured for 4 days in DMEM medium containing ITS and B27 supplements, FGF10, follistatin and forskolin with pH adjusted to 6.0. Cells were divided into 6 groups and supplemented with ZnCl2 (group I), ZnCl2 and wortmannin (group II), IGF-1, EGF and Exendin-4 (group III), ZnCl2, IGF-1, EGF and Exendin-4 (group IV), ZnCl2, wortmannin, IGF-1, EGF and Exendin-4 (group V). Group VI was used as a control sample. Finally, cells were cultured for 3 days in CMRL medium containing 5% FCS, JNK inhibitor, GSK-3 inhibitor, fibronectin, Exendin-4, HGF, IGF, NGF and nicotinamide. Successful differentiation was evaluated by IRMA assessment of human C-peptide.
Results: Non-endocrine cells formed islet-like cell clusters (ILCC) within 3 days. ILCC contained mainly cytokeratin-19 positive cells and were negative for insulin. Small cellular buds began to spread out of the ILCCs upon the lowering of pH. While the expression of PDX-1 and NeuroD genes was significantly higher in groups I and V in comparison with the other groups, the highest level of insulin gene expression was observed in groups III and VI. The expression of Neurogenin-3 was observed only in the samples from group IV. In accordance with the results from RT-PCR, the highest number of C-peptide containing cells was observed in group VI and III (9.1 ± 3.2% and 12.2 ± 3.2%) after the immunofluorescence staining. In response to glucose stimulation (5 and 20 mM) ILCC secreted 0.24 and 0.91 pmol C-peptide/μg DNA.
Conclusion: Modulation of pH in the culture medium and further stimulation of PI3K by growth factors and regulators of signaling kinases induce reproducible differentiation of endocrine progenitors derived ILCCs into the insulin producing cells.
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IPITA-O-8.11

Expression of pancreatic endocrine markers by prolactin treated rat bone marrow mesenchymal stem cells

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Background: The search for a renewable source of cells with insulin-producing capacity to replace β cells destroyed by the autoimmune process in Type 1 Diabetes Mellitus, is a very interesting subject and a promising therapeutic alternative for this disease. Mesenchymal stem cells (MSC) have a great potential in cell therapy, due to its relative ease of isolation, expansion and differentiation. Previous studies have demonstrated the ability of prolactin to induce an increase in β-cell mass and maturation (glucose sensitivity) both *in vitro* and *in vivo*, suggesting that its use in MSC differentiation protocols could exert a beneficial effect. Our intention in this work was to evaluate the expression of endocrine differentiation markers in rat MSC treated with prolactin *in vitro*.
Methods: MSC from bone marrow of Wistar rats were isolated, characterized (by cytochemistry and FACS) and *in vitro* expanded. MSC differentiation was performed in medium containing high glucose concentration (25 mM), nicotinamide, 2-Mercaptoethanol and exendin-4, in the presence or abscence of 500 ng/ml of rat recombinant prolactin. Expression of Pax6, neurogenin-3, Isl1, NeuroD1, Nkx2.2, Nkx6.1, pancreatic polypeptide, somatostatin, insulin-1 and 2, glucagon and prolactin receptor genes were evaluated by real-time PCR and compared between culture stages and the presence or abscence of prolactin in culture medium. Insulin, somatostatin, glucagon and PDX-1 expression were also evaluated by immunofluorescence microscopy.
Results: Isolated cells were CD29 and CD73 positive, CD45 negative and differentiated into adipocyte, chondrocyte and osteocyte lineages, confirming their MSC origin. Pax6, neurogenin-3, Isl1, NeuroD1, Nkx2.2 and Nkx6.1 showed varied expression during culture stages, while insulin, glucagon and pancreatic polypeptide were not expressed. Long-form prolactin receptor mRNA was significantly induced ($p < 0.05$) in prolactin treated cultures. Somatostatin gene was significantly induced ($p < 0.05$) in early stages of differentiation, and its expression was induced by prolactin as confirmed by immunofluorescence.
Conclusion: Culture of rat bone marrow MSC with differentiation media induced expression of pancreatic endocrine-specific genes, and somatostatin and prolactin receptor expression was also induced by prolactin.

IPITA-O-8.12

Comparison of adipocyte and bone marrow-derived mesenchymal stem cell as an immune modulator in pancreatic islet transplantation

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Allogeneic pancreatic islet transplantation has the potential to cure type 1 diabetes. One of the barriers to islet transplantation is the alloreactive T-cell response between donors and recipients. Adult mesenchymal stem cells (MSCs) were recently found to suppress effector T cell responses and to have beneficial effects in various immune disorders. Previous our experiment have proven that autologous BM-derived MSCs (BM-MSCs) as an immune modulator to suppress T-cell and inflammatory responses and to induce generation of antigen-specific regulatory T cells in rodent allogeneic islet transplantation. As a source of these cells, the adipose tissue might be easier compared with bone marrow in clinical application. However, the *in vivo* function of human adipose derived mesenchymal stem cells (hAD-MSCs) has never been compared with bone marrow-derived mesenchymal cells (MSC) in transplant model. hAD-MSCs were isolated and characterised by adherence to plastic, appropriate expression of surface markers, and differentiation capability *in vitro*. The immunosuppressive properties of hAD-MSCs were determined *in vitro* in mixed lymphocyte reactions. The hAD-MSCs showed a homogenous population of cells with high expression levels of CD73 and CD90 and absence of CD31, CD34, and CD45. The stem cell potential of the hAD-MSCs was verified by differentiation into adipogenic, osteogenic, neurogenic, myogeneic and chondrogeneic cells. We compared the immunosuppressive properties of hAD-MSCs with BM-MSCs in terms of immunosuppressive properties. hAD-MSCs did not provoke an *in vitro* xenoreactivity in MLR using rat splenocytes and, rather suppressed mixed lymphocyte reaction (MLR). In rodent islet allotransplantation, hAD-MSCs injection alone did not prolong allograft survival. However, combined treatment of hAD-MSCs and CsA (5 mg/kg; for 2 weeks) significantly prolonged allograft survival compared with CsA alone ($>56.5 \pm 21.9$ days vs 9.2 ± 4.02 days). These findings support that hAD-MSCs as well as BM-MSCs has immunosuppressive function in islet transplantation model. From this result, we can deduce that hAD-MSCs could be a better alternative source for immune modulator than BM-MSCs in islet allograft. We are performing further study to dissect the mechanism of hAD-MSCs in prologation of islet allograft survival.

IPITA-O-8.13

Expression of human CD39 on mouse islets protects against chemically-induced diabetes

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Background: Islet transplantation is a potential cure for type 1 diabetes mellitus; however a gradual loss of graft function and decline in insulin independence occurs post transplantation. Detrimental factors include blood-mediated inflammatory reaction of the body to the transplanted islets, recurrent autoimmunity and metabolic exhaustion. CD39 is an ectoenzyme that works in concert with CD73 to hydrolyse pro-inflammatory extracellular nucleotides to anti-inflammatory adenosine (ADO). We have previously shown that overexpressing human CD39 (hCD39) protects against chemically-induced diabetes.

Aim: To examine the effect of hCD39 expression in a chemically induced model of diabetes. To validate a syngeneic islet transplant model using spontaneously diabetic "IRC" mice as recipients.

Methods: Diabetes was induced in CD39KO (KO), wild-type (WT) and hCD39 transgenic (TG) mice using multiple low dose streptozotocin (MLDS) (50 mg/kg for 5 days) and followed for 90 days. Adoptive transfer studies were done to delineate the site of hCD39 expression that mediates protection. Reducing numbers of WT islets were transplanted subcapsularly into IRC mice.

Results: KO mice developed overt diabetes (BGL 24.5 ± 1.6 mM) at day 8, while WT mice were hyperglycaemic (BGL 19.4 ± 1.5 mM) at day 12 and progressed to an overtly diabetic state at day 42. In contrast, TG mice were euglycemic (BGL 13.3 ± 0.8 mM) throughout the 90-day study. Histologically, leucocytes were observed in islets of KO mice at the onset of diabetes and extensive apoptosis was evident. There was significantly less apoptosis in the TG islets. Irradiated TG mice reconstituted with either WT (TGWTBM) or KO (TGKOBM) bone marrow remained euglycemic following MLDS (TGWTBM-BGL 10.2 ± 1.2 mM; TGKOBM-BGL 10.8 ± 0.6 mM).

As few as 200 WT islets can restore euglycemia in IRC mice following subcapsular islet transplantation.

Conclusions: The level of hCD39 expression correlates inversely with severity of hyperglycemia. hCD39 expression on tissues (specifically islets) alone is sufficient to protect against diabetes induced in the MLDS model. A subcapsular islet transplant model has been validated and will be used to determine the minimum TG islet mass required to restore euglycemia.

IPITA-O-8.14

Reduced soluable CD30 serum levels early post-transplant correlated with graft survival in islet allograft recipients

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Soluble CD30 (sCD30) is a TNF-family member expressed on T cells and is proteolytically cleaved from its membrane bound form into serum following T cell activation. While detectable levels of sCD30 have been reported in healthy subjects, elevated sCD30 levels have been shown following infections, rheumatoid arthritis, lymphoma, or allosensitization. Recent studies suggest that increased sCD30 both pre- and post-transplant correlate with higher incidence of rejection in kidney, heart and lung transplants. A recent study demonstrated that higher pre-transplant sCD30 indicated higher risk of islet allograft rejection. In this study we evaluated the levels of sCD30 posttransplant in islet allograft recipients and correlated those to graft outcome. We examined serum samples from 22 islet allograft recipients receiving different immunusuppressive induction therapies: hOKT3g-1 (ala, ala) (non-depleting anti-CD3), antithymocyte globulin (ATG) and ATG + daclizumab (anti-IL2Rα). sCD30 levels were determined by ELISAs pre and posttransplant. ATG Induction (alone or with daclizumab) demonstrated a sharp decrease in sCD30 levels within 7 days posttransplant whereas induction with hOKT3g-1 (ala, ala) led to an increase in sCD30. These data suggest that hOKT3g-1 activated T cells early posttransplant as compared to ATG which primarily functions by depleting lymphocytes. The decrease in sCD30 was maintained posttransplant in recipients who received ATG or ATG + daclizumab but returned to baseline levels within 28 days post-transplant in hOKT3g-1 group. We next examined correlation between sCD30 levels and graft rejection. Islet graft functionality was grouped as graft survival (insulin independent $>$d365) and graft rejection (insulin dependent within 365 days). Of recipients receiving ATG + daclizumab, there was no correlation of sCD30 levels with graft function; both groups showed similar decreases in sCD30 levels. In the group receiving ATG induction alone, an increase in sCD30 levels was observed by d180 posttransplant that correlated with graft rejection. Interestingly, increase in sCD30 was clearly observed early (d7) and late (d180) posttransplant in graft rejection group. Taken together, our data demonstrates that increase in sCD30 levels posttransplant are indicative of islet graft rejection, and may be an indicator of long-term islet graft function.

IPITA-O-8.15

Combination of anti-LFA-1 and anti-CD154 monoclonal antibodies induces dominant and CD4+ T cell dependent tolerance to neonatal porcine islet xenografts

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Background: A variety of transient therapies directed against T lymphocyte activation and function result in long-term islet allograft survival. However,

there are relatively few examples of durable islet xenograft survival using similar short-term approaches in phylogenetically disparate xenograft models. Our previous study demonstrates that combined anti-LFA-1 and anti-CD154 monoclonal antibody (mAb) therapy induces tolerance to NPI xenografts. The aim of this study therefore, was to examine whether tolerance to NPI xenografts is dominant and if so, which subtype of T cells is important in this tolerance.
Methods: Streptozotocin-induced diabetic C57BL/6J (B6) mice were transplanted with 2000 neonatal porcine islets (NPI) under the left kidney capsule and were treated with short-term intraperitoneal injections of a combination of anti-LFA-1 and anti-CD154 mAbs. Blood glucose levels were monitored for >150 days post-transplantation.

At this time, spleen cells were isolated and 25×106 or 50×106 unpurified spleen cells or 10×106 purified CD4+ or CD8+ T cells alone or in combination with naive B6 mouse spleen cells were injected into normoglycemic B6 *rag*$^{-/-}$ mice with established NPI xenografts. Blood glucose levels of these mice were monitored for 60 days after cell reconstitution or until the graft was rejected. Islet grafts were harvested to determine the presence of NPI as well as immune cells by immunohistochemistry staining.
Results: Short-term administrations of a combination of anti-LFA-1 and anti-CD154 mAbs resulted in prolonged (>150 days) NPI xenograft survival in 39/40 B6 mouse recipients. All B6 *rag*$^{-/-}$ mice that received 25 or 50×106 unpurified spleen cells, or 10×10^6 purified CD4+ or CD8+ T cells from tolerant B6 mice (n=5 in each group) did not reject the graft for >60 days post-cell transfer. The majority of B6 *rag*$^{-/-}$ mouse recipients of NPI that received the combination of 25 or 50×10^6 unpurified spleen cells or 10×10^6 purified CD4+ but not CD8+ T cells from tolerant B6 mice combined with immune cells from naïve B6 mice (n=5 in each group) also accepted the graft for >60 days post-cell transfer.
Conclusion: These results show that short-term administrations of a combination of anti-LFA-1 and anit-CD154 mAbs induce dominant and CD4+ T cell dependent tolerance to NPI xenografts.

IPITA-O-8.16

Identification of a molecular hierarchy whereby NF-κB blockade prior to transcriptional induction of A20 results in impaired islet function and allograft rejection

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Background: The transcription factor NF-κB mediates cytokine-induced islet-graft dysfunction and apoptosis. We examined the effect of NF-kb blockade upon islet allografts.
Methods: NF-κb activity was blocked by recombinant adenovirus (rAd.) mediated expression of IKBα or by pyrrolidine dithiocarbamate (PDTC). Islet inflammatory responses and allograft survival were analysed.
Results: rAd. GFP-expressing islets (H-2d) provided normal metabolic control immediately after transplantation into diabetic allogeneic (H-2b) recipients. Both rAd. GFP-expressing grafts and non-transduced were rejected with a similar tempo (MST ~14 days, n≥6). Transduction of islets with rAd. IKBα prevented NF-κb activation, e.g. suppression of IL-1β-induced CCL2/MCP-1. Surprisingly, rAd. IKBα-transduced grafts struggled to restore euglycaemia in the immediate post-transplantation period. Further, allograft survival was not improved (MST ~15 days, n≥10). Similarly, PDTC treatment effectively blocked NF-κb activation but also impaired islet allograft function. Microarray analysis was next used to identify cytokine-induced and NF-κb-dependent early-immediate response genes that might impact upon islet graft survival. This analysis identified the cytoprotective gene A20 as a early-immediate response gene in islets. Significantly, cytokine-dependent induction of A20 was reduced by ~90% in rAd. IKBα expressing islets. We next tested the effects of A20 over-expression on islet allografts. Transduction of islets with rAd. A20 prevented cytokine-induced activation of NF-κb and expression of inflammatory chemokines CCL2, CXCL2, CXCL1 and CXCL10. In marked contrast to rAd. IKBα transduced grafts, A20-expressing islet allografts maintained normal metabolic control post transplantation. Further, ~20% of A20-expressing islet allografts (n=20) exhibited improved allograft survival. These long-term surviving (≥100 days) allografts exhibited strong insulin labelling and were surrounded by Foxp3+ mononuclear cells.
Conclusion: These data demonstrate a molecular hierarchy whereby inhibiting NF-κb with A20, a physiologic target and regulator of NF-κb, dampens islet pro-inflammatory responses, preserves islet graft function and promotes long-term engraftment. Dampening NF-κb activation and islet apoptosis may favour islet allograft tolerance.

Wednesday, October 14, 2009

Special Symposium, "Transplantation in the Hyperimmunized Patient"

Session I: Immunological Concepts and Diagnostic Tools

The hyperimmunized patient
J. Grinyò
Servei de Nefrologia, Hospital Universitari de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain

B-cell immunobiology
K. Wood
Transplantation Research Immunology Group, Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford, UK

What can we learn from ABO incompatible cardiac transplantation in children?
L. West
Department of Pediatrics, Alberta Diabetes Institute, University of Alberta, Edmonton, Alberta, Canada

The humoral theory of transplantation
P. Terasaki
Terasaki Foundation, Los Angeles, CA, USA

New diagnostic tools
A. Zachary
Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD, USA