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# Primary islet graft function and its impact on the success of pancreatic islet transplantation

Mikaël Chetboun

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Pour l'obtention du titre de

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Discipline : Chirurgie Viscérale et Digestive

Présentée par  
***Mikaël CHETBOUN***

**Rôle de la fonction primaire du greffon d'îlots pancréatiques et  
impact sur le succès de la transplantation d'îlots pancréatiques**

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# Abbreviations

Islet Transplantation (IT)

Type 1 Diabetes (T1D)

Islet Transplantation Alone (ITA)

Simultaneous Islet and Kidney transplantation (SIK)

Islet-After-Kidney transplantation (IAK)

Serious Adverse Events (SAEs)

Estimated Glomerular Filtration Rate (eGFR)

Primary islet Graft Function (PGF)

Continuous Glucose Monitoring Systems (CGMS)

# Part 1: Introduction

## 1.1 Overview

Pancreatic islet transplantation is an established  $\beta$ -cell replacement therapy strategy for the treatment of severe type 1 diabetes. Modern developments in islet isolation, cell culture, immunosuppression and transplantation have significantly enhanced patient safety and improved long-term clinical outcomes<sup>1,2</sup>.

For type 1 diabetes patients, islets isolated from a pancreas procured from a deceased donor associated with intrahepatic allogeneic transplantation under immunosuppression allow restoration of endogenous insulin secretion associated with glucagon counter-regulation<sup>3</sup>. The recovery of endogenous secretion of insulin significantly reduces and sometimes even discontinues the use of exogenous insulin therapy, decreases or even stops the occurrence of severe hypoglycemia by stabilizing glycemic lability and maintaining an optimal glycemic balance, with a better quality of life<sup>2,4-6</sup>. Most often, recipients receive several islet infusions from several human pancreases. This multiple islet infusion from several pancreas donors approach differs mainly from allogeneic pancreas organ transplantation.

The development of alternative sources of insulin-secreting cells for diabetes  $\beta$ -cell replacement therapy is also a growing field of research with ongoing clinical trials and promises to expand the availability of islet transplantation in the future treatment of diabetes<sup>7</sup>.

Nevertheless, the function of transplanted allogeneic human islets decreases over time, and this failure is multifactorial and due to the characteristics of the donor, recipient, isolation process, cell culture and islet transplantation itself in the context of immunosuppressive therapy.

Our work focused on the optimization of islet transplantation, and in particular on the predictive role of the primary islet graft function on the long-term outcome of allogeneic islet transplantation.

## 1.2 Pancreatic islet transplantation

While islet transplantation (IT) is now considered as a standard of care approach to restore glucose control in patients with severe type 1 diabetes (T1D) in many countries, this approach, which is complementary to whole-organ pancreas transplantation, has benefited from many developments.

The earliest preclinical reports on murine diabetic models, published in the early 1970s<sup>8-10</sup>, confirmed that IT was effective in achieving glucose control under both syngeneic and allogeneic conditions, and that transplantation into the liver route was an effective transplant site<sup>10-12</sup>.

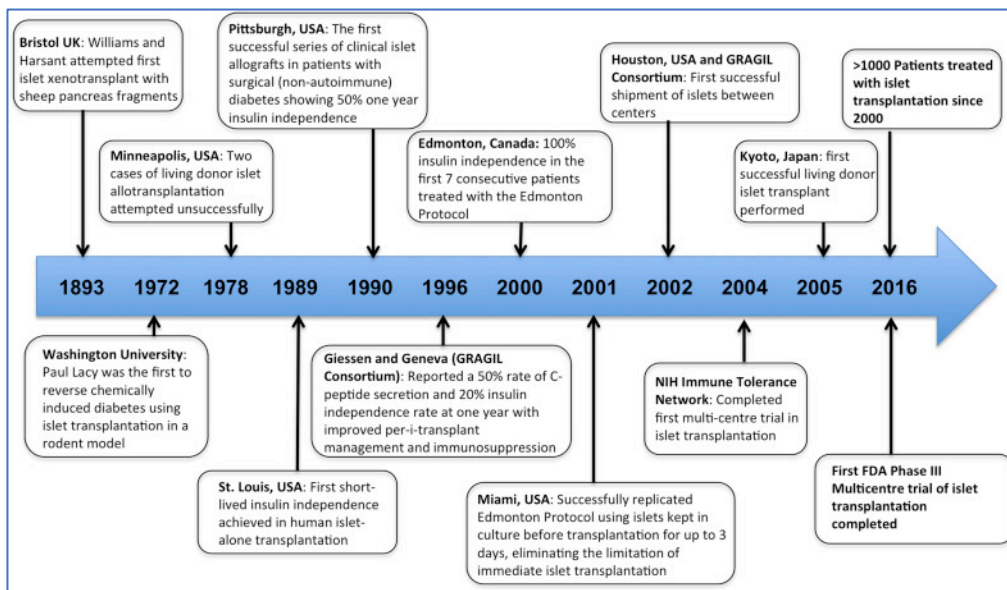
This proof of concept of using the liver as a transplantation site for isolated islets through infusion of cells via the portal vein opened the way for the development of IT for cell therapy of T1D patients. Attempts to improve the islet purification procedure, which represents less than 5% of the total pancreas volume, and to improve human islet preparation quality through viability of the isolated islets during the isolation procedure by some groups<sup>13,14</sup> led to the diffusion in 1988 of an automated method of human islet isolation by Ricordi and colleagues<sup>15,16</sup> which today, more than thirty years later, still stands as the “state-of-the-art” method even if it has benefited from some refinements since then<sup>17,18</sup>. This method of mechanical and enzymatic purification using collagenase has allowed the success of allogeneic IT leading for the first time in 1990 to a brief period of insulin independence<sup>19</sup>. Finally, in 2000, the Edmonton group reported seven consecutive patients with T1D all achieved insulin independence after IT with a glucocorticoid-free immunosuppression regimen<sup>20</sup>.

The so-called Edmonton immunosuppression regimen involved immunosuppression induction with an interleukin-2 receptor antagonist monoclonal antibody (initially Daclizumab, but which is no longer marketed and will later be replaced by Basiliximab) and a maintenance regimen using Sirolimus (m-TOR inhibitor agent) and Tacrolimus (Calcineurin inhibitor agent) without the use of glucocorticoid, which was (and still is) the gold-standard in kidney transplantation.

If finally, today, in the field of IT, the non-use of corticosteroids seems rational, given the direct toxicity on  $\beta$ -cells and the resulting insulin resistance, it is since the release of the Edmonton immunosuppression regimen that the development of numerous clinical research programs has appeared worldwide.

In 2005, the Minnesota group proposed several modifications to the management of the peritransplant period including the use of T-cell depleting agent (anti-thymocyte globulin) with the use of TNF alpha antagonist, and reported the achievement of insulin independence in eight consecutive T1D patients who benefited from IT isolated from a single pancreas donor<sup>21</sup>.

Figure 1 shows the key steps of pancreatic IT developments (illustrated from Juliet A.Emamaullee<sup>22</sup>).



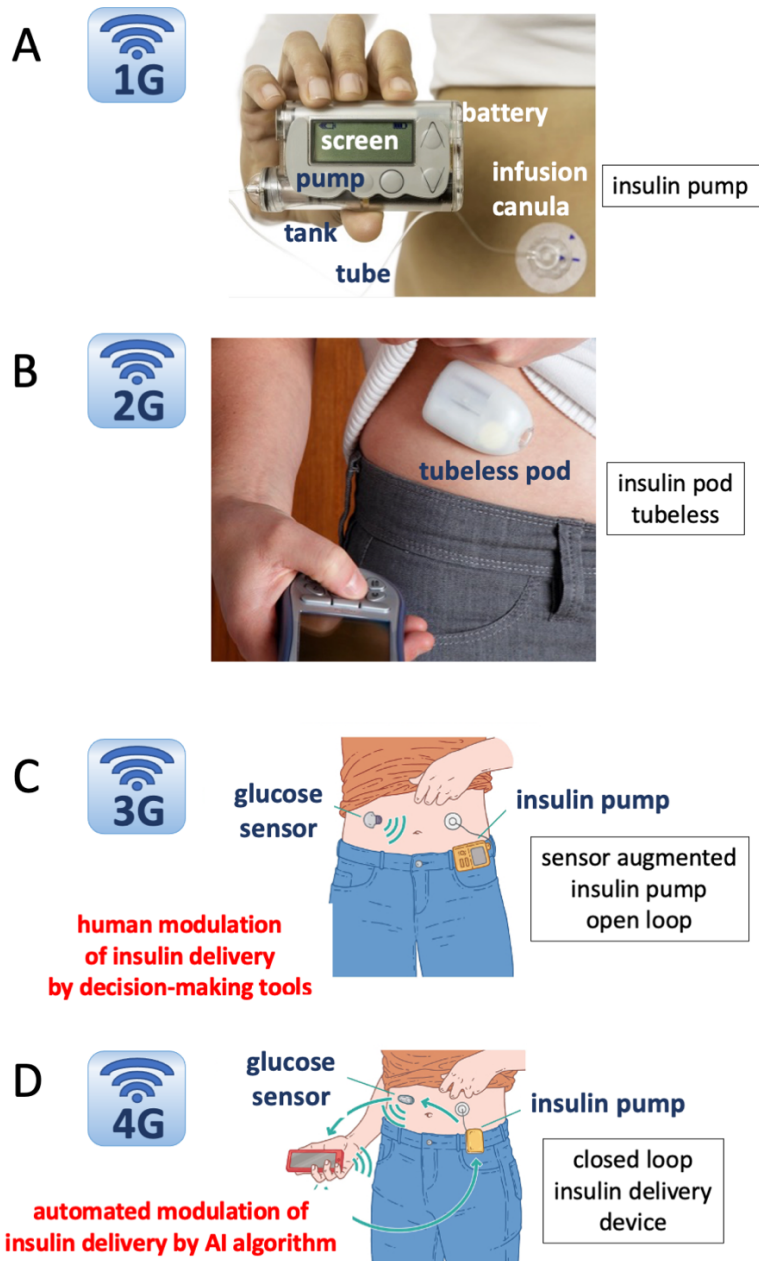
*Figure 1: Key steps of pancreatic islet transplantation developments (adapted from Emamaullee and colleagues<sup>22</sup>).*



## 1.3 Indication of islet allotransplantation for the treatment of type 1 diabetes

Since the invention of insulin more than 100 years ago, many innovative technologies have been developed to improve therapeutic education, adherence and glycemic control in diabetic patients. Figure 2 shows the different generations of systems allowing the subcutaneous release of insulin, from the first pump generation that delivers a planned basal dose of insulin with bolus initiated by patients according to his diet and physical activity, to the latest generation where the interstitial glucose level measured by the glucose sensor informs a computer that modulates the release of insulin according to artificial intelligence algorithms. Since the publication of the Edmonton protocol and its associated multicenter trial<sup>20,23</sup>, many clinical trials have been developed and have proposed allogeneic IT for the treatment of the most severe T1D patient, i.e. those with severe hypoglycemia unawareness and significant glucose lability leading to major alteration of the quality of life with numerous stays in hospital and/or in intensive care units<sup>24</sup>, or in patients who are already under immunosuppressive regimen because of a pre-existing kidney transplant and whose glucose control is not achieved<sup>25,26</sup>.

The management of hypoglycemic events in diabetic patients under insulin should be supervised by a diabetologist to: 1) promote educational interventions related to the optimization of insulin therapy through the use of continuous subcutaneous insulin delivery systems (insulin pumps), 2) assess glucose variability and lability through continuous glucose monitoring devices (CGMS), and 3) introduce composite systems combining "smart" pumps and continuous monitoring.



*Figure 2: Different generations of insulin delivery systems. First generation (A), second generation with tubeless pumps (B), third generation with glucose sensor augmented pump, called open loop when help the decision (C), closed-loop automated delivery systems (D) (adapted from "Fédération Française des Diabétiques"<sup>27</sup>)*

The assessment of glucose lability, perception and severity of hypoglycemia symptoms can be evaluated using specific tools such as the HYPOscore<sup>28</sup> (scale for quantifying the severity of hypoglycemia), the lability index (LI)<sup>29</sup> and the Clarke score<sup>30</sup> (scale for quantifying the degree of perception of hypoglycemia).

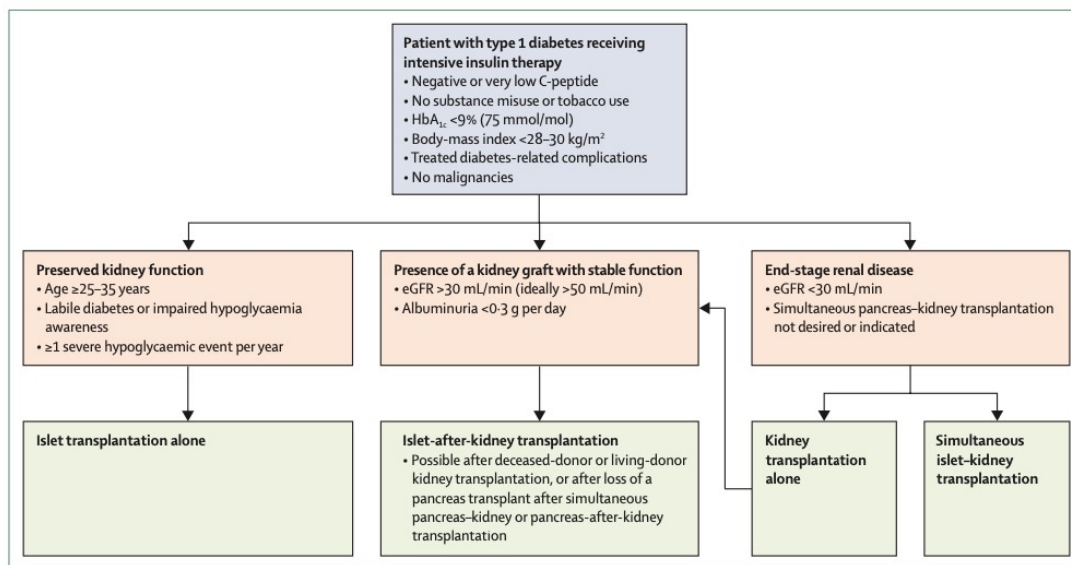
Thus, after failure of educational and technological approaches, islet transplantation alone (ITA) under long-term immunosuppressive therapy can be recommended for adult T1D patients with severe hypoglycaemia unawareness (HYPOscore  $\geq$  800, Clarke score  $\geq$  4, or LI  $\geq$  400) after failure of optimal medical therapy<sup>31</sup>.

Simultaneous islet and kidney transplantation (SIK) or islet-after-kidney transplantation (IAK) is indicated in unbalanced T1D adult patients (HbA1c > 7%) with end-stage renal disease for whom kidney transplantation is recommended among patients contraindicated for pancreas transplantation (recused to major abdominal surgery for morbid conditions or when the recipient's arteries compromise pancreas transplantation) or after a first failure of pancreas transplantation.

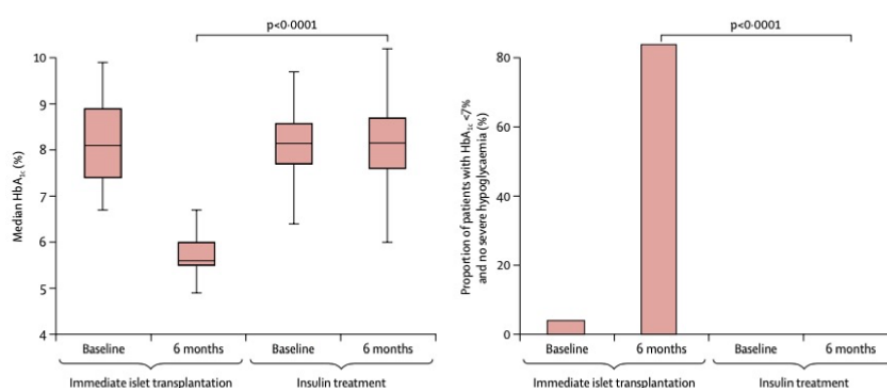
In these very selected patients with severe forms of T1D, the most physiological approach to achieve and maintain an optimal glucose control and to decrease the occurrence of hypoglycemia and improve their sensitivity, is therefore to restore the endogenous mass of  $\beta$ -cells by allogeneic transplantation of pancreatic islets under long-term immunosuppression, without or with a project of kidney transplantation. (Figure 3).

As mentioned above, if the first clinical trials were able to demonstrate the proof of concept, the feasibility and the safety of intrahepatic IT, it is only later that many teams were able to demonstrate its superiority compared to the use of intensive insulin therapy.

Thus, a randomized trial<sup>32</sup> and various controlled studies evaluating IT versus intensive insulin therapy have reported better overall metabolic balance and prolonged decrease of the incidence of severe hypoglycemic events after IT<sup>33–37</sup>.



*Figure 3: Indications for allogeneic islet  $\beta$ -cell replacement therapy in type 1 diabetes with preserved kidney function, end-stage renal disease or previous kidney transplantation (adapted from Vantyghem and colleagues<sup>2</sup>)*



*Figure 4: Metabolic outcomes at the baseline and 6 months in islets transplanted recipients or with insulin therapy (adapted from Lablanche and colleagues<sup>32</sup>).*

## 1.4 Islet Transplantation is Standard of Care in brittle type 1 diabetes

Intraportal IT is widely available and is a standard of care in most countries, with 69 IT programs since its widespread use, covering 84 centers worldwide and 5 transplantation networks. Islet transplantation is covered in many countries with full reimbursement by the health-care system in 9 countries: Australia, Belarus, Canada/Province of Alberta, Finland, France, Iran, Poland, Sweden, Switzerland and the United Kingdom. Reimbursement is partially covered by the health-care system in Belgium, Czech Republic, Germany, Italy, Japan and Norway <sup>38</sup>.

The Collaborative Islet Transplant Registry <sup>4</sup>, which is the most comprehensive international registry for the follow-up of patients with allogeneic or autologous IT, now includes the results of more than 1,300 islet allogeneic transplant recipients (80% ITA recipients / 20% IAK recipients). Almost 20 years after the landmark papers of the Edmonton group, the latest registry report has clearly confirmed the global spread of IT and the reproducibility of IT outcomes.

The reproducibility over time could be explained in particular by the standardization of procedures, including pancreatic procurement, islet isolation and purification, cell culture, transplantation and immunosuppression strategies.

Allogeneic IT in T1D patients does not require HLA matching, but a careful look at the occurrence of anti-HLA antibodies in the recipient and requires ABO blood group match. Islet transplantation involves pancreas organ graft mostly procured from deceased donors, even if

the organ donation from dead donors due to controlled circulatory arrest is currently under development <sup>39</sup>.

Following agreement between the recipient's transplant team and the organ procurement coordination team, the pancreas is procured from the abdominal floor after the liver and before the kidneys. The procurement of the pancreas does not modify the conventional steps of multi-organ procurement. Crucial for the success of the procedure is the preservation of the integrity of the peripancreatic membrane that will allow optimal distension by collagenase during the isolation procedure. The main pancreatic duct is then catheterized in the middle of the pancreatic gland in the region of the pancreatic neck, which will enhance the infusion of the collagenase for pancreatic digestion (Figure 5) <sup>6,40</sup>.

The duration between the time of clamping (when the systemic circulation stops in the donor and is replaced by preservation solution) and the time when the pancreas starts to be dissociated by the collagenase is called the cold ischemia time. The cold ischemia time should be as short as possible in order not to alter islet viability and ideally isolation should be started after a cold ischemia time of 8-12 h maximum<sup>41</sup>. Enzyme dissociation is initiated by infusion of collagenase at 4°C directly into the main pancreatic duct (Wirsung duct) and optimal distension of the pancreatic parenchyma are key elements during this step. Then, the pancreas is shipped to the isolation facility, sectioned and placed in the Ricordi chamber, which is connected to a system containing collagenase heated to 37 °C to finish islet dissociation from exocrine cells (Figure 5). After isolation, islets are purified from the exocrine contents by Ficoll™ gradient of different densities (Figure 6). Islets can then be transplanted directly as the original Edmonton protocol, but for organizational reasons, the vast majority of transplantation centers have instituted an overnight culture in a human albumin medium before IT <sup>42</sup>.

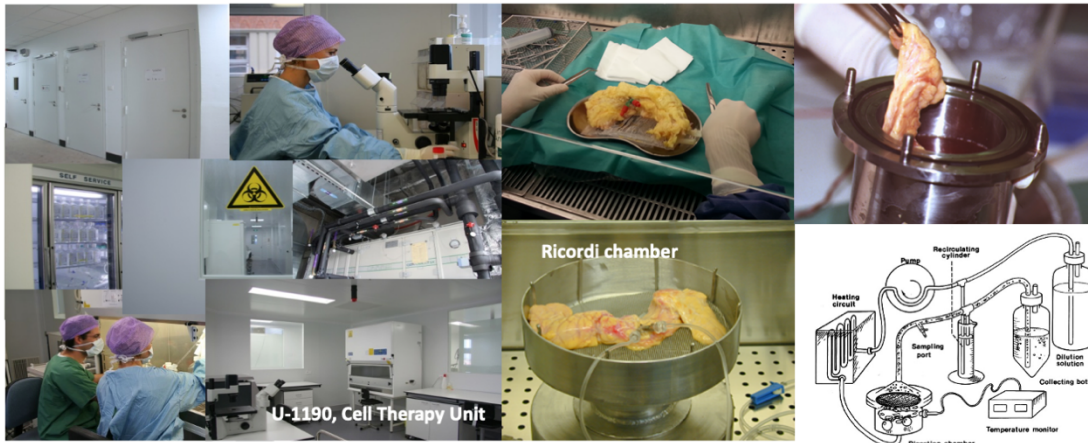


Figure 5: Islet cell isolation with digestion of pancreas using mechanical and collagenase dissociation in the Ricordi chamber (adapted from U-1190 Cell Therapy Unit).

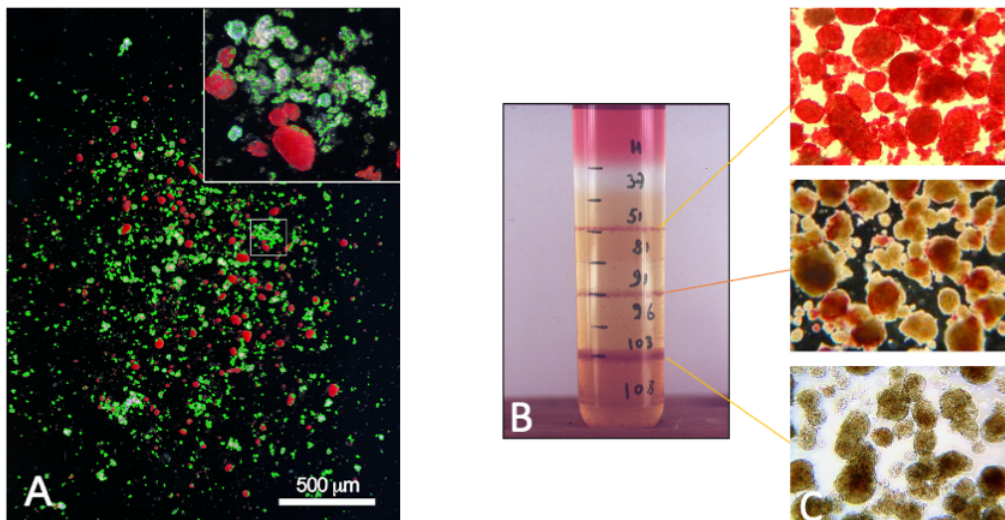
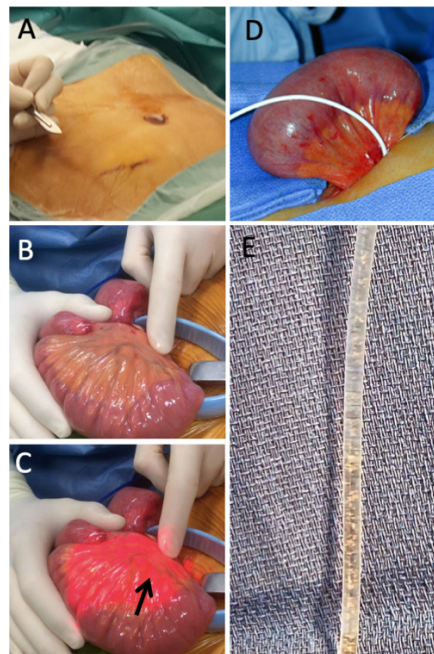


Figure 6: Islet purification: The diagram on the left represents a solution of digested pancreas (islet cells in red and exocrine/ductal cells in green) and on the right after purification procedure by Ficoll™ gradient of different densities, release of rings of islet of different purity, the purest fraction (top ring) will be transplanted (adapted from U-1190 Cell Therapy Unit).

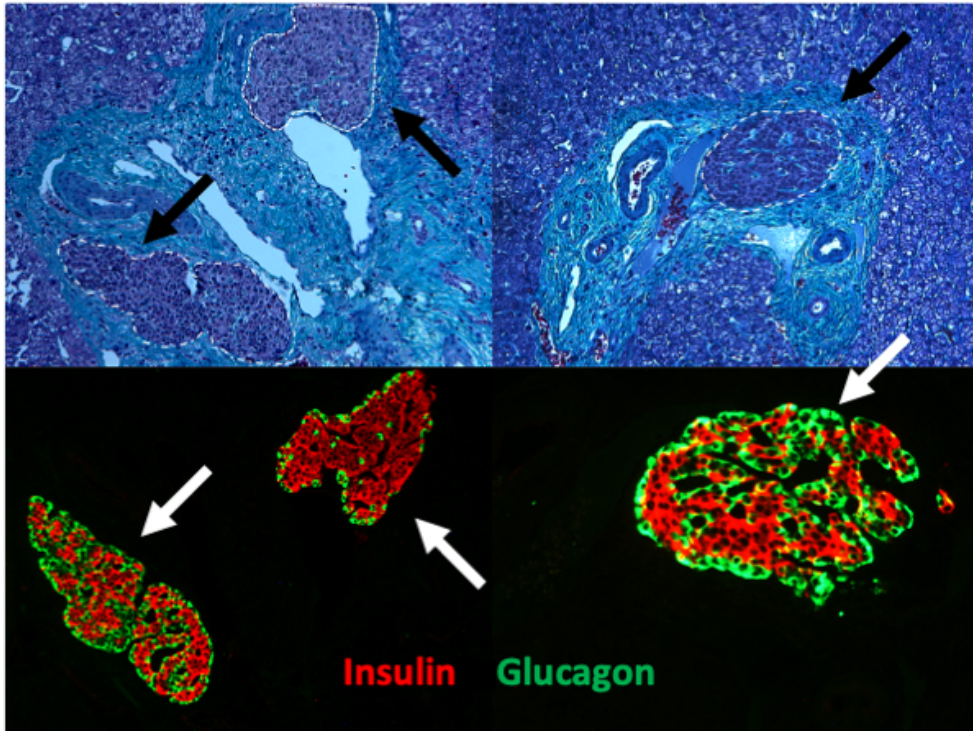
Human islet preparation is usually infused under general anesthesia into the portal trunk, either by percutaneous radiological trans-hepatic approach or by trans-mesenteric surgical approach, after exposure of the last intestinal loop by minimally invasive laparotomy (Figure 7).

After radiological or surgical route performed, islets are infused into the portal flow in the transplantation solution (CMRL cell culture media with human albumin) previously supplemented with heparin (70 IU / kg of the recipient's weight), a procedure that lasts about 30 minutes under continuous monitoring of portal pressure and blood glucose in the recipient. The islets travel into the recipient's liver through the sinusoidal capillaries to engraft with the donor liver (Figure 8).



*Figure 7: Surgical islet transplantation by mini-invasive laparotomy. Under general anesthesia, a 5 cm incision is made in the operating room (A). The last intestine loop is found (B) and the vessels are visualized with a vein scanner device (C, black arrow), then a catheter is inserted in the vein (D) and moved to the portal trunk. The islets are then infused into the patient's liver. Images of the slow infusion of the islets (E) (adapted from U-1190, Cell Therapy Unit).*





*Figure 8: Islets engrafted in the liver, one year following intraportal islet transplantation. On the lower picture we visualize the islets after immunostaining of alpha cells (anti-glucagon antibody immunostaining, green) and beta cells (anti- insulin antibody immunostaining, red) (adapted from U-1190 Cell therapy Unit)*

## 1.5 Complications of islet transplantation

Complications following IT are scarcer and less severe than those following pancreas organ transplantation <sup>43-45</sup>.

However, according to the last report of the CITR, approximately 11% of ITA recipients and 14% of IAK recipients experienced a serious adverse event (SAEs) within the first 30 days after transplantation <sup>4</sup>. In this report, there was a sharp decline in the occurrence of SAEs after 2010, with 15% of the patients experiencing SAEs at baseline, compared with about 5% in 2015-2018.

The most common transplantation-related adverse events are transient elevation of liver transaminases, portal vein thrombosis, liver hematoma and hemorrhage. Portal vein thrombosis is more frequent in islet autologous than allogeneic transplantation, firstly as it occurs after major pancreatectomy and secondly because islets are mostly unpurified. Even though the total islet mass transplanted is often lower than in allotransplantation, the total cell volume is often higher. Consequently, portal vein thrombosis occurs in islet autologous transplantation in 6-10% of adults and in 2% of children <sup>46-49</sup>. Portal vein thrombosis occurs mostly in a segmental branch of the portal vein <sup>47</sup> (2/3 of cases).

In the literature, the rate of portal vein thrombosis in allogeneic IT is about 3-4% <sup>6,47,49</sup>. Caiazzo and colleagues showed that early surgical adverse events affect early function and long-term islet graft survival in islet transplant recipients <sup>50</sup>.

With regard to immunosuppression-related adverse events, they are common to all organ transplants and depend on the specific agent used and edema, oral aphtosis,

neutropenia/lymphopenia, opportunistic infections are the most common SAEs, with a mortality rate less than 2%<sup>1,2</sup>.

As for kidney function, previous studies have reported impaired glomerular filtration rate in islet transplant recipients<sup>51</sup>, but these results have not been confirmed by a prospective study comparing T1D patients treated with IT or insulin therapy<sup>52</sup> or in a prospective study that compares ITA and IAK recipients<sup>6</sup>.

The impact on long-term kidney function in islet transplanted recipients under prolonged immunosuppression with sustained islet graft survival is still debated but a very recent study of the Edmonton group's largest single-center cohort, which compared kidney function in islet transplant patients with and without sustained islet graft survival during a 20-year follow-up seems to allow to set some speculations<sup>53</sup>.

This study showed that first, patients in both groups had a significant decrease in the estimated glomerular filtration rate (eGFR) after IT during the follow-up. Second, the significant decrease in eGFR was more pronounced in the prolonged graft survival recipients with a higher incidence of stage 3 chronic kidney disease, which could be explained by prolonged exposure to immunosuppression. This higher rate of stage 3 chronic kidney disease was not associated with an increased mortality rate in these patients.

Finally, recipients with stage 3 chronic kidney disease before transplantation and sustained graft survival had delayed progression to end-stage renal disease compared with recipients without sustained graft survival.

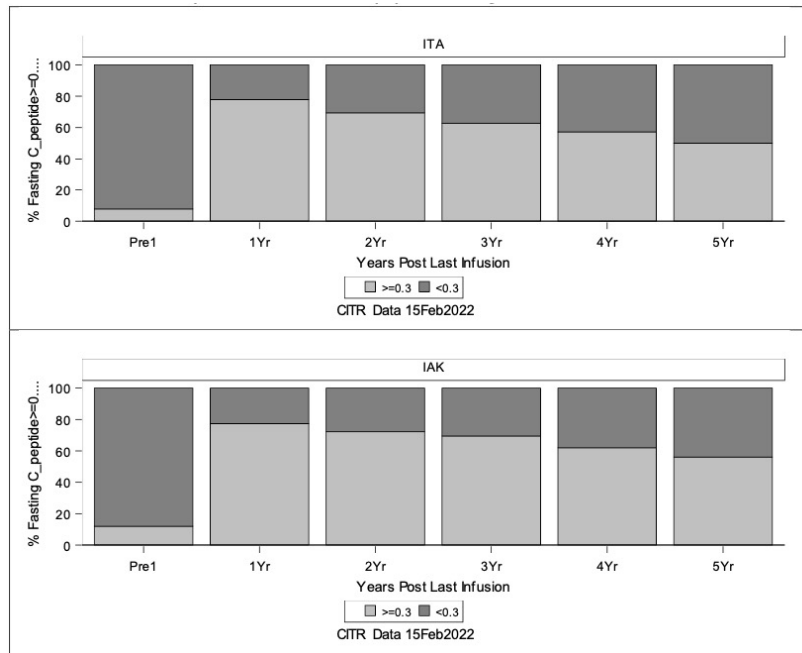
Other transplant-related events may be observed such as alloimmunization, but these are not specific to islet allotransplantation and the relationship between the occurrence of preformed or de novo donor specific anti-HLA antibodies (and their quantity) and their specific impact on islet graft survival and function are still debated<sup>54-56</sup>.

## 1.6 Long-term outcome and multifactorial decline in functional beta cell mass following islet transplantation

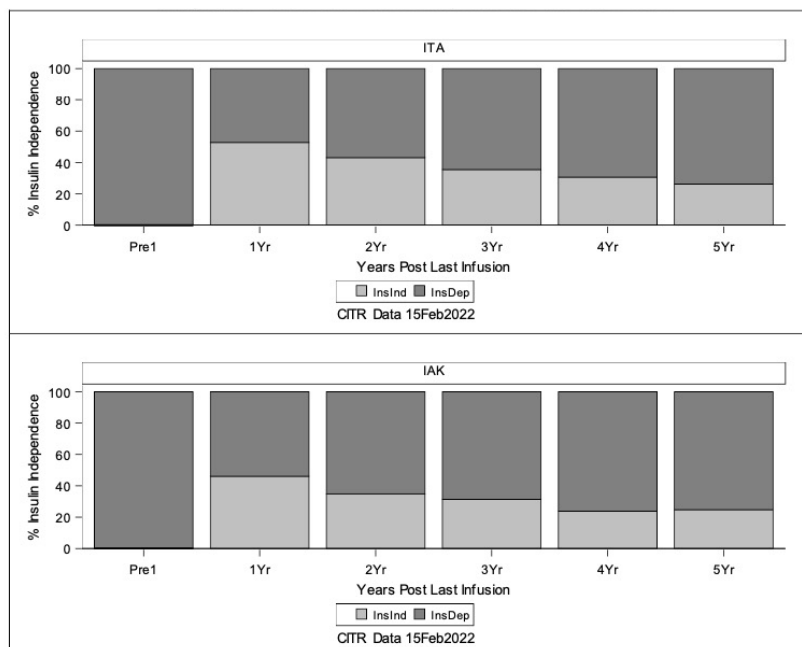
Islet transplantation has emerged as a proven treatment option for restoring endogenous insulin secretion in patients with T1D and severe hypoglycaemia, and/or who have previously benefited from a kidney transplant. The Collaborative Islet Transplant Registry now compiles data from 1108 patients who have received islet transplantation alone and 291 patients with islet and kidney transplantation (simultaneously or not) reaching altogether 2832 human islet infusions from the isolation of 3326 human pancreases.

Long-term ( $\geq 10$  years) reports of IT are now increasingly present in the literature.

Thus, two prospective cohort studies<sup>6,43</sup> and several retrospective cohort studies<sup>37,53,57</sup> reported 86-100% patient survival and 52-78% graft survival at 10 years, as well as improved glycemic control and lability and a significant reduction in severe hypoglycemic event<sup>53</sup>. Unadjusted prevalence of graft function (i.e retention of serum C-peptide level  $\geq 0.3$  ng/mL) and insulin independence post last infusion during a 5-year follow-up are presented in Figures 9 and 10 respectively<sup>4</sup>. However, all these mono-centric cohorts and the international registry demonstrated a progressive decrease in islet graft function and insulin independence in recipients over time, which persisted respectively in only 45% and 25% of patients after 5 years in the global registry<sup>4</sup>.



***Figure 9: Prevalence of graft function (serum C-peptide level  $\geq 0.3$  ng/mL) post last infusion during 5 years in ITA (top diagram) and IAK recipients (bottom diagram) in the CITR cohort (adapted from eleventh CITR report)<sup>4</sup>.***



***Figure 10: Prevalence of insulin Independence post last infusion during 5-years in ITA (top diagram) and IAK recipients (bottom diagram) in the CITR cohort (adapted from eleventh CITR report)<sup>4</sup>.***

The decrease in graft function observed over time is probably multifactorial as shown by the Edmonton International Trial, where, considering the same immunosuppression protocol, the functional results of the transplanted patients were heterogeneous one year after transplantation in different centers <sup>23</sup>.

The Figure 11 illustrates the different mechanisms that could be involved in the progressive failure of the transplanted islets. An obvious component of this process is the allogeneic and/or autoimmune rejection of transplanted islets by the recipient's immune system <sup>58</sup>. The Figure 12A shows islet infiltration (anti-chromogranin A antibody immunostaining, red) by T cells (mononuclear cells with anti-CD3 antibody immunostaining, blue), in a liver biopsy performed in a patient following an intraportal IT (48 hours after transplantation, pre Edmonton era)). This reaction is currently prevented or at least limited by long-term immunosuppression, induced and maintained by the combination of several treatments <sup>4,59</sup>. A large fraction of the transplanted islets is lost during the first days following intraportal IT. Indeed, under appropriate glycemic conditions, 60% of the transplanted islets were lost 3 days after syngeneic transplantation in a mouse model as a result of cell death by apoptosis and necrosis. This mechanism was even more significant in islets exposed to chronic hyperglycemia <sup>60</sup>.

A study was able to visualize with PET imaging labelled islets after intraportal infusion in the liver of T1D recipients and estimated that 50% of the islets mass was lost within a few hours after transplantation <sup>61</sup>

Many authors have theorized that early islet loss was due to innate immunity involving both a non-specific inflammatory response and activation of the coagulation and complement systems, called the Instant Blood-Mediated Inflammatory Response (IBMIR) <sup>62,63</sup>. Pancreatic cells activate coagulation early and intensely, leading to activation of thrombin, which

activates platelets and leads to portal thrombosis and stimulation of polynuclear cells, which release cytokines and damage the endothelial wall (Figure 12B).

Consequently, we routinely administer anticoagulation simultaneously to human islets preparation to prevent portal thrombosis and optimize islet engraftment.

In IT, the functional islet graft mass is not easily measurable for the reasons mentioned above and because a recipient is usually a combination of several grafts from heterogenous donors. The functional islet mass and its decrease is therefore multifactorial, because it depends on the interactions between the donor characteristics, the procurement and preservation of the pancreas organ, the quality of islet isolation and residual islet viability, and islet culture. In addition to these pre-transplant conditions, transplantation and immunosuppression strategies and the recipient (sex, insulin resistance, body mass index) are also relevant and impact the functional islet mass following IT<sup>6</sup>.

Thus, many teams have tried to estimate the functional islet mass following IT<sup>64-66</sup> and especially to associate it to long-term transplantation outcomes.

In the field of solid organ transplantation, there is a well-established relationship between early graft function and long-term functional outcomes, which appears to be unrelated to immune mediated mechanisms. Thus, Terasaki and colleagues<sup>67</sup> showed that the survival of living donor kidney transplants from (MHC unmatched) spouses was excellent at 3 years and comparable to that of living donors from the same family and far superior to cadaveric kidney donors. Figure 13 also shows that the 3-year survival of kidney grafts from patients transplanted with deceased donors was better when patients had optimal primary graft function (diuresis on the 1st day) than suboptimal primary graft function (no diuresis on the 1st day), regardless of the number of mismatches. Primary graft function had a greater impact on survival than MHC matching.

Indeed, early kidney graft dysfunction, defined as the use of dialysis during the first week after transplantation (a non-standardized but relatively consensual definition), is associated with limited graft survival, even in the absence of acute immune rejection <sup>68</sup>.

On the analysis of 588 kidney recipients after transplantation from deceased donors. We can see on Figure 14 that patients who received a kidney graft with delayed graft function ( i.e. using dialysis during the first 15 days after transplantation) and treated with a hypothermic machine perfusion benefited from a better graft survival <sup>69</sup>.

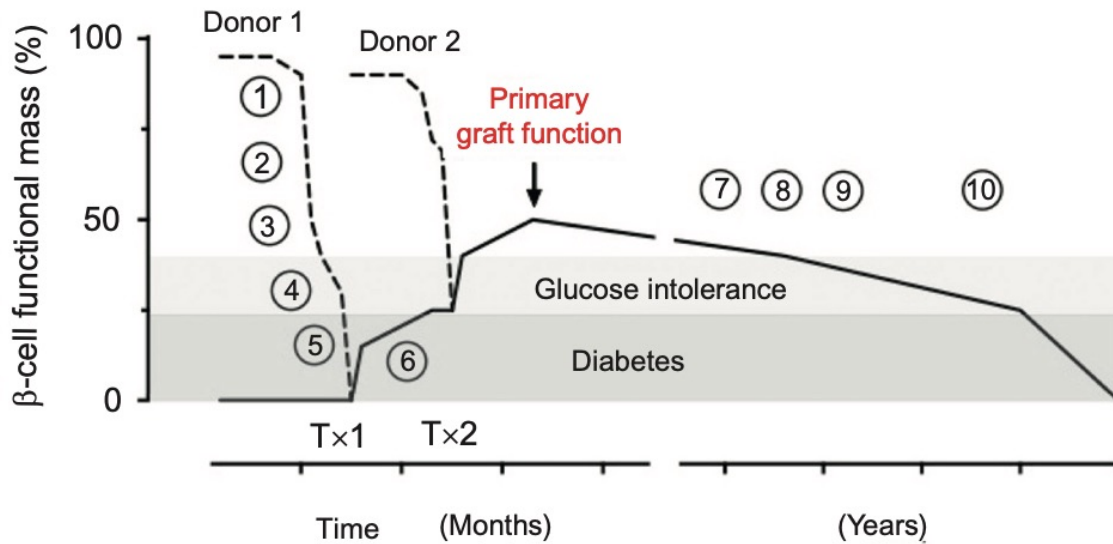
Thus, in this trial where grafts were randomized with or without the use of hypothermic machines, primary graft function optimized by hypothermic perfusion improved long-term survival of kidney grafts compared to those treated without hypothermic machine perfusion, regardless of the level of MHC matching.

In liver transplantation, early graft function can be measured by combining liver biomarkers (bilirubin, INR and transaminases; non-standardized definition). Early graft dysfunction is also associated with decreased patient and liver graft survival in the long term <sup>70,71</sup>.

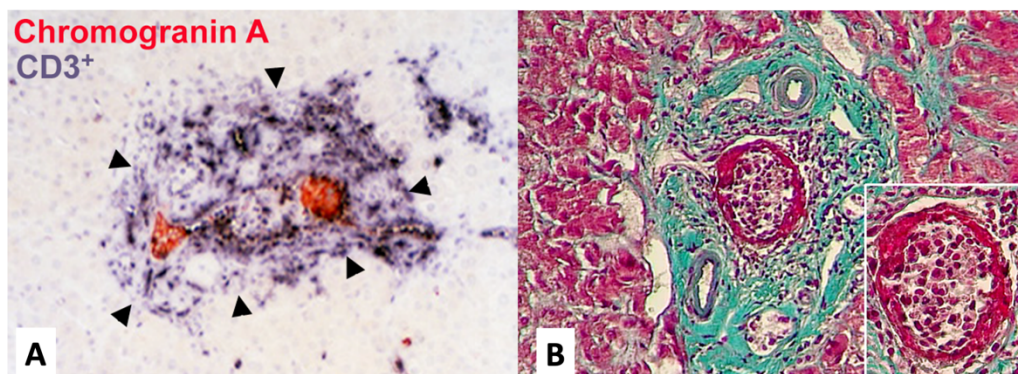
In IT, the Lille team previously reported that primary islet graft function (PGF) could be measured using a validated composite categorical index , the Beta-score, measured from 0 to 8, one month after the last islet infusion with stimulated serum C-peptide, fasting blood glucose, HbA1c and the need for exogenous insulin <sup>65,72</sup> . The authors reported in this single-center cohort of ITA recipients with standardized procedures of islet isolation, culture, immunosuppression, and transplantation that optimal PGF (Beta-score of 7 or 8) was associated with significant prolonged islet survival and longer duration of insulin independence compared to patients who benefited from suboptimal PGF (Beta-score < 7) at 3 years (Figure 15).



- |                    |                      |                 |                      |
|--------------------|----------------------|-----------------|----------------------|
| 1. Organ donation  | 4. Hypoxia           | 7. Alloimmunity | 10. Glucotoxicity    |
| 2. Islet isolation | 5. Innate immunity   | 8. Autoimmunity | and islet exhaustion |
| 3. Culture         | 6. Revascularization | 9. Xenobiotics  |                      |



*Figure 11: Outline representation of early- and long-term factors involved in beta-cell functional mass failure (adapted from Chetboun and colleagues<sup>73</sup>)*



*Figure 12: Islet cell (anti-chromogranin A Ab immunostaining, red) infiltration by T cells (mononuclear cells with anti-CD3 Ab immunostaining, blue), in a liver biopsy performed in a patient following intraportal islet transplantation (12A). Pancreatic cells activate coagulation, leading to activation of platelets and to portal thrombosis and stimulation of polynuclear cells, that damage the endothelial wall (12B). (Pre Edmonton era, adapted from U-1190, Cell Therapy unit).*

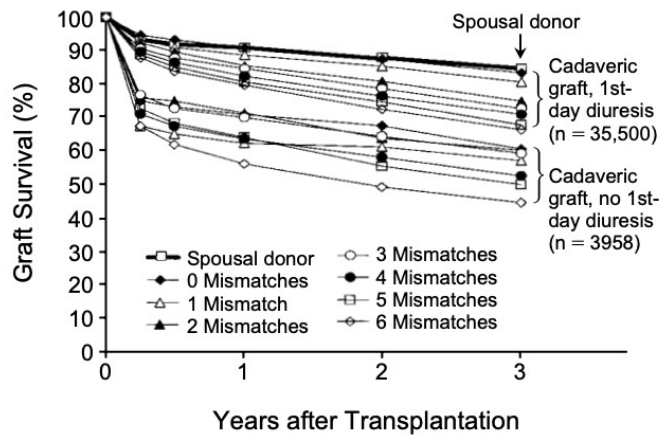


Figure 13: Survival of cadaveric kidney grafts according to the number of HLA-A, B, and DR mismatches and according to the urine flow on the first day post transplantation. The unmatched spousal donor (living donors of kidney transplants) group expected the highest survival rates and similar to that of parental donor kidneys (adapted from Terasaki and colleagues.<sup>67</sup>).

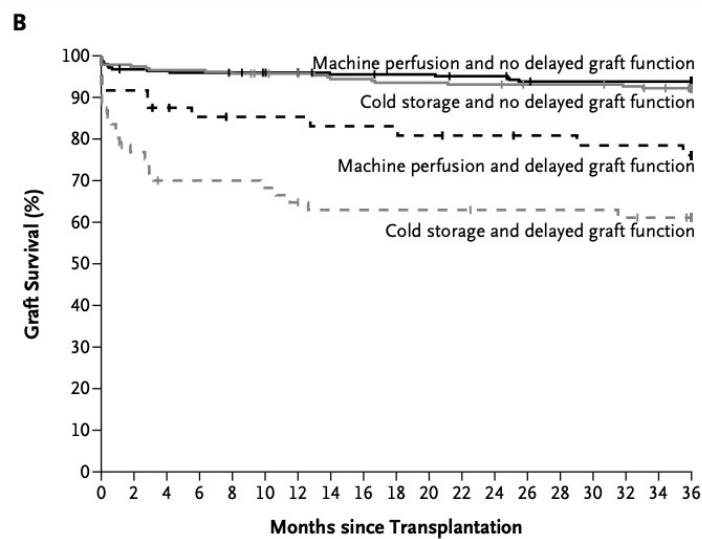
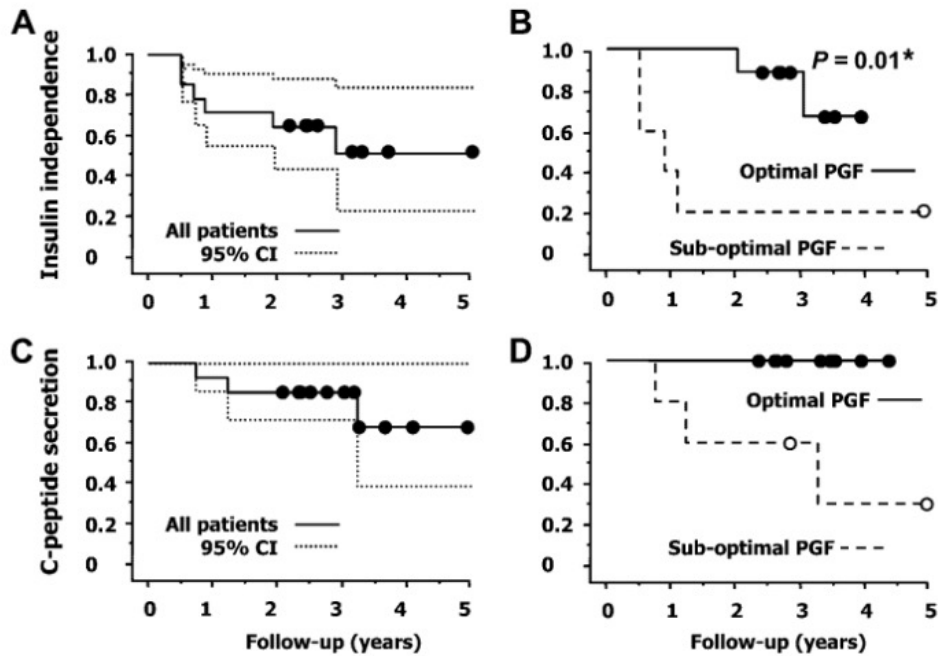


Figure 14: Analysis of 588 kidney recipients after transplantation from deceased donors. Patients who received a kidney graft with delayed graft function (i.e. using dialysis during the first 15 days after transplantation) and treated with hypothermic machine perfusion benefited from a better graft survival PGF optimized by hypothermic perfusion improved long-term survival of kidney grafts, regardless of the level of MHC matching (adapted from Moers and colleagues<sup>69</sup>).



*Figure 15: Kaplan-Meier (with CI 95%, dotted lines) estimates of the prevalence of insulin independence with good glucose control ( $HbA1c \leq 6.5\%$ ) (top panels) and graft survival (fasting serum C-peptide  $\geq 0.5$  ng/mL) (bottom panels) in the whole cohort of 14 consecutive islet transplant alone recipients (left panels) and in patients expected optimal vs suboptimal primary graft function following islet transplantation (adapted from Vantyghem and colleagues<sup>72</sup>)*

## 1.7 Objectives

The concept of primary islet graft function was firstly described in 2009 on 14 consecutive islet transplant alone recipients in Lille and has suggested its impact on transplantation success, i.e. islet graft survival and insulin independence at 3 years with IT.

The objectives of our work were to firstly explore the effect of the donor, recipient, transplantation, and PGF on IT outcomes in islet transplant alone and islet after kidney transplant recipients at 5 years on the Lille single-center cohort of patients under the Edmonton immunosuppressive regimen (article 1) and to confirm this impact on the long-term follow-up (10 years) in this cohort (article 2).

The second part of our work consisted in a collaborative study to refine the definition of IT success and in particular by studying the contribution of continuous glucose monitoring measures (IglS 2.0 criteria) (article 3).

Finally, the third and last part of our work was to demonstrate on the CITR registry, the largest international cohort with various transplantation and immunosuppression strategies, the impact of primary islet graft function on the 5-year success of allogeneic IT by using in particular the refine definition of success of IglS 2.0 criteria (article 4). In addition, we characterized the nature of the relationship between PGF and 5-year success, including the independence of PGF from other confounders known to impact islet outcomes and by characterizing its dose-response relationship on IT success.

We then focused on deriving and validating algorithms to predict the risk of the incidence of unfavorable IT outcomes based on the measurement of the primary islet graft function. We therefore developed an online web application to estimate the predicted risk of IT outcome

to assist the clinician in the decision to perform a new islet infusion based on the measure of the PGF, one month after the last islet infusion.

## Part 2: Methods and Results

## 2.1 Article 1: Optimizing primary graft function in islet allotransplantation: the Lille experience

Islet allotransplantation is a validated therapy to restore endogenous insulin secretion in patients with T1D and severe hypoglycemia, and/or following a previous kidney transplantation.

The international registry documented a progressive loss of islet graft survival and insulin independence over time

This origin is multifactorial involving donor factors, pancreas procurement, islet isolation and culture, transplantation and immunosuppression strategies and recipient related factors.

In this study, between 2003 and 2012, 28 adult T1D recipients with undetectable serum C-peptide levels benefited from islet transplantation at the Lille University Hospital: islet transplantation alone (ITA) was indicated for patients with severe hypoglycemia unawareness (n=14 patients, trial NCT00446264) and islet-after-kidney transplantation (IAK) (n=14 patients, trial NCT01123187).

The median islet mass (IQR) of the 28 transplanted patients was 13.5 (10.9-15.3) kIEQ/kg, in 3 (2-3) intraportal islet infusions, corresponding to a total of 74 infusions, mostly performed by minimally invasive laparotomy in 57/74 (77%) patients.

PGF, the exposure of the study, measured with the Beta-score at 1 month from the last islet infusion, was optimal in 18 patients (64%) (Beta-score of 7 or 8) and suboptimal in 10 patients (36%) (Beta-score < 7). Baseline patient and graft characteristics did not differ significantly between recipients with optimal or suboptimal PGF.

In this study, using an univariate Kaplan-Meier survival model, 82% (95% CI, 62-92) of patients had sustained graft survival (C-peptide  $\geq$  0.3 ng/mL) and 39% (95% CI, 22-57) were insulin-independent at 5 years. Optimal PGF recipients had a better 5-year graft survival rate than suboptimal PGF recipients with 100% vs 50% (18-75), respectively ( $P < 0.001$ ). Similarly, the proportion of insulin-independent patients was 56% (31-75) vs. 10% (1-36), ( $P < 0.001$ ) at 5 years, respectively. Only 24 % of the patients with suboptimal PGF vs. 81 % with optimal PGF recipients experienced IT success according to the IGLS 1.0 definition ( $P < 0.001$ ).

In conclusion, this prospective mono-centric study performed in Lille in 14 consecutive ITA and 14 IAK recipients, treated with standardized isolation, culture, transplantation and immunosuppression (Edmonton) procedures, showed that no recipient or donor factors were significantly associated with transplantation success (graft survival or insulin independence). There was a trend toward sustained insulin independence when increased total islet mass transplanted, in female recipients and with longer diabetes history in the recipient and a trend toward sustained graft survival in donors with shorter cold ischemia time.

On the other hand, patients experienced suboptimal PGF had a 6-fold higher risk of insulin reintroduction, and a 13-fold higher risk of graft failure at 5 years.

**This chapter (pages 637-643) “Optimizing primary graft function in islet allotransplantation: The Lille experience” edited by Giuseppe Orlando, Lorenzo Piemonti, Camillo Ricordi, Robert J. Stratta, and Rainer W.G. Gruessner was published as first author in 2020 in the following book “Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas (Academic Press).**



## Optimizing primary graft function in islet allotransplantation: The Lille experience

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### OUTLINE

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### Introduction

Islet allotransplantation is today a validated therapeutic option for restoring endogenous insulin secretion in patients with type 1 diabetes and severe hypoglycemia,<sup>1</sup> and/or a previous kidney transplantation.<sup>2</sup> The Collaborative Islet Transplant Registry (CITR)<sup>3</sup> now combines the results of more than 1000 transplanted patients (80% islet transplantation alone or ITA/20% islet after kidney or AK). Nearly 20 years after the landmark papers from the Edmonton group,<sup>4</sup> the latest report of the registry clearly confirmed the worldwide spread of islet transplantation and the reproducibility of its early success. On the other hand, the registry also documented the progressive decrease in time of islet graft survival with C-peptide secretion and insulin independence persisting respectively in only 45% and 25% of patients after 5 years. Fig. 1 illustrates the various mechanisms

that contribute to the progressive decline of transplanted islets.

One obvious factor for this decline is the allogenic and autoimmune rejection of islets by the recipient immune system.<sup>5</sup> This reaction is currently prevented or at least limited with immunosuppression, induced and maintained with a combination of several immunosuppressive drugs. Furthermore, a PET study imaging labeled islets following their intraportal infusion in a type 1 diabetic patients suggested that nearly 50% of islets are lost within only few hours.<sup>6</sup> Some authors proposed that this early islet loss is due to innate immunity involving both nonspecific inflammation reaction and the activation of coagulation and complement systems, so-called instant blood mediated inflammatory reaction (IBMIR).<sup>7,8</sup> Most teams are currently administrating high doses of heparin simultaneously to the islets to prevent portal thrombosis and optimize islet engraftment.<sup>9</sup> Finally, like for any

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|--------------------|----------------------|--------------------------------------|-------------------|
| 1. Organ donation  | 4. Hypoxia           | 7. Alloimmunity                      | 10. Glucotoxicity |
| 2. Islet isolation | 5. Innate immunity   | 8. Autoimmunity and islet exhaustion |                   |
| 3. Culture         | 6. Revascularization | 9. Xenobiotics                       |                   |

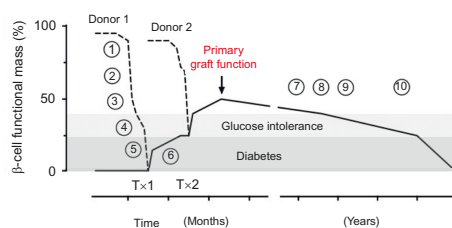


FIG. 1 Outline representation of early- and long-term factors involved in beta-cell functional mass failure. Tx, transplantation.

other allogenic transplantation, the initial function of the graft is essential. In islet transplantation this functional mass depends not only on donor characteristics and the conditions of organ procurement but also on the outcome of islet isolation and/or culture prior to their transplantation. It is therefore highly heterogeneous. For that reason we hypothesized that increasing “primary islet graft function” by optimizing islet quality and quantity could improve long-term cell survival and function. Noteworthy, most successful cases of islet transplantation for type 1 diabetes were initially described after the transplantation of islets combined from several donors, that were by chance simultaneously available.<sup>10</sup> For that reason, we proposed to favor the deliberate sequential and repeated infusion of islets from several donors. This implied that under efficient immunosuppression, islet transplantation from multiple and non-MHC-matched donors would be superior to the strategy of exclusive transplantation with islets from a single, well MHC-matched donor. Likewise, the better outcomes of kidney grafts preserved with continuous perfusion<sup>11</sup> or obtained from nonrelated living donors,<sup>12</sup> demonstrated that the influence of the quality of the graft outstripped that of MHC matching in kidney transplantation. We first demonstrated the safety of sequential multiple islet infusions through a percutaneous catheter surgically placed in the portal vein in a minipig preclinical model.<sup>13</sup> This technique was then initially translated in three T1D patients who received sequential infusions of islets isolated from up to four donors within 10 days.<sup>14</sup> These first clinical cases confirmed the relevance of this strategy to significantly increase primary graft function. Based on a similar sequential islet infusions but with an optimized, corticoid free, immunosuppression regimen, the outstanding results reported by the Edmonton team<sup>4</sup> convinced us to adopt this deliberate multiple infusion strategy for the upcoming clinical trials in Lille. In the present report we specifically analyzed the association

between primary graft function and long-term outcome of islet allotransplantation in 28 consecutive T1D patients.

## Methods

### Patients

Between 2003 and 2012, 28 consecutive T1D patients aged 18–65 years, with blood arginine-stimulated C-peptide level  $<0.2$  ng/mL, received an islet transplantation at Lille University Hospital (islet transplantation alone (ITA) for severe hypoglycemia in 14 patients—NCT00446264; and islet after kidney (IAK) transplantation in 14 patients with a functioning kidney graft—NCT01123187). The overall long-term results outcome of these patients has been recently reported elsewhere.<sup>15</sup> In the present report we analyzed more specifically the impact of primary graft function on 5-year outcome.

### Transplantation

Islet transplantation consisted of two or three fresh sequential islet infusions to reach 10,000 IEQ/kg of the recipient body weight. Human islets were isolated from ABO-compatible pancreata from deceased donors, and transplanted in case of negative cross-match with satisfactory islet viability and number. The success criterion was adequate metabolic control without exogenous insulin. We applied the steroid-free immunosuppression provided by Edmonton/University of Alberta,<sup>4</sup> consisting of tacrolimus, target trough levels 3–6 ng/mL, sirolimus, target trough levels 12–15 ng/mL for 3 months and at 7–10 ng/mL thereafter. A five-dose induction course of daclizumab (1 mg/kg) was also administered bimonthly, beginning 1 h before the first infusion as previously described.<sup>15</sup>

### Sequential multiple infusions in Lille

Each islet preparation should contain at least 4000 IEQ/kg with a purity of at least 30% and a tissue volume of less than 0.15 mL/kg of the recipient's body weight. Heparin (35 units/kg) was added to the final human islet preparation, gently infused by gravity under portal pressure monitoring, in 21 (15–25) min. Islet infusions were initially performed with percutaneous radiological catheterization of a peripheral portal branch under ultrasound guidance. An alternative surgical route was first developed to limit the risk of bleeding in IAK recipients who were treated chronically by aspirin.<sup>16</sup> This approach was progressively adopted as the main procedure in all patients.

## Results

### Patient and graft characteristics

The 28 patients received a median (IQR) islet mass of 13.5 (10.9–15.3) kIEQ/kg, in 3 (2–3) intraportal islet infusions, corresponding to a total of 74 islet infusions, predominantly performed surgically (57/74 (77%).<sup>16</sup> The median portal pressure postinfusion remained stable at the end of the first, second, and third infusions with 11 (8–13), 12 (8–15), and 11 (8–15) mmHg, respectively

(Fig. 2A). The median portal pressure before second: 10 (7–13) and third: 8 (7–12) islet infusions did not increase significantly from the first islet infusion: 9 (6–11) mm Hg (Fig. 2A).

Plasma c-peptide at day 7 postinfusion increased significantly from undetectable to 0.9 (0.5–1.3) ng/mL after first infusion to 1.4 (1–2) ng/mL ( $P = 0.0191$ ) after the second and 1.5 (1–1.8) ng/mL ( $P = 0.0116$ ) after the third infusion (Fig. 2B). Fig. 2C shows the Kaplan-Meier estimates of insulin withdrawal following first infusion. All 28 patients could stop insulin following islet transplantation. The median time to quit exogenous insulin following the first infusion was 68 days with 75% of the patients who had discontinued insulin by 3 months (Fig. 2C and D).

### Primary graft function

Primary graft function (PGF) was estimated by measuring HbA1C, fasting glucose, daily insulin dose, and plasma c-peptide, 1 month after the last infusion (Fig. 3). The Beta-score was calculated as previously described.<sup>17</sup> PGF was considered optimal in 18 patients (64%) who had a Beta-score of 7 or 8 at 1 month and suboptimal in 10 patients (36%) when Beta-score was less than or equal to 6. Baseline patient and graft characteristics did

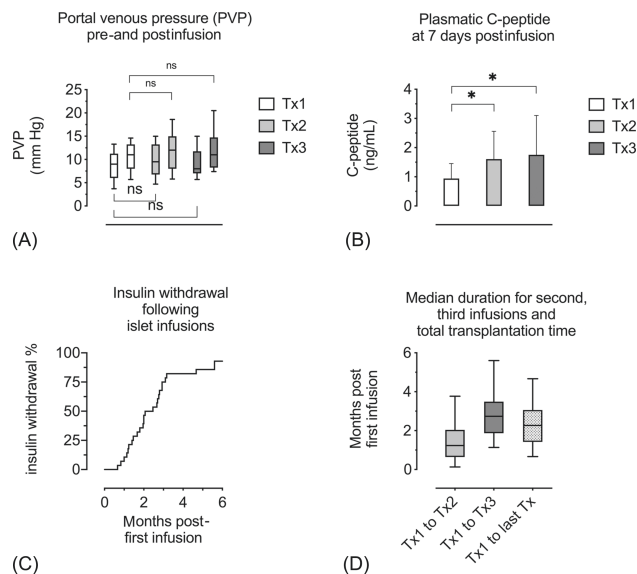
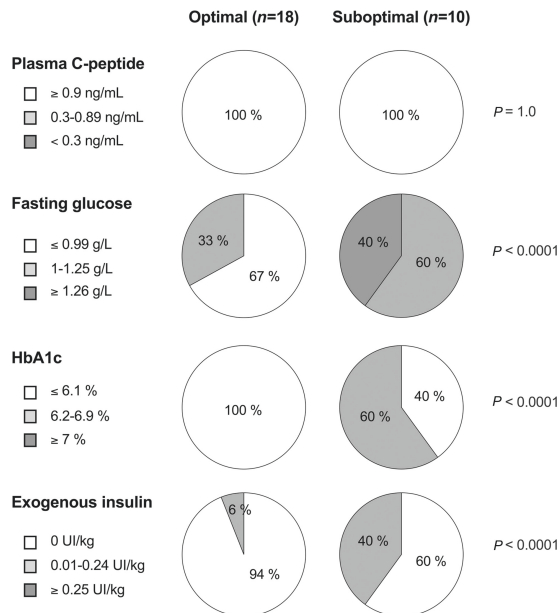


FIG. 2 Portal venous pressure monitoring in the first, second, and third islet infusion (A), C-peptide increase at day 7 postinfusion (B, one-way ANOVA), insulin withdrawal postislet infusion (C), and median time from first infusion to second, third transplantations, and total transplantation duration (D). \*,  $P$ -value < 0.05; ns, nonsignificant; Tx, transplantation.



**FIG. 3** Details of beta-score determinants measured in the optimal ( $n=18$ ) and suboptimal ( $n=10$ ) PGF recipients. Primary graft function (PGF) was evaluated 1 month after the last islet infusion with the beta-score, a previously validated composite index ranging from 0 (no graft function) to 8 (excellent graft function). This score gives two points for normal fasting glucose ( $\leq 0.99$  g/L or  $\leq 5.5$  mmol/L), HbA1c  $\leq 6.1\%$  (43 mmol/mol), stimulated and/or basal C-peptide ( $\geq 0.9$  ng/mL or  $\geq 0.3$  nmol/L), and absence of insulin or oral hypoglycemic agent use. No point is awarded if fasting glucose is in the diabetic range ( $\geq 1.26$  g/L or  $\geq 7$  mmol/L), HbA1c is  $\geq 7\%$  (53 mmol/mol), C-peptide secretion is  $< 0.3$  ng/mL ( $< 0.1$  nmol/L), or daily insulin use is  $\geq 0.25$  units/kg. One point is given for intermediate values. Graft function was considered optimal when the beta score was seven or eight, suboptimal when the beta score was four to six, and poor when the beta score was three or less. Optimal and suboptimal recipients were compared with Chi-square or Fisher exact tests.

not differ significantly between recipients with optimal and suboptimal PGF (Table 1).

Primary graft function (PGF) was estimated by measuring HbA1C, fasting glucose, daily insulin dose, and plasma c-peptide, 1 month after the last infusion (Fig. 3). The Beta-score was calculated as previously described.<sup>17</sup> PGF was considered optimal in 18 patients (64%) who had a Beta-score of 7 or 8 at 1 month and suboptimal in 10 patients (36%) when Beta-score was less than or equal to 6. Baseline patient and graft characteristics did not differ significantly between recipients with optimal and suboptimal PGF (Table 1).

Fig. 4 shows the rate of graft survival (C-peptide  $\geq 0.3$  ng/mL) and insulin independence (associated with a glycated hemoglobin less than or equal to 6.5%) following islet transplantation in the 28 patients. At 5 years, graft function and insulin independence were maintained respectively in 82% (62–92) and 39% (22–57) of patients (Fig. 4A and B). Patients with optimal PGF experienced a better graft survival rate at 5 years than recipients with suboptimal PGF with 100% vs 50% (18–75), respectively

( $P < 0.01$ ) (Fig. 4C). The proportion of patients remaining insulin independent with HbA1c inferior to 6.5% at 5 years was also superior in recipients who experienced optimal PGF as compared to those with suboptimal PGF: 56% (31–75) vs 10% (1–36), respectively ( $P < 0.001$ ) (Fig. 4D).

The association between patient (gender, age, BMI, diabetes duration, autoimmunity, metabolic balance with continuous glucose monitoring, number of severe hypoglycemic events, and kidney function) and graft characteristics at baseline (donor characteristics, human islet quality proxies, and islet mass infused) was studied in a Cox proportional-hazards model for insulin reintroduction and graft loss. As illustrated in Fig. 5, PGF was significantly associated with both outcomes. Overall, patients with suboptimal PGF had a 6-fold higher risk of insulin reintroduction at 5 years, and a 13-fold higher risk of graft loss. Donor's characteristics, autoimmunity status at baseline, IAK or ITA status and time to achieve infusions did not seem to impact long-term insulin-independence. Noteworthy, these results were

TABLE 1 Patients and transplantation characteristics at baseline in optimal and suboptimal recipients

	Optimal PGF patients, <i>n</i> = 18	Suboptimal PGF patients, <i>n</i> = 10	<i>P</i> -value
<b>Patient characteristics at baseline</b>			
IAK recipient	9 (50%)	5 (50%)	1
Gender female	11 (61)	4 (40)	0.433
Age (years)	43 (37–51)	43 (37–49)	0.891
BMI (kg/m <sup>2</sup> )	22.8 (21.3–24.4)	23.5 (22.5–25.1)	0.491
Diabetes duration (years)	30 (25–36)	24 (16–28)	0.059
GAD/ICA/IA2 autoantibody	8 (44)/2 (12)/1 (6)	4 (44)/3 (30)/0	1/0.326/1
Exogenous insulin requirements (UI/day/kg)	0.5 (0.4–0.6)	0.7 (0.5–0.8)	0.099
HbA1c (%)	8 (7.3–8.9)	8.6 (7.3–9.2)	0.593
HbA1c (mmol/mol)	64 (56–74)	70 (56–77)	0.593
Mean glucose (CGM, mg/dL)	144 (125–203)	148 (137–220)	0.306
SD of mean glucose (CGM, mg/dL)	56 (42–77)	72 (61–87)	0.260
Time spent with glycemia <70 mg/dL (CGM, % of total time)	11 (3–16)	9 (2–17)	0.710
Severe hypoglycemia events (per year)	2 (1–5)	1.5 (1–7)	0.950
eGFR (mL/min/1.73 m <sup>2</sup> )	63 (58–74)	73 (63–88)	0.291
<b>Graft characteristics</b>			
Donor's age (years)	46 (37–52)	43 (37–52)	0.7372
Donor's BMI (kg/m <sup>2</sup> )	28.9 (25.7–31.8)	25.3 (24.1–26.9)	0.125
Cold ischemia time (min)	354 (307–382)	313 (283–368)	0.3623
Time between first and last infusion (days)	71 (50–88)	67 (42–140)	1
Total islet mass (10 <sup>3</sup> IEQ/kg)	14.1 (10.34–15.67)	13.32 (12.22–13.88)	0.6316
Exclusive surgical transplantation	12 (67)	4 (40)	0.2425

PGF, primary graft function; IAK, islet after kidney recipient; BMI, body mass index; CGM, continuous glucose monitoring; SD, standard deviation; IEQ, islet-equivalent; No., number; eGFR, estimated glomerular filtration rate. Variables are presented with *n* (%) or Median (Quartile1–Quartile3); optimal and suboptimal recipients were compared with Wilcoxon-Mann-Whitney's test and Fisher's Exact test when appropriate.

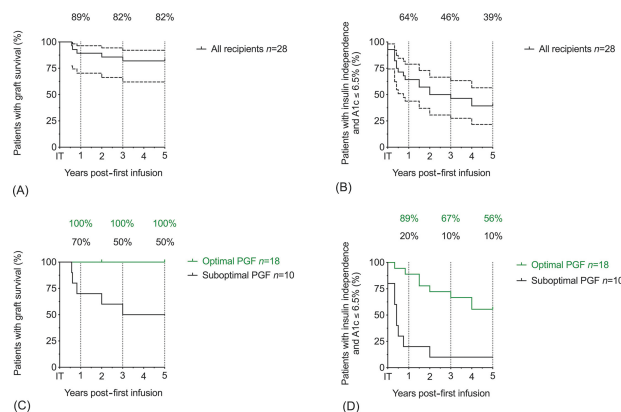


FIG. 4 Kaplan-Meier estimate of graft survival (A) (C-peptide  $\geq 0.3$  ng/mL) and insulin independence plus HbA1c  $\leq 6.5\%$  (B) in the whole cohort of 28 recipients and in optimal (*n* = 18) and suboptimal (*n* = 10) PGF recipients (C and D). PGF, primary graft function.

#### B. Islet allo-transplantation

Donor's and graft characteristics	Insulin reintroduction		Graft loss	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Age (years)	0.97 (0.95-1.006)	0.5747	0.97 (0.812-1.081)	0.3714
BMI (kg/m <sup>2</sup> )	1.105 (0.907-1.348)	0.3216	1.182 (0.807-1.73)	0.3904
Cold ischemia time (min)	1.0 (0.995-1.009)	0.8649	1.004 (0.999-1.009)	0.0918
Time between first and last infusion (days)	0.996 (0.907-1.025)	0.4161	0.989 (0.905-1.1214)	0.389
Total islet mass (IEQ/kg)	0.852 (0.721-1.006)	0.0585	1.104 (0.821-1.485)	0.5131
Exclusive surgical transplantation	0.492 (0.189-1.281)	0.1464	0.487 (0.078-2.799)	0.4045
Optimal PGF	0.169 (0.002-0.461)	9e-04	0.079 (0.01-0.277)	0.0025

Recipient's characteristics at baseline		Insulin reintroduction		Graft loss	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	P-value
ITA recipient	1.023 (0.394-2.655)	0.9628	1.474 (0.246-8.825)	0.6707	
Female gender	0.41 (0.155-1.088)	0.0733	0.202 (0.023-1.816)	0.1535	
Age (years)	0.981 (0.934-1.031)	0.4497	0.986 (0.905-1.075)	0.7518	
BMI (kg/m <sup>2</sup> )	0.963 (0.862-1.077)	0.5119	0.822 (0.618-1.092)	0.176	
Diabetes duration (years)	0.957 (0.911-1.005)	0.0794	0.95 (0.866-1.043)	0.2827	
Diabetes autoimmunity*	1.323 (0.587-3.391)	0.3872	1.719 (0.287-10.29)	0.5528	
Exogenous insulin requirements (U/day/kg)	7.288 (0.676-111.987)	0.1562	0.48 (0.004-39.276)	0.7562	
HbA1c (%)	1.185 (0.753-1.865)	0.4625	1.327 (0.564-3.121)	0.5167	
Mean glucose (CGM, mg/dL)	1.002 (0.99-1.015)	0.7735	0.993 (0.972-1.015)	0.5282	
SD of mean glucose (CGM, mg/dL)	1.013 (0.981-1.024)	0.8142	0.997 (0.906-1.037)	0.8627	
% of time spent in hypoglycemia (<70 mg/dL, CGM)	0.992 (0.942-1.045)	0.7649	1.015 (0.828-1.111)	0.7393	
Severe hypoglycemia events (per year)	1.059 (0.827-1.21)	0.3963	0.947 (0.712-1.259)	0.7067	
eGFR (mL/min/1.73m <sup>2</sup> )	1.01 (0.98-1.041)	0.5138	1.042 (0.879-1.189)	0.199	

FIG. 5 Donor, graft, and recipient characteristics associated with insulin reintroduction and graft loss at 5 years postislet transplantation represented with Forest plot. The impact of donor and graft characteristics were analyzed using a Cox proportional hazard model on insulin reintroduction and graft loss at 5 years posttransplantation in 28 consecutive islet recipients, expressed with their respective hazard ratio and 95% confidence intervals. \*At least one diabetes autoantibody present at baseline. BMI, body mass index; CGM, continuous glucose monitoring; SD, standard deviation; kIEQ, 10<sup>3</sup> islet-equivalent; PGF, primary graft function; ITA, islet transplantation alone recipient; eGFR, estimated glomerular filtration rate (MDRD).

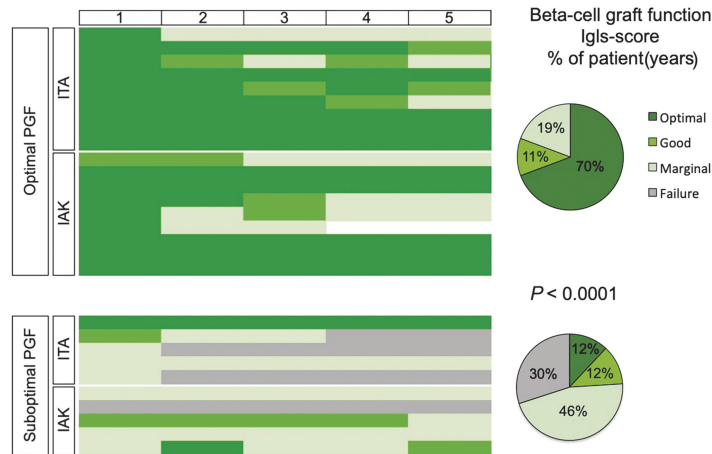


FIG. 6 Longitudinal follow-up diagram of beta-cell function according to the EPITA-IPITA IglS-score (optimal, good, marginal, and failure) in optimal and in suboptimal primary graft function recipients. For each optimal and suboptimal group, results are presented in % of total time. The proportions of time spent were significantly different between optimal and suboptimal primary graft function recipients ( $P < 0.0001$ , Chi-square test). ITA, islet transplantation alone recipient; IAK, islet after kidney transplantation recipient; PGF, primary graft function.

confirmed when analyzed at 10 years,<sup>15</sup> as well as the protective impact, although to a lesser extent, of female recipient gender, long history of diabetes previous to islet transplantation, and higher total infused islet mass from insulin reintroduction.

Fig. 6 summarizes the 5-year individual evolution of beta cell function according to the IPTA-EPITA IglS

score,<sup>18</sup> in patients with optimal or suboptimal PGF. According to this new classification, successful beta cell replacement (i.e., optimal or good graft function) was maintained during 81% of time when PGF was optimal vs 24% when PGF was suboptimal ( $P < 0.0001$ ). There was no difference between recipients of islet alone and islet after kidney transplantation.

B. Islet allo-transplantation

### Discussion and perspectives

This observational cohort study confirmed that long-term successful beta cell replacement can be obtained with islet transplantation when optimal PGF is initially achieved.<sup>19</sup> Our results suggest that, in addition to the development of better tolerated strategies to combat auto-immune and allogenic reactions, optimizing the primary function is crucial for improving the overall outcome of islet transplantation. In islet transplantation, the first and obvious determinant of PGF is the functional mass of islets available for transplantation. We, like many others, have deployed in past decades many efforts to identify optimal donors,<sup>20</sup> to improve pancreas harvesting and preservation from available donors<sup>21</sup> and to refine the procedures used to isolate and culture islets.<sup>22</sup> However, despite all these improvements, the outcome of islet isolation has remained most often insufficient to achieve optimal PGF after the transplantation of islets obtained from only one donor. On the other hand, the benefit of the proposed strategy of initial repeated islet infusion is hampered by donor pancreas availability. Transplanting islets from multiple donors also carries the risk of allogenic immunization that could limit future access to renal transplantation. For these reasons, the ultimate goal remains the description of an alternative and less limited source of insulin-secreting cells. In the meantime, further improvements in islet preparation from deceased donors are still needed to significantly increase the number and/or function of islets isolated from a single pancreas, and thus avoid the need for repeated islet infusions. Other advances, in alternative transplant sites or peritransplant adjuvant therapies, may eventually further contribute to increase islet engraftment and foster PGF. Eventually, our results also suggest that PGF, as a relevant proxy of long-term outcome of islet allotransplantation, could serve as a valuable early endpoint in future clinical trials.

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## 2.2 Article 2: Ten-year outcome of islet alone or islet after kidney transplantation in type 1 diabetes: a prospective parallel-arm cohort study

In 2019, the long-term outcome ( $\geq 10$  years) of allogeneic IT was largely unknown with no evidence of prospective cohort exploring long-term metabolic results in IT recipients.

This report explored in a parallel cohort design 28 T1D recipients who were treated with islet transplantation either alone (14 ITA) or after kidney transplantation (14 IAK) according to the Edmonton protocol and followed up to ten years.

Patients received two or three intraportal allogeneic islets infusions administered within 68 days (43-92).

28% (13-45) of patients remained insulin independent 10 years after IT, respectively (KM estimates, 95% CI). Graft function was sustained in 78% (57-89) of recipients after 10 years, respectively,

Optimal primary graft function, assessed 1 month after the last islet infusion with the Beta-score (optimal PGF : Beta-score of 7 and 8), was significantly associated with sustained graft function and insulin independence and was associated with improved glycemic control, decreased need for exogenous insulin and a marked decrease in severe hypoglycemic events after transplantation.

There was no difference between ITA and IAK recipients in metabolic outcome in the follow-up. In this report in an univariate Cox regression analysis, increased total islet mass transplanted, female recipients and longer history of diabetes recipients before



transplantation confirmed to be associated with sustained insulin independence during the follow-up.

**This article “Ten-year outcome of islet alone or islet after kidney transplantation in type 1 diabetes: a prospective parallel- arm cohort study” was published as first co-author in 2019 in Diabetes Care journal.**



# Ten-Year Outcome of Islet Alone or Islet After Kidney Transplantation in Type 1 Diabetes: A Prospective Parallel-Arm Cohort Study

<https://doi.org/10.2337/dc19-0401>

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CLIN CARE/EDUCATION/NUTRITION/PSYCHOSOCIAL

## OBJECTIVE

The long-term outcome of allogenic islet transplantation is unknown. The aim of this study was to evaluate the 10-year outcome of islet transplantation in patients with type 1 diabetes and hypoglycemia unawareness and/or a functioning kidney graft.

## RESEARCH DESIGN AND METHODS

We enrolled in this prospective parallel-arm cohort study 28 subjects with type 1 diabetes who received islet transplantation either alone (ITA) or after a kidney graft (IAK). Islet transplantation consisted of two or three intraportal infusions of allogenic islets administered within (median [interquartile range]) 68 days (43–92). Immunosuppression was induced with interleukin-2 receptor antibodies and maintained with sirolimus and tacrolimus. The primary outcome was insulin independence with A1C  $\leq$  6.5% (48 mmol/mol). Secondary outcomes were patient and graft survival, severe hypoglycemic events (SHEs), metabolic control, and renal function.

## RESULTS

The primary outcome was met by (Kaplan-Meier estimates [95% CI]) 39% (22–57) and 28% (13–45) of patients 5 and 10 years after islet transplantation, respectively. Graft function persisted in 82% (62–92) and 78% (57–89) of case subjects after 5 and 10 years, respectively, and was associated with improved glucose control, reduced need for exogenous insulin, and a marked decrease of SHEs. ITA and IAK had similar outcomes. Primary graft function, evaluated 1 month after the last islet infusion, was significantly associated with the duration of graft function and insulin independence.

## CONCLUSIONS

Islet transplantation with the Edmonton protocol can provide 10-year markedly improved metabolic control without SHEs in three-quarters of patients with type 1 diabetes, kidney transplanted or not.

The demonstration in 2000 that  $\beta$ -cell replacement with allogenic islet transplantation could restore endogenous insulin secretion and near-normal glucose homeostasis was an important landmark for the treatment of type 1 diabetes (1). Since then, islet transplantation has been offered worldwide in >1,000 patients with type 1

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M.-C.V. and M.C. contributed equally to this work.

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diabetes and hypoglycemia unawareness and/or a kidney graft for end-stage renal disease (2). The favorable early benefit-risk profile of islet transplantation has been reported by numerous single and multicenter studies (3–10), and confirmed in the international Collaborative Islet Transplantation Registry (CITR) (11). Furthermore, islet transplantation appeared superior to optimized medical treatment in several case-control studies (12–15), and a multicenter randomized controlled trial recently demonstrated that islet transplantation was associated with better glucose control at 6 months (16). Other studies also suggest that islet transplantation improves quality of life (16,17) and may favorably impact chronic diabetic complications (18–22). On the other hand, islet graft function may decline with time (4,11), and chronic immunosuppression has been associated with serious adverse events (SAEs) and a decrement in renal function (4,9,11,12). Moreover, the persistence of the early benefit of islet transplantation beyond 5 years can only be speculated from a few series of selected cases (23–28).

Therefore, the aim of the current study was to evaluate the 10-year outcome, in intention to treat, with islet transplantation in patients with type 1 diabetes and hypoglycemia unawareness and/or a functioning kidney graft initially included in two clinical trials. The secondary objectives were to explore the determinants of long-term successful  $\beta$ -cell replacement with islet transplantation.

## RESEARCH DESIGN AND METHODS

### Study Design

This observational, prospective, parallel-arm, cohort study was designed to evaluate the long-term outcome of allogeneic islet transplantation in patients with type 1 diabetes. We enrolled all participants from two single-arm, single-center, phase 2 studies initiated in 2003 at Lille University Hospital to evaluate the 1-year outcome of islet transplantation, performed either alone (ITA) in nonuremic patients (NCT00446264) or after a kidney graft (IAK) in uremic patients (NCT01123187). Study protocols were approved by the institutional review board, and a signed informed consent was obtained from each patient, as previously described (10). The 28 consecutive participants in these two studies received islet transplantation between

13 March 2003 and 1 December 2012. As initially planned for each study, the enrollment was interrupted when the primary outcome (80% insulin independence with adequate glucose control after 1 year) was confirmed in the first 14 participants (sequential triangular design). Participants gave written informed consent to pursue follow-up beyond the 1st year and attended at least yearly routine hospital visits up to 10 years after islet transplantation. The database was frozen on 22 December 2017.

### Patients

Enrolled subjects had type 1 diabetes documented for  $>5$  years at the time of islet transplantation and arginine-stimulated C-peptide  $<0.3$  ng/mL. Nonuremic patients had hypoglycemia unawareness and/or documented metabolic lability and an estimated glomerular filtration rate (eGFR)  $>60$  mL/min/ $1.73$  m<sup>2</sup>. Uremic patients had a kidney graft with stable renal function, no episode of kidney graft rejection, and blood pressure in the normal range whatever the use of antihypertensive drugs. In these patients, simultaneous pancreas transplantation had been refused because of age  $>45$  years, severe macroangiopathic complications, or by patient's choice, or performed but followed by a nonimmune complication requiring pancreas graft explantation. In all cases, exclusion criteria included an age  $<18$  or  $>65$  years, a BMI  $\geq 28$  kg/m<sup>2</sup>, albuminuria  $>300$  mg/24 h, unstable arteritis or heart disease, active infection, insulin daily requirements  $>1.2$  units/kg, history of malignancy, smoking, desire for pregnancy, psychiatric disorders, and lack of compliance.

### Islet Transplantation

Islet transplantation consisted of up to three sequential islet infusions within 3 months, with the aim of reaching adequate metabolic control without exogenous insulin. Islets were isolated from pancreata harvested in ABO blood type-compatible deceased donors with a negative cross-match (10). The access to the portal vein was gained under general anesthesia by percutaneous catheterization of a peripheral portal branch under ultrasound guidance or by surgical catheterization of a small mesenteric vein. In all cases, heparin (35 units/kg)

was added to the final islet preparation, gently infused by gravity with portal pressure monitoring.

### Immunosuppression

The immunosuppression consisted of tacrolimus (Prograf; Astellas, Paris, France), target trough levels at 3–6 ng/mL, and sirolimus (Rapamune; Wyeth Pharmaceuticals, Paris, France), target trough levels at 12–15 ng/mL for 3 months and at 7–10 ng/mL the first year and 5–6 ng/mL thereafter. A five-dose induction course of daclizumab (1 mg/kg) (Zenapax; Roche, Welwyn Garden City, U.K.) was administered biweekly beginning 1 h before the first infusion. For IAK, the median (interquartile range) elapsed time between kidney and islet transplantation was 22 months (18–38). The kidney transplantation had been performed with a standard-of-care protocol, i.e., in most cases antithymocyte antibodies, mycophenolate, and tacrolimus with an initial bolus of 1 g of prednisolone. Steroids had been progressively tapered over 3–9 months until complete discontinuation if there was no sign of kidney rejection. About 12 months after kidney transplantation, mycophenolate was progressively switched to sirolimus to reach blood trough sirolimus levels of 7–10 ng/mL and tacrolimus levels around 5 ng/mL. The blood pressure and renal function had to be normal. When an islet preparation was available, a course of anti-interleukin-2 receptor antibody was performed, repeated for each of the two or three islet injections performed over 3 months.

### Follow-up

A comprehensive clinical and biological evaluation was performed before islet transplantation and each year after the first islet infusion, with intermediate routine clinical visits at least twice per year. Daily exogenous insulin requirements, antidiabetic treatments, and adverse events were recorded at each visit. Exogenous insulin was reintroduced when A1C increased above 6.5% (48 mmol/mol) on two consecutive measurements. The following parameters were analyzed using standardized methods unless otherwise indicated: daily glucose profile (mean glucose, SD around mean glucose and percentage of time spent in hypoglycemia  $<70$  mg/dL) assessed with continuous glucose

monitoring (CGM; Medtronic MiniMed, Northridge, CA) for three consecutive days, fasting and postprandial blood glucose and C-peptide (RIA-coat C-peptide; Mallinckrodt, Paris, France) (detection threshold 0.2 ng/mL), plasma creatinine, A1C, and tacrolimus and sirolimus trough levels. The presence and type of autoantibodies GAD, islet cell antibody (ICA), and IA2 were evaluated before transplantation, after each islet infusion, yearly during the follow-up, and, in case of graft loss, 3 months after discontinuation of immunosuppression.

#### Study Outcomes

The primary outcome was insulin independence, defined as the absence of exogenous insulin therapy associated with  $A1C \leq 6.5\%$  (48 mmol/mol). Secondary outcomes were patient survival, yearly incidence of severe hypoglycemic events (SHEs), graft function defined as fasting plasma C-peptide  $\geq 0.3$  ng/mL, metabolic control assessed by A1C, the CGM daily glucose profile, and the daily exogenous insulin requirement. Primary graft function was evaluated 1 month after the last islet infusion with the  $\beta$ -score, a previously validated composite index ranging from 0 (no graft function) to 8 (excellent graft function) (29,30). This score gives two points for normal fasting glucose ( $\leq 5.5$  mmol/L),  $A1C \leq 6.1\%$  (43 mmol/mol), stimulated and/or basal C-peptide ( $\geq 0.3$  nmol/L), and absence of insulin or oral hypoglycemic agent use. No point is awarded if fasting glucose is in the diabetic range ( $\geq 7$  mmol/L),  $A1C$  is  $\geq 7\%$  (53 mmol/mol), C-peptide secretion is undetectable on stimulation, or daily insulin use is  $\geq 0.25$  units/kg. One point is given for intermediate values. Graft function was considered optimal when the  $\beta$ -score was 7 or 8, suboptimal when the  $\beta$ -score was 4–6, and poor when the  $\beta$ -score was 3 or less.

We also analyzed renal function with the eGFR calculated with the MDRD formula. Adverse events were classified according to the National Cancer Institute common terminology criteria for adverse events (version 3.0). SAEs (grades 3–5) were monitored and classified as most likely related to the islet transplantation procedure, immunosuppression, or diabetes complications.

#### Statistical Analysis

All results available at each time point were analyzed in intention to treat (i.e., including patients who had lost graft function and stopped immunosuppression) and expressed as medians (and interquartile range) for continuous variables and as frequencies (and percentages) for categorical variables, without any imputation. Continuous variables were compared between groups with the Mann-Whitney *U* test. Discrete variables were compared with Fisher exact tests. To test the effect of time on the evolution of metabolic and renal measurements, a linear mixed model was applied with the “patient” effect considered as a random effect. Graft function and insulin independence survival rates were estimated with the Kaplan-Meier model. The impact of patient and graft characteristics on these survival rates were estimated with a Cox proportional hazards regression model. A *P* value  $< 0.05$  was considered significant. All statistical analyses were performed with SAS Studio Statistics (version 3.71) and Prism GraphPad (version 8.0.0) software.

## RESULTS

#### Patient Characteristics

A total of 28 patients (14 nonuremic and 14 uremic) were enrolled. The patient characteristics prior to transplantation are presented in Table 1. Three uremic patients had received previous pancreas transplantation (two simultaneously to and one after a kidney graft) and experienced a nonimmunological failure of the pancreas. Each patient initially received two ( $n = 10$ ) or three ( $n = 18$ ) infusions delivered within 68 days (43–91), and, overall, 74 islet infusions were performed. No supplementary islet infusion was performed during the follow-up. At baseline, the clinical and biological characteristics of patients and grafts were not different between uremic and nonuremic patients, except for renal function and BMI (Table 1). Primary graft function, calculated 1 month after the last islet infusion (see RESEARCH DESIGN AND METHODS), was optimal in 18 patients (64%) and suboptimal in 10 patients (36%).

#### Patient Follow-up

The median follow-up duration was 11.5 years (8.9–12.9), corresponding to a total of 298 patient-years. One IAK patient

with a previous leg amputation died of a stroke 35 months after islet transplantation, with functioning islet and kidney grafts, and 27 patients were alive at the time of this analysis. The overall mortality rate was 0.3% per 100 patient-years. One ITA patient who had lost graft function declined follow-up after the 5-year visit, and one IAK patient moved from the region with a functioning islet graft after the 6-year visit. All other participants had attended each yearly visit, and at the time of this analysis, 27 (96%) and 20 (71%) of the patients initially enrolled completed the 5- and 10-year visits, respectively (Table 2).

#### Primary Outcome

After islet transplantation, exogenous insulin could be interrupted in all 28 patients, 91 days (61–115) after the first islet infusion. Overall, the Kaplan-Meier estimates of patients remaining off insulin with  $A1C \leq 6.5\%$  (48 mmol/mol) were 39% (22–57) at 5 years and 28% (13–45) at 10 years (Fig. 1A). These figures did not differ significantly between ITA and IAK recipients (Fig. 1B). Among the five patients that were insulin independent at 10 years, three patients had received oral antidiabetic medications after 5, 7, and 8 years. In a Cox proportional hazards univariate regression analysis, optimal primary graft function, female sex, longer history of diabetes, and total islet mass infused were associated with retention of insulin independence with  $A1C \leq 6.5\%$  after 10 years (Supplementary Table 1).

In patients who experienced optimal primary graft function, the median duration of insulin independence associated with  $A1C \leq 6.5\%$  (48 mmol/mol) was 6 years (1.9–10) vs. 0.4 years (0.2–1.1) in those with suboptimal primary graft function (hazard ratio [HR] 0.19 [0.08–0.48],  $P = 0.0004$ ) (Fig. 1C and Supplementary Table 1).

#### Secondary Outcomes

At last follow-up, graft function persisted in 20 patients (10 ITA and 10 IAK). Six patients lost their graft function while they were still under immunosuppression, 7, 15, 35, and 89 months after ITA and 7 and 10 months after IAK.

The Kaplan-Meier estimates of graft survival were 82% (62–92) and 78% (57–89) after 5 and 10 years, respectively, in the entire study group (Fig. 1D).

**Table 1—Baseline patient and graft characteristics of the entire study group and comparison of ITA and IAK recipients before islet transplantation**

	All recipients (n = 28)	ITA recipients (n = 14)	IAK recipients (n = 14)	P value, ITA vs. IAK
Sex male	13 (46)	7 (50)	6 (43)	1
Age (years)	43 (37–50)	42 (36–51)	44 (40–49)	0.6130
BMI (kg/m <sup>2</sup> )	22.9 (21.3–24.6)	24.6 (22.9–25.9)	22.6 (20.2–22.9)	0.0012
Diabetes duration (years)	28 (24–31)	28 (17–31)	30 (24–34)	0.3749
Exogenous insulin requirements (IU/kg per day)	0.57 (0.41–0.74)	0.6 (0.42–0.73)	0.54 (0.39–0.74)	0.5757
No. of severe hypoglycemia events in previous year	2 (1–5)	3 (1–7)	2 (0–3)	0.4084
No. of autoantibodies	1 (0–2)	1 (1–2)	2 (0–2)	0.6749
Glycated hemoglobin (%) (mmol/mol)	8.15 (7.3–8.95) 66 (56–74)	8.45 (7.3–8.9) 69 (56–74)	7.9 (7.3–9.2) 63 (56–77)	0.7789
Mean glucose (CGM) (mg/dL)	146 (131–208)	159 (136–210)	139 (129–186)	0.3613
SD of mean glucose (CGM) (mg/dL)	63 (45–77)	60 (41–87)	68 (53–77)	0.4908
Time below range (<70 mg/dL) (CGM) (%)	9 (3–16)	14 (3–21)	9 (3–13)	0.5053
eGFR (mL/min/1.73 m <sup>2</sup> )	68 (59–84)	84 (73–89)	59 (49–64)	<0.0001
No. of islet infusions	3 (2–3)	3 (2–3)	3 (2–3)	0.6970
Total tissue volume (mL)	12.3 (8.8–15.2)	12.5 (10–14)	11.8 (8.7–16.3)	0.7743
Total islet mass (10 <sup>3</sup> IEQ/kg)	13.45 (10.93–15.28)	12.07 (10.64–14.65)	13.83 (12.79–15.43)	0.4025
Islet viability (%)	93 (90–96)	94 (91–95)	93 (89–97)	0.7988
Islet function (GSIS)	2.08 (1.57–2.45)	2.03 (1.48–2.52)	2.26 (1.62–2.38)	0.5683
Time from first infusion to insulin independence (days)	91 (61–115)	91 (62–115)	91 (56–111)	0.8678
Optimal primary graft function	18 (64)	9 (64)	9 (64)	1

Values expressed as medians (IQR) or frequencies (percentages). GSIS, glucose-stimulated insulin secretion; IEQ, islet-equivalent.

The Kaplan-Meier estimates of graft survival were not significantly different after 5 years (79% [47–93] vs. 86% [54–96]) and after 10 years (71% [41–88] vs. 86% [54–96]) in ITA and IAK recipients, respectively (HR 0.55 [0.1–3],  $P = 0.4877$ ) (Fig. 1E and Supplementary Table 1).

In patients who experienced optimal primary graft function, the median duration of graft survival was 10 years (8–10) vs. 4.5 years (0.8–10) in those with suboptimal primary graft function (HR 0.07 [0.01–0.64],  $P = 0.0184$ ) (Fig. 1F and Supplementary Table 1).

In a Cox proportional hazards univariate regression analysis, optimal primary graft function and a longer history of diabetes were associated with higher graft survival at 10 years (Supplementary Table 1).

The median incidence of SHEs per year significantly decreased from 2 (1–5) events per year prior to islet transplantation to 0 (0–0) events at 5 ( $P < 0.0001$ ) and 10 years ( $P < 0.0001$ ), respectively (Table 2).

All metabolic parameters A1C, daily exogenous insulin requirement, mean glucose, SD around mean glucose, and percentage of time spent in hypoglycemia were improved durably over time.

These parameters slightly deteriorated with time but remained significantly improved at 10 years (Table 2).

#### Immunosuppression

Immunosuppressive drugs were stopped progressively in three out of the six ITA patients who lost graft function, within 3.6 months (2.8–5.8) after C-peptide became undetectable. One patient chose to stop his immunosuppressive treatment after reintroduction of insulin became necessary, despite detectable C-peptide. The last two patients are currently under progressive discontinuation. Immunosuppression was maintained after islet graft loss in two IAK patients with functioning kidney graft. Overall, 6 out of 28 patients (21%; 1 ITA and 5 IAK) had to be switched from sirolimus to mycophenolate after 26.1 months (11.5–43.2), for intolerance.

#### Adverse Events

All SAEs occurring during and beyond the 1st year are summarized in Supplementary Table 2. Each SAE was classified as most likely related to the infusion procedure, immunosuppression, or complications of type 1 diabetes. During the 1st year posttransplantation, 11 SAEs related to

the infusion procedure were observed, 6 of them involving bleeding, including 3 potentially life-threatening events after percutaneous islet infusion. Five SAEs (hematological disorders, nonopportunistic infections, and diarrhea) were related to immunosuppression. One toe amputation was related to diabetic complications. After 1 year and until 10 years postislet transplantation, eight SAEs related to immunosuppression occurred: four infections (two opportunistic and two nonopportunistic) and four skin carcinomas (two squamous and two basal cell carcinomas). Three of these skin carcinomas, all successfully treated with local excision, occurred in IAK recipients. Eleven diabetes-related macroangiopathic events occurred, nine of them >5 years after the first islet transplantation: five symptomatic events, four of them in the IAK recipients (one stroke in the IAK patient who later died as mentioned above, one myocardial infarct, one pulmonary edema, and two amputations), and six totally asymptomatic events, found by systematic yearly screening, two of them in IAK recipients. The six silent myocardial ischemic episodes were treated by coronary angioplasty stenting in five cases and surgical coronary bypass in the remaining case.

**Table 2—Metabolic and renal long-term outcomes in the entire study group**

	1 year	<i>P</i> value vs. baseline	5 years	<i>P</i> value vs. baseline	10 years	<i>P</i> value vs. baseline
Patients followed	28		27		20	
No. of severe hypoglycemia events in previous year	0 (0–0)	<0.0001	0 (0–0)	<0.0001	0 (0–0)	<0.0001
Glycated hemoglobin (%) (mmol/mol)	5.9 (5.5–6.7) 41 (37–50)	<0.0001	6.9 (6.1–7.5) 52 (43–58)	<0.0001	6.7 (6.1–8) 50 (43–64)	0.0009
Exogenous insulin requirements (IU/kg per day)	0 (0–0.04)	<0.0001	0 (0–0.36)	<0.0001	0.28 (0–0.43)	<0.0001
Mean glucose (CGM) (mg/dL)	112 (102–133)	<0.0001	126 (110–144)	<0.0001	118 (113–154)	0.0007
SD of mean glucose (CGM) (mg/dL)	22 (15–41)	<0.0001	29 (17–52)	<0.0001	40 (18–54)	<0.0001
Time below range (<70 mg/dL) (CGM) (%)	0 (0–5)	<0.0001	1 (0–3)	<0.0001	3 (0–9)	0.0012
eGFR (mL/min/1.73 m <sup>2</sup> )	68 (55–81)	0.8883	64 (51–80)	0.7926	54 (43–91)	0.252

Values expressed as medians (IQR) or frequencies (percentages).

### Kidney Function

Renal function differed between ITA and IAK at baseline (Table 1). As illustrated in Fig. 2, a slight decrease of eGFR was observed in both groups with time:  $-1.1$  mL/min/1.73 m<sup>2</sup> per year ( $-2.5$  to  $0.1$ ) in ITA and  $-0.9$  mL/min/1.73 m<sup>2</sup> per year ( $-2.2$  to  $0.8$ ) in IAK. This reduction, however, did not reach statistical significance, even after 10 years ( $P = 0.52$  in ITA and  $P = 0.38$  in IAK, Wilcoxon matched-pairs signed rank test between 10 years and baseline) (Table 2). One IAK patient, who received islet transplantation 45 months after kidney transplantation, while eGFR had decreased to 30 mL/min/1.73 m<sup>2</sup>, remained insulin independent 10 years after islet transplantation. From the three patients referred after pancreas graft failure, one who had received a kidney from a twin living donor lost islet graft function after 10 months. His eGFR was 40 mL/min/1.73 m<sup>2</sup> at 10 years after the islet transplantation. The second patient remained insulin independent at the last follow-up 8 years after islet transplantation. The third one died with a functioning islet graft as mentioned above.

### CONCLUSIONS

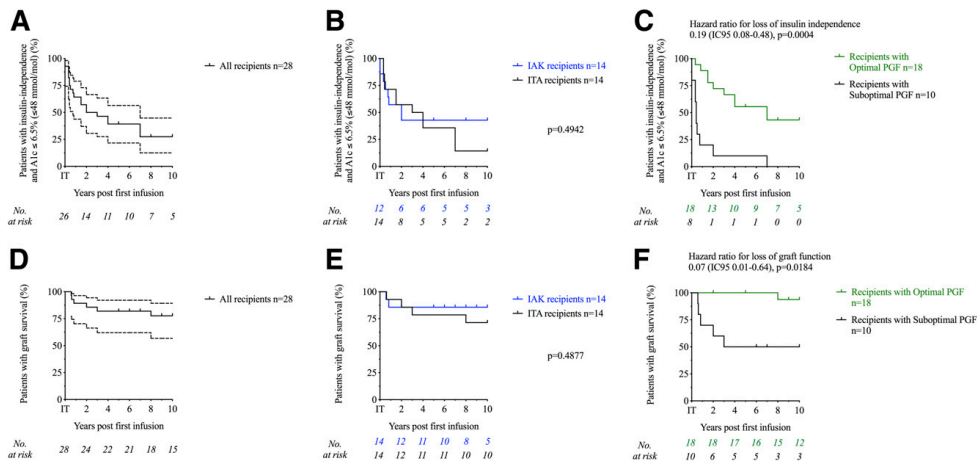
In the current study, we evaluated the long-term outcome of allogenic islet transplantation in patients with type 1 diabetes and hypoglycemia unawareness and/or a previous kidney graft. After 10 years, graft function was maintained in 75% of patients, and 28% percent of patients met the study primary outcome: insulin independence with A1C  $\leq 6.5\%$  (48 mmol/mol).

In contrast to previous long-term reports of a single case or a small series of selected patients (23–28), we analyzed in this prospective study the 10-year outcome of an entire cohort, with minimal attrition and no secondary rescue islet infusion. Overall, the 10-year results appear comparable to those reported after pancreas transplantation when proposed for the same indications (31,32). Furthermore, half of our patients still maintained A1C level  $<7\%$  without SHEs, the alternative end point considered for licensure of islet transplantation in the U.S. (9).

We also confirmed that long-term outcomes were first related to the primary graft function, evaluated 1 month after the last islet infusion (33). However, the precise determinants of early islet graft function remain to be clarified. Indeed, this early proxy reflects not only the mass and quality of transplanted islets but also their initial engraftment. In the present cohort, we deliberately optimized primary graft function by initially administering two or three sequential islet infusions. All patients reached insulin independence, an early outcome that was also associated with longer retention of islet graft function in the CTR (2). In the current study, an optimal primary graft function was associated with prolonged graft function and a median duration of insulin independence with A1C  $\leq 6.5\%$  of 6 years. Since partial graft function is sufficient to prevent severe hypoglycemia (30), alternative and less stringent composite end points have been proposed to define success in islet transplantation, based on glucose

control and avoidance of severe hypoglycemia, independently of insulin independence (34). Nevertheless, in the current study, suboptimal graft function was associated with shorter overall islet graft survival. This is in line with the association between initial achievement of insulin independence, another proxy for good primary graft function, and long-term islet graft survival in the CTR (2). Second, we found that the duration of insulin independence was longer in female recipients, independently of their lower body mass. Although the underlying mechanisms remain unclear, recent studies argue for a favorable effect of estrogens on glucose metabolism (35,36).

Importantly, we observed equivalent results when islet transplantation was performed after a kidney graft, in patients with more vascular complications and who had often been refuted for simultaneous pancreas-kidney transplantation. Preexisting immunosuppression and a lower BMI may have contributed to these favorable results. Another key aspect was the stringent selection of the study participants, who had not experienced any acute rejection, uncontrolled hypertension, or macroalbuminuria after kidney transplantation. A progressive switch from mycophenolate to sirolimus was warranted prior to the registration on the islet waiting list, as well as a tapering of steroids. Finally, a previous nonimmunological loss of a pancreas transplant in three patients did not seem to have impaired the results of islet transplantation. Taken together, our results suggest that in uremic patients with

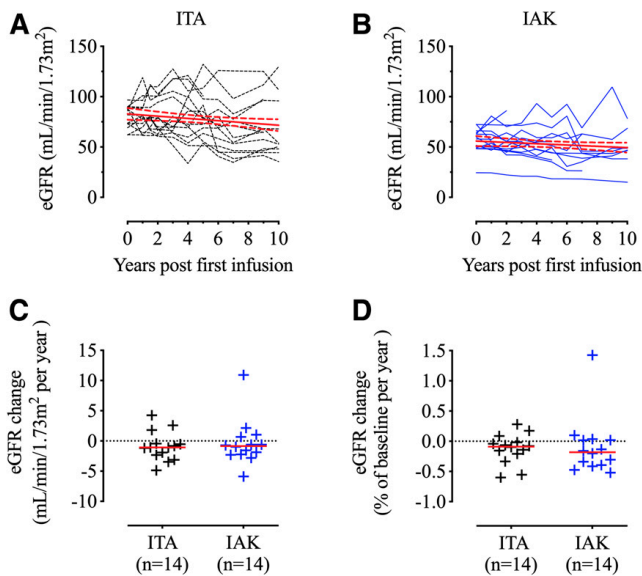


**Figure 1**—Ten-year Kaplan-Meier estimates of insulin independence with A1C ≤ 6.5% (≤ 48 mmol/mol) and graft survival in the entire cohort in ITA and IAK recipients and in islet recipients with optimal and suboptimal primary graft function (PGF). Insulin independence with A1C ≤ 6.5% (48 mmol/mol) in the entire cohort (95% CIs in dotted black lines) (A), in ITA and IAK recipients (B), and in islet recipients with optimal and suboptimal PGF (C). Graft survival in the entire cohort (95% CIs in dotted black lines) (D), in ITA and IAK recipients (E), and in islet recipients with optimal and suboptimal PGF (F). (A high-quality color representation of this figure is available in the online issue.)

type 1 diabetes, the option of a pancreas or an islet transplantation should be discussed prior to kidney transplantation

to propose the best strategy according to patient characteristics and local possibilities (32,37).

As expected (2), islet infusion was associated with a significant risk of complications (Supplementary Table 2). However, the overall risk profile of intraportal islet infusion observed in the current study appears lower than reported after pancreas transplantation (31,32). All other complications were related to chronic immunosuppression and/or to diabetes. The overall mortality rate observed here (0.3% per 100 patient-years) was equivalent to the mortality rate observed in the Diabetes Control and Complications Trial (DCCT) in patients with type 1 diabetes with little or no complications, and in absence of any immunosuppressive treatment (38). In contrast, the mortality rate reported in patients with characteristics similar to those of the participants enrolled in the current study (i.e., with frequent SHEs or a functioning kidney graft), but non islet transplanted, is three to four times higher and mostly related to SHE or ischemic heart disease (37,39,40). The yearly screening of macroangiopathic diabetes-related complications proposed in this study was more stringent than usually recommended. Likewise, 6 out of 11 events (54%) were detected in absence of any symptoms. Meanwhile, the five symptomatic cardiovascular events occurred >5 years after islet transplantation, and all in IAK



**Figure 2**—Baseline to 10 years follow-up of kidney function in islet transplantation in ITA and IAK recipients. Individual evolution of eGFR changes over the 10 years of follow-up in ITA (A) and IAK (B) recipients with linear regression (red line) and 95% CI (dotted red lines). Absolute change per year (C) and proportion of change from baseline value (D) in ITA and IAK recipients (red lines summarize the median value). (A high-quality color representation of this figure is available in the online issue.)

patients initially refuted for combined kidney-pancreas transplantation because of preexisting severe diabetes-related complications.

Importantly, the mean decline of eGFR in the entire cohort was similar to the rate expected in the general population >40 years old ( $-2$  mL/min/1.73 m<sup>2</sup> per year). This was also true for patients with a previous renal graft. Our study, which is in line with some other results (25) but in contrast to earlier ones (41), suggests that improved metabolic control obtained after islet transplantation may exert a favorable effect on kidney function in type 1 diabetes, such as after pancreas transplantation (5,42,43).

One limitation of this study is the lack of a control group of patients receiving optimized insulin therapy or a pancreas transplant. Therefore, whether the improved metabolic control resulting from islet transplantation is balancing the associated risks remains to be demonstrated. Another limitation is the sample size of our study, which was calculated according to its primary metabolic end point. This limits the conclusions that can be drawn about kidney function and macroangiopathic complications. One may also remain cautious when interpreting the difference in early graft function because all participants initially received the same intervention. Moreover, the proposed strategy of initial repeated islet infusion for optimizing primary graft function can be hampered by donor pancreas availability. Finally, we could not explore the impact of the immunosuppression regimen on the islet transplantation long-term outcome. Noteworthy, all participants in our study received low-dose tacrolimus and sirolimus, a drug combination associated with a favorable outcome in the CTR (2). In contrast, immunosuppression was induced here with anti-interleukin-2 receptor antibodies, and not T-cell depletion or TNF- $\alpha$  inhibitors (2,9).

To conclude, the current study provides direct evidence that islet transplantation performed alone or after a kidney graft in patients with type 1 diabetes can markedly improve metabolic control and suppress SHEs during 10 years.

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**Author Contributions.** M.-C.V. contributed to study design, patient enrollment, patient follow-up, data interpretation, and writing of the manuscript. M.C. contributed to figure conception, data interpretation, analysis, and writing of the manuscript. V.G., N.D., and J.K.-C. contributed to islet isolation and writing of the manuscript. A.J., S.E., K.L.M., V.R., M.F., E.V.B., P.P., and M.H. contributed to patient follow-up. F.M. contributed to data interpretation and analysis. T.H. and R.C. contributed to organ procurement and islet transplantation. C.N. contributed to study design, patient enrollment, patient follow-up, and data interpretation. F.P. contributed to study design, transplantation, data interpretation, and writing of the manuscript. M.-C.V. and F.P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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## 2.3 Article 3: Examination of the Igl's criteria for defining functional outcomes of $\beta$ -cell replacement therapy: Ipita symposium report

The first version of the Igl's criteria was developed in Igl's, Austria in 2017 to provide a definition of success for  $\beta$ -cell replacement therapy (i.e. in islet and pancreas transplant recipient) in T1D patient during a workshop of the International Pancreas and Islet Transplant Association (IPITA) and the European Pancreas and Islet Transplant Association (EPITA). In July 2019, during the IPITA World Congress a symposium was dedicated to investigating its criteria after 2 years of clinical practice.

The Igl's 1.0 four categories of the success of  $\beta$ -cell replacement therapy was compared in IT, pancreas transplantation alone and simultaneous pancreas-kidney transplantation cohorts and through blood glucose targets derived from continuous glucose monitoring (CGMS) in a ten years prospective islet alone and after kidney transplantation cohort.

CGMS monitoring data were collected in the Lille cohort of 55 patients with T1D before and after transplantation for 10 years in ITA (n = 39) or IAK (n = 16) recipients, representing a follow-up of 302 patients-years and compared to the Igl's 1.0 categories of success at any time points during annual visit from the first islet infusion.

These results allowing to validate that CGMS data were in accordance with the actual definition and could allow to discriminate each category of this definition.

Following IT, the median (IQR) time spent in the glucose range recommended by the American Diabetes Association (i.e. 70 to 180 mg/dL) was gradually and significantly improved in patients with failure, marginal, good and optimal categories of the Igl's 1.0 criteria of success

with 58% (44-73), 75% (64-89), 90% (78-97), and 100% (95-100), respectively and compared with 54% (44-71) before transplantation (1-way ANOVA,  $P < 0.0001$ ).

Similarly, the median (IQR) time spent below the glucose recommended range (i.e.  $< 70$  mg/dL) was significantly and gradually decreased in those categories with 7% (3-13), 2% (0-7), 0% (0-5), and 0% (0-1) compared with 9% (3-15) before transplantation ( $P < 0.0001$ ).

Finally, this study allowed us to refine the definition of success and to define the IglS 2.0 criteria which included data from continuous glucose monitoring to discriminate the different categories.

**This recommendation article “Examination of the IglS criteria for defining functional outcomes of  $\beta$ -cell replacement therapy: Ipita symposium report” was published as first co-author in 2021 in the Journal of Clinical Endocrinology & Metabolism.**



Reports and Recommendations

## Examination of the Igls Criteria for Defining Functional Outcomes of $\beta$ -cell Replacement Therapy: IPITA Symposium Report

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**Abbreviations:** AP, artificial pancreas; CGM, continuous glucose monitoring; CTR, Collaborative Islet Transplant Registry; HbA1c, glycated hemoglobin; IAK, islet after kidney; IPITA, International Pancreas & Islet Transplant Association; IPTR, International Pancreas Transplant Registry; ITA, islet transplant alone; PRO, patient-reported outcome; PTA, pancreas transplant alone; SHE, severe hypoglycemia event; SPK, simultaneous pancreas-kidney; TBR, time below range; T1D, type 1 diabetes; TIR, time in range.

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### Abstract

**Context:** The Igls criteria were developed to provide a consensus definition for outcomes of  $\beta$ -cell replacement therapy in the treatment of diabetes during a January 2017 workshop sponsored by the International Pancreas & Islet Transplant Association (IPITA) and the European Pancreas & Islet Transplant Association. In July 2019, a symposium at the 17th IPITA World Congress was held to examine the Igls criteria after 2 years in clinical

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practice, including validation against continuous glucose monitoring (CGM)-derived glucose targets, and to propose future refinements that would allow for comparison of outcomes with artificial pancreas system approaches.

**Evidence acquisition:** Utilization of the criteria in various clinical and research settings was illustrated by population as well as individual outcome data of 4 islet and/or pancreas transplant centers. Validation against CGM metrics was conducted in 55 islet transplant recipients followed-up to 10 years from a fifth center.

**Evidence synthesis:** The Igls criteria provided meaningful clinical assessment on an individual patient and treatment group level, allowing for comparison both within and between different  $\beta$ -cell replacement modalities. Important limitations include the need to account for changes in insulin requirements and C-peptide levels relative to baseline. In islet transplant recipients, CGM glucose time in range improved with each category of increasing  $\beta$ -cell graft function.

**Conclusions:** Future Igls 2.0 criteria should consider absolute rather than relative levels of insulin use and C-peptide as qualifiers with treatment success based on glucose assessment using CGM metrics on par with assessment of glycated hemoglobin and severe hypoglycemia events.

**Key Words:** pancreas transplantation, islet transplantation, type 1 diabetes,  $\beta$ -cell replacement, continuous glucose monitoring

The aim of  $\beta$ -cell replacement therapy is to achieve near-normal glycemic control in the absence of clinically significant hypoglycemia for patients with diabetes and  $\beta$ -cell failure experiencing severe hypoglycemia, hypoglycemia unawareness, and/or marked glycemic lability, and for patients with diabetes already committed to immunosuppression in support of another organ transplant. Current options for  $\beta$ -cell replacement include whole pancreas (1) or isolated islet transplantation (2), both of which can restore endogenous insulin secretion and improve glycemic control and stability, ameliorate clinically significant hypoglycemia, and reduce diabetes-related complications (3). As an alternative to restoration of endogenous insulin secretion, the artificial pancreas (AP) uses continuous glucose monitoring (CGM) to automate exogenous insulin delivery (4). Despite varying uses and options for  $\beta$ -cell replacement therapy, there had been a lack of clear and standardized definitions for graft function and clinical success, as well as poor alignment of glycemic control metrics used to evaluate AP systems impeding comparison of outcomes with cellular and technological approaches to therapy (5). To that end, in January 2017, the International Pancreas and Islet Transplant Association (IPITA) and the European Pancreas and Islet Transplant Association held a 2-day workshop in Igls, Austria, to develop a standardized definition for functional and clinical outcomes of  $\beta$ -cell replacement therapy, now known as the Igls criteria (6, 7).

The Igls criteria define  $\beta$ -cell graft function as optimal, good, marginal, or failure, based on glycated hemoglobin

( $HbA_{1c}$ ); severe hypoglycemia events (SHEs); insulin requirements; and C-peptide levels (Table 1). A SHE is defined as an event associated with loss of consciousness or requiring third-party assistance for recovery (8). Optimal graft function requires near-normal glycemic control defined by  $HbA_{1c} \leq 6.5\%$  (48 mmol/mol), absence of SHE, insulin independence (including absence of other antihyperglycemic therapy), and a C-peptide increase over pretransplant measurement. Good  $\beta$ -cell graft function requires on-target glycemic control defined by  $HbA_{1c} < 7.0\%$  (53 mmol/mol), absence of SHE, a reduction in insulin requirements of more than 50% compared with pretransplant (or use of noninsulin antihyperglycemic therapy), and a C-peptide increase over pretransplant measurement. Marginal graft function is defined by either  $HbA_{1c} \geq 7.0\%$  (53 mmol/mol), occurrence of any SHE, or a reduction in insulin requirements of less than 50% in the presence of a C-peptide increase from pretransplant. When C-peptide measures less than 0.5 ng/mL (0.17 nmol/L), or lower than the patient's baseline before transplantation, the graft is considered to have functionally failed (6, 7). Optimal and good function are considered clinically successful outcomes, whereas marginal and failure are not.

In July 2019, a daylong symposium was held as part of the 17th IPITA World Congress in Lyon, France, to examine implementation of the Igls criteria after 2 years of use in clinical practice. The aims included evaluating the utility and limitations of the current criteria in assessing  $\beta$ -cell graft function, identifying possible areas

**Table 1.** Igls definition of functional and clinical outcomes for  $\beta$ -cell replacement therapy (6, 7) (joint publication)

$\beta$ -cell graft functional status	HbA <sub>1c</sub> , % (mmol/mol) <sup>a</sup>	Severe hypoglycemia, events per y	Insulin requirements, U·kg <sup>-1</sup> ·d <sup>-1</sup>	C-peptide	Treatment success
Optimal	≤6.5(48)	None	None	>Baseline <sup>b</sup>	Yes
Good	<7.0(53)	None	<50% baseline <sup>c</sup>	>Baseline <sup>b</sup>	Yes
Marginal	Baseline	<Baseline <sup>d</sup>	≥50% baseline	>Baseline <sup>b</sup>	No <sup>e</sup>
Failure	Baseline	Baseline <sup>f</sup>	Baseline	Baseline <sup>g</sup>	No

Baseline, pretransplant assessment (not applicable to total pancreatectomy with islet autotransplantation patients).

<sup>a</sup>Mean glucose should be used to provide an estimate of the glycated hemoglobin, termed the glucose management indicator, in the setting of disordered red blood cell life span.

<sup>b</sup>Should also be > 0.5 ng/mL (>0.17 nmol/L) fasting or stimulated.

<sup>c</sup>Should also be < 0.5 U·kg<sup>-1</sup>·d<sup>-1</sup>; might include the use of noninsulin antihyperglycemic agents.

<sup>d</sup>Should severe hypoglycemia occur following treatment, then continued benefit may require assessment of hypoglycemia awareness, exposure to serious hypoglycemia (<54 mg/dL [3.0 mmol/L]), and/or glycemic variability/lability with demonstration of improvement from baseline.

<sup>e</sup>Clinically, benefits of maintaining and monitoring  $\beta$ -cell graft function may outweigh risks of maintaining immunosuppression.

<sup>f</sup>If severe hypoglycemia was not present before  $\beta$ -cell replacement therapy, then a return to baseline measures of glycemic control used as the indication for treatment (6, 7) may be consistent with  $\beta$ -cell graft failure.

<sup>g</sup>May not be reliable in uremic patients and/or in those patients with evidence of C-peptide production before  $\beta$ -cell replacement therapy.

for improvement, and proposing further refinements to the original criteria. Five experienced transplant centers illustrated of the usefulness of the Igls criteria in various clinical and research settings, and the symposium included discussion of limitations and recommendations for paving the way toward future implementation of the Igls criteria to compare outcomes of  $\beta$ -cell replacement therapies with AP system approaches to diabetes management.

## Methods

### Utilization of the Igls criteria

To illustrate the various uses of the Igls criteria, patient data from 4 transplant centers were examined. Usefulness in a clinical setting on a population level was demonstrated by data from center A. All consecutive data on patients that had completed at least 1 year of follow-up after either an islet or solitary pancreas transplant in this center were included. In addition, all patients were included who received a simultaneous pancreas-kidney (SPK) transplant in the year 2014 to provide at least 4 years of follow-up. Igls criteria were assessed at 6 months and 1, 2, and 4 years posttransplantation and are presented as a percentage of the population for each of the three  $\beta$ -cell replacement therapy groups (ie, islet transplantation, solitary pancreas transplantation, and SPK transplantation).

Usefulness of the criteria in a clinical setting on an individual level was illustrated by centers B and C using data from islet and pancreas transplant recipients who had completed at least 2 years of follow-up. For center B, patients were assessed at 6 months and 1 and 2 years posttransplantation, longitudinally describing individual

patients' graft function according to the Igls criteria using all functional categories. For center C, individual patients' graft function was longitudinally delineated using the dichotomous Igls criteria definition of treatment success (optimal or good  $\beta$ -cell graft function) and treatment failure (marginal or failed  $\beta$ -cell graft function).

Usefulness of the criteria in a research setting was illustrated by data of center D, describing consecutive islet transplant recipients included in a research study of a novel immunosuppressive approach that avoided calcineurin inhibitors as previously reported (9), followed now over a 10-year period.

### Comparison with CGM metrics

To address whether CGM metrics should be included as part of functional criteria that would better align glycemic control metrics with the AP field, validation of the Igls criteria against standard CGM metrics of glycemic control was provided using data from another transplant center, experienced with CGM in islet transplant recipients.

All CGM data collected during annual posttransplant follow-up in a cohort of patients with type 1 diabetes (T1D) before and after islet transplantation in center E were analyzed (10). CGM metrics were assessed using a blinded system (Medtronic MiniMed, Northridge, CA) for 3 to 5 consecutive days during usual daily life activities and diet as previously described (11). The percentages of glucose time in range (TIR) 70 to 180 mg/dL (3.9-10 mmol/L) and time below range (TBR) <70 mg/dL (3.9 mmol/L) were categorized according to the Igls criteria as optimal, good, marginal, and failure based on 146, 36, 90, and 30 patient assessments, respectively, and evaluated using 1-way ANOVA.

## Results

The Igl's criteria provide the ability to present and compare data on multiple clinically important levels. On a population level, the Igl's criteria are useful to cross-sectionally present and compare functional outcomes of different  $\beta$ -cell replacement modalities (Fig. 1). Using the Igl's criteria, functional outcomes of 36 islet transplant recipients (30 islet after kidney [IAK] (12), 4 islet alone [ITA], 2 islet-after-lung (13)), 29 solitary pancreas transplant recipients (26 pancreas after kidney, 3 pancreas transplant alone [PTA]), and 23 SPK recipients from center A were evaluated at 6 months and 1, 2, and 4 years posttransplantation. Good and marginal  $\beta$ -cell graft function is experienced most often with islet transplantation, and optimal and failure with solitary pancreas transplantation, such that treatment success (optimal or good) is experienced by ~60% of recipients over the first 2 years, with more durable function in the pancreas than islet group at 4 years. The highest rate of treatment success is seen with SPK.

The Igl's criteria can also be used for individual longitudinal description of  $\beta$ -cell graft function over time. Graft function in individual patients following islet transplantation (1 IAK (14), 2 ITA, 3 simultaneous islet-kidney, 1 simultaneous islet-liver-lung-kidney (15)), 1 solitary pancreas transplantation (PTA), and 8 SPK was assessed at 6 months and 1 and 2 years posttransplantation by center B (Fig. 2A). Islet transplant recipients more often experienced good and marginal functional outcomes with high fluctuation between functional categories, whereas pancreas transplant recipients showed either optimal function or graft failure. Describing  $\beta$ -cell graft function using the binary Igl's criteria outcome measure of treatment success (optimal or good) vs treatment failure (marginal or failed) in 7 individuals following ITA and 7 following PTA from center C (Fig. 2B) shows that achieving treatment success is

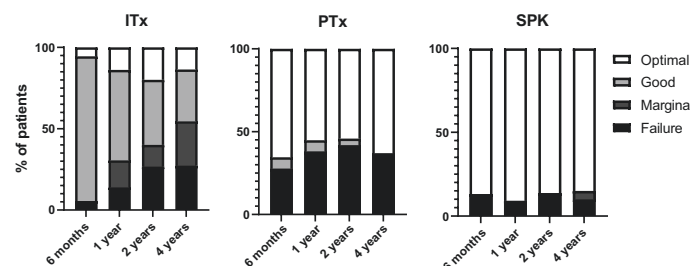
less fluctuant and follows similar patterns in ITA compared with PTA recipients.

Apart from clinical settings, the Igl's criteria can also be used in a research setting to describe and compare  $\beta$ -cell graft function. Graft function according to the Igl's criteria was structurally assessed in 10 consecutive ITA recipients from center D that received islet transplantation under protocols evaluating belatacept or efalizumab (Fig. 3). A switch in graft function from treatment success to treatment failure according to the Igl's criteria always predated the clinical decision to perform a supplemental islet infusion (16, 17) or subsequent pancreas transplant (18).

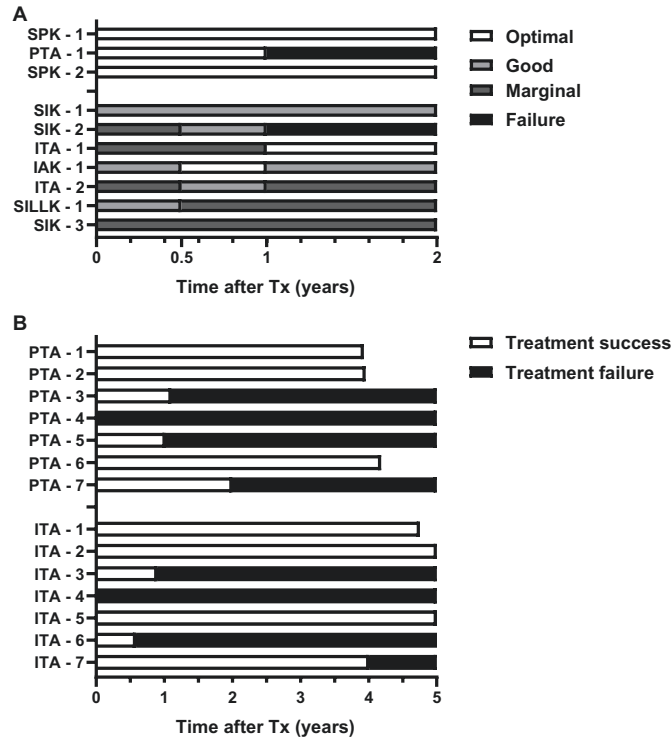
CGM data were collected in a cohort of 55 patients with T1D before and after ITA (n = 39) or IAK (n = 16) in center E, providing >302 patient-years based on individual follow-up periods of 1 to 10 years (10). After islet transplantation, median (interquartile range) TIR was incrementally improved at 100% (95-100; optimal function), 90% (78-97; good function), 75% (64-89; marginal function) and 58% (44-73; failure) compared with 54% (44-71) pretransplant ( $P < 0.0001$ , Fig. 4A). Similarly, TBR was 0% (0-1; optimal function), 0% (0-5; good function), 2% (0-7; marginal function) and 7% (3-13; failure), compared with 9% (3-15) pretransplant ( $P < 0.0001$ , Fig. 4B).

## Discussion

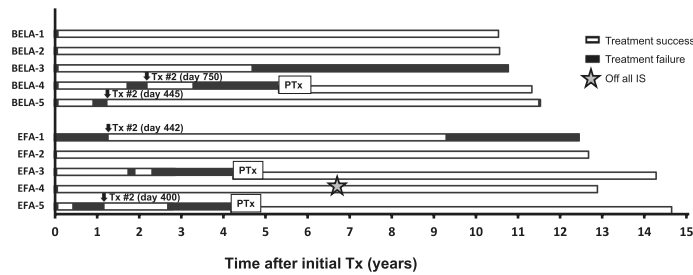
The Igl's criteria represent an important step forward in the process of standardizing the assessment of outcomes for  $\beta$ -cell replacement therapy, allowing for individual patient monitoring and the comparison of outcomes by different treatment approaches (ie, islet and pancreas transplantation). Illustrated by outcome data of experienced transplant centers, the criteria have shown versatility to capture information on different levels in a clinical (at both a



**Figure 1.** Igl's criteria in a clinical setting on a population level. Illustration of the Igl's criteria utility for cross-sectional comparison between  $\beta$ -cell replacement modalities, illustrated by consecutive data from center A at 0.5, 1, 2, and 4 years posttransplantation. Igl's criteria functional categories are presented as a percentage of each population for islet transplantation (ITx; n = 36), solitary pancreas transplantation (PTx; n = 29), and SPK (n = 23), respectively. Describing the natural course posttransplantation according to current clinical practice, this includes 17 islet transplant recipients that received a subsequent islet infusion by the 2-year assessment, and 1 pancreas transplant recipient with a failed graft at 1 and 2 years receiving a subsequent whole pancreas transplant with optimal graft function at 4 years. SPK, simultaneous pancreas-kidney transplantation.



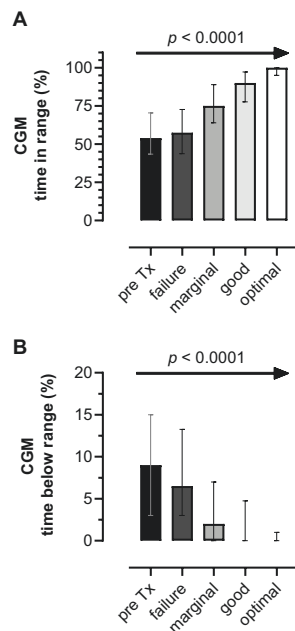
**Figure 2.** Igl's criteria in a clinical setting on an individual level. Illustration of the Igl's criteria utility for individual longitudinal description of  $\beta$ -cell graft function over time for individual patients after ITA, IAK, simultaneous islet-kidney (SIK), simultaneous islet-liver-lung-kidney (SILLK), PTA, and SPK. (A) Illustrated by data of patients from center B followed-up at 0.5, 1, and 2 years posttransplantation, using all functional categories of the Igl's criteria. (B) Illustrated by data of patients from center C, using the binary Igl's criteria outcomes of treatment success (optimal or good  $\beta$ -cell graft function) vs treatment failure (marginal or failed  $\beta$ -cell graft function). IAK, islet after kidney; ITA, islet alone; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.



**Figure 3.** Igl's criteria in a research setting. Illustration of the Igl's criteria utility for individual longitudinal description of  $\beta$ -cell graft function over time in a research setting, illustrated by 10 structurally and consecutively followed patients that received islet transplantation under protocols investigating belatacept (BELA) or efalizumab (EFA) from center D. Both islet and pancreas transplants were applied in these patients. The binary Igl's criteria outcomes of treatment success (optimal or good  $\beta$ -cell graft function) vs treatment failure (marginal or failed  $\beta$ -cell graft function) were used. IS, immunosuppression.

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**Figure 4. Igl's criteria and continuous glucose monitoring metrics of glycemic control.** Continuous glucose monitoring (CGM) metrics of glycemic control categorized according to the Igl's criteria for scoring  $\beta$ -cell graft function as optimal, good, marginal, or failure, using data from a cohort of islet transplant recipients ( $n = 55$ ) followed-up to 10 years from center E. CGM parameters incrementally improved with each consecutive category of Igl's classification following islet transplantation ( $P < 0.0001$  for both, 1-way ANOVA test for linear trend). Values are represented as median (interquartile range). (A) Glucose time-in-range (TIR, %) 70-180 mg/dL (3.9-10 mmol/L) for each of the functional categories of the Igl's criteria. (B) Glucose time-below-range (TBR, %)  $< 70$  mg/dL ( $< 3.9$  mmol/L) for each of the functional categories of the Igl's criteria.

treatment group and at an individual patient level) as well as in a research setting.

Existing registries for pancreas (International Pancreas Transplant Registry [IPTR]) and islet (Collaborative Islet Transplant Registry [CITR]) transplantation have used different definitions for functional graft outcomes. IPTR previously defined pancreas graft failure or success by whether insulin was used or not, irrespective of glucose regulation. Recently, this definition has been revised to insulin requirements  $\geq 0.5$  units/kg per day (19), which remains limited as an outcome in the absence of glucose criteria. In addition to insulin requirements, CITR requires reporting of measures for glucose regulation ( $HbA_{1c}$ , fasting glucose, severe hypoglycemia events) and C-peptide levels, with primary outcomes defined for insulin independence,  $HbA_{1c} \leq 6.5\%$

(48 mmol/mol), absence of SHE, and C-peptide  $\geq 0.3$  ng/mL (0.10 nmol/L) (20). Similar metrics are being collected by CITR for a registry of patients undergoing total pancreatectomy with islet autotransplantation (21). Thus, CITR is positioned to implement assessment by the Igl's criteria across both allogeneic and autologous islet transplantation, and IPTR could expand its data reporting requirements for pancreas transplant recipients. By combining measures of glucose regulation and  $\beta$ -cell graft function, the Igl's criteria allow for treatment success of whole pancreas, isolated islet, or future stem cell-derived islet transplantation with ongoing insulin use, provided goals for glycemic control and elimination of severe hypoglycemia are met, and clinically significant endogenous insulin secretion (C-peptide) has been restored.

#### Limitations of the Igl's criteria

The basis of  $\beta$ -cell graft functional categories on the achievement of  $HbA_{1c}$  targets, absence of SHE, reduction in insulin requirements, and restoration of clinically significant C-peptide production is currently limited by the requirement for baseline measures before transplantation. In addition, although the thresholds used for defining a successful graft outcome are unavoidably arbitrary, the rationale for glycemic control metrics (ie,  $HbA_{1c}$  and severe hypoglycemia events) is stronger than that for those reflecting graft function to secrete insulin (ie, insulin use and C-peptide levels).

The requirement for good  $\beta$ -cell graft function of a 50% reduction in insulin use (which should also be  $< 0.5$  units/kg per day) is based on expert opinion (22). Insulin requirements are, however, highly variable and depend on factors that not only vary day to day but are also independent of  $\beta$ -cell graft secretory capacity, such as dietary habits, physical activity, insulin sensitivity, kidney function, and the use of noninsulin antihyperglycemic agents. Patient requirements for glucocorticoid therapy, particularly the maintenance of supraphysiologic dosing in combined islet and lung transplants for individuals with  $\beta$ -cell failure because of cystic fibrosis (13, 15), may result in higher insulin requirements from steroid-induced insulin resistance despite all other criteria being optimal/good. Thus, when insulin requirements are the only component leading to classification of marginal  $\beta$ -cell graft function with glycemic control targets being met, it may be difficult to conclude that the treatment is not clinically successful.

For patients with chronic pancreatitis undergoing total pancreatectomy with islet autotransplantation, the assessment for a reduction in insulin requirements or an increase in C-peptide levels relative to baseline prior to intervention (prepancreatectomy) is not possible. Thus, good  $\beta$ -cell

graft function that is required to meet criteria for treatment success depends on the presence of insulin requirements  $<0.5$  units/kg per day and C-peptide levels that are  $>0.5$  ng/mL (0.17 nmol/L) fasting or stimulated. In the absence of a stimulated C-peptide, a recent validation study of the IglS criteria in autologous islet recipients substituted a fasting C-peptide  $\geq 0.2$  ng/mL (0.07 nmol/L) that was highly predictive of a stimulated C-peptide  $>0.5$  ng/mL (0.17 nmol/L) when both measures were available for analysis (23). Measurement of C-peptide provides an estimate of the contribution of engrafted islets to glycemic control, enabling determination of whether improvements in HbA<sub>1c</sub> are due to changes in insulin dosing or to effective secretory function of the  $\beta$ -cell graft.

### Incorporation of CGM metrics

At the time of the IPITA/European Pancreas and Islet Transplant Association Opinion Leaders Workshop in 2017, consensus targets for CGM-derived metrics of glycemic control had not been established. Since then, the use of CGM has increasingly expanded in clinical practice. The use of CGM metrics such as TIR may identify changes in glycemia sooner than a change in HbA<sub>1c</sub>, allow simultaneous assessment of hypoglycemia from TBR, and would allow for more direct comparison of  $\beta$ -cell replacement with AP system outcomes (24, 25). In addition, glucose variability has gained increasing importance as both a therapeutic target and an outcome measure in diabetes clinical trials (26), including of islet transplantation (27), where improvement in glucose variability may be related to improvements in measures of neuropathy (28).

The IglS criteria were well-correlated to CGM parameters in the allogeneic islet transplant recipients reported here, with similar findings recently demonstrated in a smaller cohort of autologous islet transplant recipients (23). These results support an approach that applies CGM metrics to the assessment of  $\beta$ -cell graft function, thus further enabling comparisons of results with AP system technology. As even a marginal  $\beta$ -cell graft function is enough to increase TIR and decrease TBR, these results further support that marginal function could still provide benefit to an individual patient by reducing the risk for experiencing future SHE (29-31).

The increasing use of CGM has led to the recent publication of an international consensus for TIR targets, which may soon be adopted as a surrogate for HbA<sub>1c</sub> (25). In the international consensus, two situations were distinguished: for adults with T1D or type 2 diabetes, TIR should be greater than 70%, TBR less than 4%, and time above range less than 25%. For older or high-risk patients, avoidance

of hypoglycemia is prioritized such that the goal is first aimed at limiting TBR to less than 1%, and decreasing the requirement of TIR to greater than 50% with time above range less than 50% (25). Although such a compromise in glycemic control is appropriate when hypoglycemia is a significant risk, the objective of  $\beta$ -cell replacement therapies to eliminate hypoglycemia should allow for the achievement of TIR  $>70\%$  to 80% even for high-risk individuals such as those with hypoglycemia unawareness or having already undergone kidney transplantation. These TIR targets are based on validation against HbA<sub>1c</sub>, whereby TIR  $>50\%$  relates to HbA<sub>1c</sub>  $<8.0\%$ , TIR  $>60\%$  to HbA<sub>1c</sub>  $<7.5\%$ , TIR  $>70\%$  to HbA<sub>1c</sub>  $<7.0\%$ , and a TIR  $>80\%$  to HbA<sub>1c</sub>  $\leq 6.5\%$  (32).

In the results from center E, and as previously reported by the same group (10, 11), those with a failed islet transplant spent only 58% TIR but with 7% TBR, and so clearly struggled with achieving even the less stringent CGM criteria for high-risk patients with T1D. Those with marginal  $\beta$ -cell graft function spent 75% TIR with only 2% TBR, and so are most often achieving adult standards for glycemic control. Those with good or optimal  $\beta$ -cell graft function spent 90 and 100% TIR, respectively, with no TBR, clearly meeting stringent glycemic control targets. Thus, there is close agreement of the IglS criteria for defining  $\beta$ -cell graft function with increasing time spent in the target glucose range and decreasing time spent with exposure to hypoglycemia. This relationship of CGM time spent both within and below the normal glucose range with the CGM-independent metrics used in IglS 1.0 should enable the adoption of CGM metrics as the most accurate approach to compare both cellular therapies and technological approaches to glycemic control.

For high-risk individuals being considered for and receiving  $\beta$ -cell replacement therapy, it is particularly important to also examine time spent with serious, clinically significant hypoglycemia  $<54$  mg/dL (3.0 mmol/L) (33) and glucose variability that more strongly relate to risk for experiencing SHE (34). Moreover, because  $\beta$ -cell replacement therapy targets near-normal glycemic control (even for high-risk patients),  $<4\%$  TBR is acceptable as long as time spent  $<54$  mg/dL (3.0 mmol/L) is negligible ( $<1\%$ ) because this amount of CGM measured hypoglycemia is present in healthy, nondiabetic individuals (35).

### Looking forward: paving the way for IglS 2.0

In summary, the IglS criteria are considered a great improvement for standardized classification of graft function and treatment success for current  $\beta$ -cell replacement therapies, including both isolated islet and whole pancreas

transplantation. Temporal assessment is important and should be included any time a clinical change in  $\beta$ -cell graft function is suspected and at the time of any additional  $\beta$ -cell transplant. Limitations include the absence of CGM metrics that preclude direct comparison of outcomes to AP systems. In addition, insulin requirements were found to be very dependent on confounding factors such as diet, exercise, and glucocorticoids rather than  $\beta$ -cell graft function, and the requirement for obtaining a stimulated C-peptide  $>0.5$  ng/mL ( $>0.17$  nmol/L) to document  $\beta$ -cell graft function in cases in which the fasting level fell below this threshold was felt too cumbersome. Additionally, the dichotomous outcome definition of treatment success and treatment failure was thought to be insensitive to the clinical benefits associated with a marginal  $\beta$ -cell graft function. Together with insulin requirements, C-peptide levels also cannot be used for comparison of cellular to technologic treatment approaches to glycemic control.

Future steps forward to improve upon the current criteria should incorporate CGM metrics to ensure comparison between  $\beta$ -cell replacement therapies and new developments in AP systems technology. We suggest that a new IglS 2.0 form composite criteria in which clinical outcome based on glucose regulation is separated from

$\beta$ -cell graft function, with the latter considered only for further qualification of  $\beta$ -cell replacement modalities (Table 2). Clinical outcome would encompass glycemic control and hypoglycemia and be sufficient for defining treatment success, and only the assessment of  $\beta$ -cell graft function would further require the addition of C-peptide and insulin use criteria. Reflecting the potential of a marginal  $\beta$ -cell function providing clinical benefit, this subdivision also would ensure the possibility for scoring treatment success, even with marginal  $\beta$ -cell graft function. Glycemic control and hypoglycemia could be assessed with or without CGM. Glucose regulation in patients with CGM could be assessed through %TIR and %TBR, whereas in those without CGM through  $HbA_{1c}$  and the occurrence of SHE.

Because insulin requirements are extremely dependent on individual lifestyle-related factors (36), and are not useful for comparison to AP systems, it was suggested to remove percent reductions for defining  $\beta$ -cell graft function in a future IglS 2.0 criteria. Furthermore, although a threshold for insulin requirements  $< 0.5$  units/kg body weight per day was felt by some to represent a reasonable expectation of a clinically successful  $\beta$ -cell graft with good function (consistent with the IPTR) (19), others felt

**Table 2.** Proposed IglS criteria 2.0

Treatment outcome	Glycemic control		Hypoglycemia		Treatment success
	$HbA_{1c}$ , % (mmol/mol) <sup>a</sup>	CGM, % time-in-range	Severe hypoglycemia, events per y	CGM, % time $< 54$ mg/dl (3.0 mmol/L)	
Optimal	$\leq 6.5$ (48)	$\geq 80$	None	0	Yes
Good	$< 7.0$ (53)	$\geq 70$	None	$< 1$	Yes
Marginal	$\leq$ Baseline	$>$ Baseline	$<$ Baseline <sup>b</sup>	$<$ Baseline	No <sup>c</sup>
Failure	$\sim$ Baseline	$\sim$ Baseline	$\sim$ Baseline <sup>d</sup>	$\sim$ Baseline	No
$\beta$ -cell graft function <sup>e</sup>	C-peptide, ng/mL (nmol/L) <sup>f</sup>		Insulin use or noninsulin antihyperglycemic therapy		
Optimal	Any		None		
Good	$>0.5$ (0.17) stimulated $\geq 0.2$ (0.07) fasting		Any		
Marginal	0.3-0.5 (0.10-0.17) stimulated 0.1- $<0.2$ (0.04- $<0.07$ ) fasting		Any		
Failure	$<0.3$ (0.10) stimulated $<0.1$ (0.04) fasting		Any		

Baseline, pretransplant assessment (not applicable to total pancreatectomy with islet autotransplantation patients).

Abbreviations: CGM, continuous glucose monitoring;  $HbA_{1c}$ , glycated hemoglobin.

<sup>a</sup>Mean glucose should be used to provide an estimate of the  $HbA_{1c}$ , termed the glucose management indicator, in the setting of disordered red blood cell life span.

<sup>b</sup>Should severe hypoglycemia occur following treatment, then continued benefit may require assessment of hypoglycemia awareness, exposure to serious hypoglycemia ( $<54$  mg/dL [3.0 mmol/L]), and/or glycemic variability/lability with demonstration of improvement from baseline.

<sup>c</sup>Clinically, benefits of maintaining and monitoring  $\beta$ -cell graft function may outweigh risks of maintaining immunosuppression.

<sup>d</sup>If severe hypoglycemia was not present before  $\beta$ -cell replacement therapy, then a return to baseline measures of glycemic control used as the indication for treatment (6, 7) may be consistent with  $\beta$ -cell graft failure.

<sup>e</sup>Categorization of  $\beta$ -cell graft function must first meet treatment outcome based on measures of glucose regulation.

<sup>f</sup>May not be reliable in uremic patients and/or in those patients with evidence of C-peptide production before  $\beta$ -cell replacement therapy.

that only insulin independence should be required for defining optimal  $\beta$ -cell graft function. By removing the amount of insulin that may be required to optimize glycemic control, these revised criteria for insulin use would also allow for more direct application of the Igl's criteria to patients undergoing total pancreatectomy with islet autotransplantation.

The treatment goal for C-peptide level as a functional measure of  $\beta$ -cell graft insulin secretion should still meet the stimulated threshold  $>0.5$  ng/mL (0.17 nmol/L) established by the Diabetes Control and Complications Trial as associated with reduced risk for experiencing severe hypoglycemia events as well as for the development and progression of microvascular complications (37). This threshold is also associated with improved glycemic control and avoidance of hypoglycemia following islet transplantation for T1D (38), where it is usually related with a fasting C-peptide of at least 0.2 ng/mL (0.07 nmol/L) (23). C-peptide below this threshold, but at least 0.3 ng/mL (0.10 nmol/L) stimulated (39) (as reported in CITR) (20) or 0.1 ng/mL (0.03 nmol/L) fasted, could be compatible with a marginal  $\beta$ -cell graft. Although lower levels of residual C-peptide detectable by high-sensitivity assays have been associated with a reduced risk of hypoglycemia in T1D (39, 40), in the phase 3 Clinical Islet Transplantation Consortium trial involving individuals with T1D complicated by hypoglycemia unawareness, only those transplant recipients who lost islet graft function defined by a stimulated C-peptide  $<0.3$  ng/mL (0.10 nmol/L) experienced a recurrence of severe hypoglycemia (41), and so should be considered failed.

It is still not known whether the Igl's criteria may predict outcomes in  $\beta$ -cell replacement therapy, nor whether the Igl's criteria may guide physicians in clinical decision making (eg, whether a shift from optimal to good function should prompt closer metabolic monitoring or immunological surveillance). Finally, given the heavy psychological burden of T1D affecting disease management (42), recent clinical trials of diabetes treatments increasingly include patient-reported outcomes (PROs), which have been recognized as a clinically meaningful outcome in T1D (24). Future updates to the criteria should also take into account PROs, including health-related quality of life, diabetes distress, fear of hypoglycemia, and patient satisfaction with their current treatment (43). It is important to note that the herewith-proposed Igl's 2.0 criteria are only preliminary. We propose that experts and practitioners in the field reconvene for another workshop to generate consensus of the incorporation of CGM metrics as proposed here, as well as considering the addition of PROs that could be applied to comparative effectiveness evaluation of both  $\beta$ -cell replacement and AP system approaches to diabetes treatment.

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## 2.4 Article 4: Relation between primary graft function and 5-year outcomes of islet allogeneic transplantation in type 1 diabetes: a retrospective cohort study in 1210 participants from the Collaborative Islet Transplant Registry

As the association between early islet graft function and sustained graft survival and insulin independence have been previously reported in our single-center cohort study, the present study was therefore designed to explore the relationship between primary islet graft function (PGF, measured with the Beta-2 score at day 28 of the last islet infusion) and 5-year clinical outcomes of IT in the Collaborative Islet Transplant Registry (CITR), adjusted for known potential confounders, including recipient and islet graft characteristics, transplant strategies, and immunosuppression regimens.

In this observational cohort study, 1210 patients with T1D from 39 transplant centers worldwide who received allogeneic islet transplantation alone or after kidney transplantation between January 19, 1999, and July 17, 2022, were included.

The primary outcome of the study was the occurrence of unsuccessful transplantation according to the Iglis 2.0 definition of success. Secondary outcomes of the study were graft exhaustion, inadequate glucose control, and the need for insulin therapy.

The cumulative incidence of unsuccessful IT was 39.9% (95% CI: 36.7-43.1) at one year and 70.7% (95% CI: 67.3-73.8) at five years. PGF was inversely related to unsuccessful IT, with an adjusted subhazard ratio of 0.77 (95% CI: 0.72-0.82) per 5-unit increase in Beta2-score

( $p < 0.0001$ ). This association was linear, resulting in a dosage-effect response. A similar relationship between PGF and graft exhaustion, inadequate glucose control, and need for insulin therapy was observed.

Finally, this study demonstrated in a large international cohort with robust statistical methodology a dose-dependent linear relationship between PGF and 5-year clinical outcomes of IT using the IgIs 2.0 refined definition of success and other current clinical outcomes of success, and independently from the number of infusions, total islet mass transplanted, and immunosuppression regimen.

A key finding of this report is to validate the role of PGF in predicting transplant success by informing on day 28 of the last infusion, the decision whether to repeat or not a new islet infusion. An online calculator is available to assist the clinician in making the decision and can predict the probability of the cumulative incidence of the 4 outcomes with good accuracy.

**This article “Relation between primary graft function and 5-year outcomes of islet allogeneic transplantation in type 1 diabetes: a retrospective cohort study in 1210 participants from the Collaborative Islet Transplant Registry” is actually submitted as first author for publication in the Lancet Diabetes and Endocrinology journal.**



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<b>Abstract:</b>	<p><b>Background:</b> Allogeneic islet transplantation (IT) is a validated therapy for severe type 1 diabetes patients, currently consists of one or more intraportal infusions of allogeneic pancreatic islets, and restores regulated endogenous insulin secretion. However, a progressive decline of islet graft function is observed with time. An association between primary islet graft function (PGF) and sustained graft survival has been reported by single center cohort studies. The primary objective of the study was to explore the relationship between PGF and 5-year clinical outcomes of IT. The secondary objective was to integrate and validate a predictive model of IT outcomes based on the measurement of PGF.</p> <p><b>Methods:</b> This observational cohort study enrolled all type 1 diabetes patients reported to the Collaborative Islet Transplant Registry, who received IT, alone or after kidney transplantation, between 01/19/1999, and 07/17/2022. Exposure was PGF, measured 28 days after the last islet infusion with the Beta2-score. The primary outcome of the study was the incidence of unsuccessful IT. Secondary outcomes were graft exhaustion, inadequate glucose control and the need for insulin therapy. A competing risk analysis was conducted to explore the relation between PGF and cumulative incidences of IT outcomes for 5 years, after adjustment on prespecified covariates and handling missing values by multiple imputations. A predictive PGF model for each IT outcome was built and the model performance was assessed and internally validated by using bootstraps resampling method (200 resamples).</p> <p><b>Findings:</b> 1210 patients in 39 transplantation centers worldwide (mean (SD) aged 47 (11) years, 712 (59.5%) females) received a total islet mass transplanted of 11.8 (8.7-15.9) thousand islet-equivalents per kg of recipient weight. Mean PGF was 14.3 (8.8). The cumulative incidence of unsuccessful IT was 70.7% (95%CI 67.3-73.8) at 5 years. PGF was inversely related to unsuccessful IT with adjusted subhazard ratio (sHR) of</p>

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	<p>0.77 (95% CI 0.72-0.82) per 5 units increase of Beta2-score (<math>p &lt; 0.0001</math>). This association appeared linear, resulting in a dose-effect response. Similar relation of PGF with graft exhaustion, inadequate glucose control and need for insulin therapy were observed. PGF predicted the probability of cumulative incidence of the four study outcomes with good accuracy with median (range) C-statistic values across imputed datasets of 0.70 (0.69-0.71); 0.76 (0.74-0.77); 0.65 (0.64-0.66); 0.72 (0.71-0.73) for unsuccessful IT, graft exhaustion, inadequate glucose control, and the need for insulin therapy, respectively.</p> <p>Interpretation: This study demonstrated in a large international cohort a linear dose-dependent relation between PGF and 5-year clinical outcomes of IT, independently of the number of infusions, total islet mass transplanted, and immunosuppression regimen. The PGF could guide current clinical practice by informing at day 28 of the last infusion, the decision whether to repeat the infusion and could serve as an early surrogate endpoint in future clinical trials.</p>
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**Relation between primary graft function and 5-year outcomes of islet allogeneic transplantation in type 1 diabetes: a retrospective cohort study in 1210 participants from the Collaborative Islet Transplant Registry.**

**Short running title:** Primary graft function and long-term outcome of islet transplantation

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## Summary

**Background:** Allogeneic islet transplantation (IT) is a validated therapy for severe type 1 diabetes patients, currently consists of one or more intraportal infusions of allogeneic pancreatic islets, and restores regulated endogenous insulin secretion.

However, a progressive decline of islet graft function is observed with time. An association between primary islet graft function (PGF) and sustained graft survival has been reported by single center cohort studies. The primary objective of the study was to explore the relationship between PGF and 5-year clinical outcomes of IT. The secondary objective was to integrate and validate a predictive model of IT outcomes based on the measurement of PGF.

**Methods:** This observational cohort study enrolled all type 1 diabetes patients reported to the Collaborative Islet Transplant Registry, who received IT, alone or after kidney transplantation, between 01/19/1999, and 07/17/2022. Exposure was PGF, measured 28 days after the last islet infusion with the Beta2-score. The primary outcome of the study was the incidence of unsuccessful IT. Secondary outcomes were graft exhaustion, inadequate glucose control and the need for insulin therapy. A competing risk analysis was conducted to explore the relation between PGF and cumulative incidences of IT outcomes for 5 years, after adjustment on prespecified covariates and handling missing values by multiple imputations. A predictive PGF model for each IT outcome was built and the model performance was assessed and internally validated by using bootstraps resampling method (200 resamples).

**Findings:** 1210 patients in 39 transplantation centers worldwide (mean (SD) aged 47 (11) years, 712 (59.5%) females) received a total islet mass transplanted of 11.8 (8.7-15.9)

thousand islet-equivalents per kg of recipient weight. Mean PGF was 14.3 (8.8). The cumulative incidence of unsuccessful IT was 70.7% (95%CI 67.3-73.8) at 5 years. PGF was inversely related to unsuccessful IT with adjusted subhazard ratio (sHR) of 0.77 (95% CI 0.72-0.82) per 5 units increase of Beta2-score ( $p < 0.0001$ ). This association appeared linear, resulting in a dose-effect response. Similar relation of PGF with graft exhaustion, inadequate glucose control and need for insulin therapy were observed. PGF predicted the probability of cumulative incidence of the four study outcomes with good accuracy with median (range) C-statistic values across imputed datasets of 0.70 (0.69-0.71); 0.76 (0.74-0.77); 0.65 (0.64-0.66); 0.72 (0.71-0.73) for unsuccessful IT, graft exhaustion, inadequate glucose control, and the need for insulin therapy, respectively.

**Interpretation:** This study demonstrated in a large international cohort a linear dose-dependent relation between PGF and 5-year clinical outcomes of IT, independently of the number of infusions, total islet mass transplanted, and immunosuppression regimen. The PGF could guide current clinical practice by informing at day 28 of the last infusion, the decision whether to repeat the infusion and could serve as an early surrogate endpoint in future clinical trials.

## **Research in context**

### **Evidence before this study**

Allogeneic islet transplantation (IT) under immunosuppression is a validated cell therapy for selected type 1 diabetes patients with severe unstable disease.

Marfil-Garza and colleagues recently reported in a large single center retrospective cohort that patients who experienced prolonged islet graft survival after IT had sustained metabolic benefits for at least 20 years. However, even when large numbers of islets are transplanted, half of the patients will experience islet graft failure within the first 5 years after IT, as reported by the international Collaborative Islet Transplant Registry (CITR).

Although Herring and colleagues recently reported four factors in the recipient or related to transplantation and immunosuppression and associated with 5-year IT success, however islet transplant failure is multifactorial and the current approach is to repeat islet infusions to increase functional islet mass in the recipient.

This strategy is therefore not always necessary and exposes to ethical, logistical and financial problems with an increased risk of complications related to the procedure and to the immunosuppression as well as alloimmunization.

It is crucial to be able to predict at an early stage, after a first islet infusion, the 5-year success of transplantation to guide the clinician's decision on the need to repeat islet infusion.

We searched in PubMed and Embase databases from Jan. 1, 2000, to Oct. 1, 2022, using the search terms “islet transplantation” AND “outcome” AND (“prediction” OR “prognostic”) AND “validation” and we did not find any validated predictors related to the 5-year outcome of allogeneic islet transplantation in type 1 diabetes recipient.

Vantyghem and colleagues reported the association of primary graft function (PGF) (calculated one month after IT with the Beta-score) and islet transplantation outcome at 10-year follow-up in a prospective parallel arm cohort study of 28 patients. Lam and colleagues recently showed that assessing PGF using the Beta-2 score was more accurate than other current tools and was related to transplant outcomes at 5 years.

### **Added value of this study**

In the present study, we demonstrated in the largest IT registry a linear dose-effect relation between PGF (measured at day 28 from the last islet infusion with the Beta2-score) and the 5-year clinical IT outcomes. PGF was an independent validated predictor of IT outcome at 5 years.

### **Implications of all the available evidence**

The PGF measure could guide clinicians by informing at an early stage (day 28 after the last islet infusion) the decision to repeat or not a new islet infusion. PGF could be used as an early surrogate endpoint in future clinical trials.

## Introduction

Allogeneic pancreatic islet transplantation (IT) is a validated treatment for type 1 diabetes associated with severe hypoglycemia unawareness and glycemic lability, or after kidney transplantation for end stage renal disease <sup>1</sup>. This beta-cell replacement strategy currently consists of one or more intraportal infusions of allogeneic pancreatic islets, aiming to restore regulated endogenous insulin secretion and improve blood glucose control <sup>2</sup>. Advances in islet processing and immunosuppression protocols have led to improved outcomes and increased success rates after IT <sup>3</sup>. One randomized trial <sup>4</sup> and several controlled studies <sup>5-9</sup> comparing IT with intensive insulin therapy, showed better metabolic control and reduced the incidence of severe hypoglycemia episodes after IT. Long-term results from two prospective <sup>10,11</sup> and several retrospective <sup>9,12-15</sup> cohort studies, showed 86-100% patient survival and 52-78% graft survival at ten years, together with improved glycemic control and lability, and a significant reduction in severe hypoglycemic event <sup>12</sup>.

In most centers, islet infusions are repeated to increase the mass of transplanted islets. However, using multiple donors limits its development in the context of organ shortage. It also increases the risk of procedures-related complications and alloimmunization. Furthermore, even when a large number of islets are transplanted, a decline in graft function is often observed with time. The underlying mechanisms remain elusive, and the respective roles of inflammatory, allogeneic, and/or autoimmune response versus the progressive metabolic exhaustion of transplanted islets are unclear.



The prolonged success of IT has been related to the primary graft function (PGF) of transplanted islets <sup>16</sup>, measured one month after the last islet infusion with Beta-score, a validated composite index of islet function based on stimulated serum C-peptide, fasting blood glucose, HbA1c and the need for exogenous insulin. In a prospective cohort study, optimal PGF was observed in 18 out of 28 patients who initially received two or three islet infusions and was associated in these patients with a Kaplan-Meier estimate of 94% (95% CI 63-99) of graft function, and 43% (95% CI 20-64) of insulin independence at 10 years <sup>10</sup>. This relation between early islet graft function and sustained graft survival has been confirmed in two other cohort studies using the Beta2-score, a simplified and continuous version of the Beta-score <sup>17,18</sup>. However, none of these studies were designed to distinguish the role of PGF from other potential confounding factors that may impact long-term outcomes including the recipient baseline characteristics, total mass of transplanted islets, number of islet infusions, as well as immunosuppression and other adjuvant treatments administered after transplantation. Clarifying the distinct impact of PGF is important for refining beta-cell replacement strategies. Measuring PGF could inform the need for repeating infusions of human islets or other insulin-secreting cells to reach expected outcomes.

Therefore, the primary objective of this study was to explore the relation between PGF and five-year clinical outcomes of IT using the Collaborative Islet Transplant Registry (CITR). This comprehensive global registry compiles all data from most islet transplant programs in North America, Eurasia and Australia <sup>3</sup>, allowing the adjustment of the analysis to known potential confounding factors. Our secondary objective was to integrate a predictive model of islet transplantation outcomes based on the measurement of primary graft function and to validate it in the CITR cohort.

## Methods

### *Study design and settings:*

This observational cohort study was designed to explore the association of primary islet graft function with the 5-year outcomes of islet transplantation. We analyzed the data from the Collaborative Islet Transplant Registry (CITR). The CITR is a single, standardized, worldwide repository of comprehensive human islet transplant data, collected since January 1999. The transplant deidentified database includes variables from pancreatic islet preparations transplanted and recipient data from consenting individuals with type 1 diabetes <sup>3</sup>. Participation in CITR is voluntary, both by the islet transplant centers and individual islet transplant recipients. As a registry, the requirements for patient enrollment and participation have been obtained per the site's institutional review board and/or country's oversight body for human research. Requirements for participation are overseen by the CITR Coordinating Center to ensure that participating islet transplant centers comply with Good Clinical Practice regarding data collection and submission. Participating transplant centers must provide annual documentation of adherence to their local Institutional Review or Ethics Board requirements. United States (US) centers must assure compliance with the Health Insurance Portability and Accountability Act (HIPAA). This study report followed the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

*Participants:*

We enrolled all type 1 diabetes patients registered in CTR after an allogeneic islet transplant alone (ITA recipient) to treat severe hypoglycemia episodes and/or impaired hypoglycemia awareness or an islet-after-kidney transplant (IAK recipient) in kidney transplant recipients required for end-stage nephropathy, if they received at least one islet infusion between January 19, 1999, and July 17, 2020 reported to CTR as of August 14, 2020. Baseline recipient characteristics (age in years, sex, race, blood type, duration of diabetes at first islet infusion in years, body weight in kg, height in cm, body mass index in kg/m<sup>2</sup>, HbA1c (glycated hemoglobin) in %, fasting and stimulated C-peptide in ng/mL, fasting blood glucose in mg/dL, daily exogenous insulin needs in IU/kg/day, number of severe hypoglycemia episodes defined as hypoglycemia requiring third party intervention to correct, presence of type 1 diabetes-associated autoantibodies) and transplantation characteristics (date of islet transplantation waiting list, date and mass of each islet infusion received per recipient in islet-equivalent and in islet-equivalent per kg of recipient, number of isolated pancreases received per patient, total islet cell volume transplanted in mL, immunosuppression regimen used) were extracted from the CTR database. We also analyzed follow-up data collected in the CTR, 28 days after islet infusion, and then annually for five years, including fasting C-peptide, fasting blood glucose, glycated hemoglobin, daily exogenous insulin needs, body weight and severe hypoglycemic episodes that occurred since the previous visit. At each new islet infusion, a new follow-up schedule was established as provided for in the CTR protocol.

*Exposure and covariates:*

The study exposure of interest was the patient's primary islet graft function (PGF) defined as the value of the Beta2-score calculated at day 28 after the last islet infusion, as previously reported <sup>19</sup>. The validated Beta2-score is a continuous variable (0 for no beta-cell function) calculated with values of fasting C-peptide (nmol/L), fasting blood glucose (mmol/L), glycated hemoglobin (%) and daily exogenous insulin needs per kg of patient weight (U/kg per day) As described by Forbes et al <sup>19</sup>, the Beta2-score was calculated using the present formula:

$$\text{BETA-2 score} = \frac{\left( \sqrt{\text{fasting C-peptide (nmol/L)}} \times (1 - \text{insulin dose [units/kg]}) \right)}{\text{Fasting plasma glucose (mmol/L)} \times \text{HbA1c(\%)}} \times 1000$$

Prespecified covariates included all variables suspected or known to impact islet transplantation outcome: recipient age, sex, body mass index, diabetes duration, daily insulin needs, baseline C-peptide level before islet transplantation, number of islet infusions, total islet mass transplanted per kg of recipient weight, total cell volume transplanted, ITA or IAK recipients, and the use of specific immunosuppression agents (interleukin 2 receptor antagonist, TNF alpha antagonist, T-cell depleting agent, calcineurin inhibitor and M-Tor inhibitor).

*Outcomes:*

Current islet transplantation outcomes were used in this study. The primary study outcome was the incidence of unsuccessful islet transplantation as defined by the IPITA/EPITA (International Pancreas and Islet Transplant Association / European Pancreas and Islet Transplant Association) IglS 2.0 consensus: with glycated hemoglobin level greater than or equal to 7.0 % (53 mmol/mol) and/or with at least one episode of severe hypoglycemia since the last visit and/or with serum fasting C-peptide secretion less than 0.2 ng/mL fasting or stimulated <sup>20</sup>. Secondary outcomes were: 1) graft exhaustion defined by stimulated C-peptide level inferior to 0.3 ng/mL, 2) inadequate glucose control using the Clinical Islet Transplantation (CIT) consortium <sup>21</sup> endpoint: with glycated hemoglobin greater than or equal to 7.0 % (53 mmol/mol) and/or occurrence of at least one episode of severe hypoglycemia since the last visit, and 3) the need for exogenous insulin therapy defined as the administration of exogenous insulin during 14 consecutive days.

*Statistical analysis, handling of missing values:*

Categorical variables were expressed as frequency (percentage). Quantitative variables were expressed as a mean  $\pm$  standard deviation in cases of normal distribution or median (interquartile range, IQR) otherwise. Normality of distributions was assessed using histograms and the Shapiro-Wilk test.

For each islet transplantation outcomes (unsuccessful IT, graft exhaustion, inadequate glucose control and the need for insulin therapy), we estimated the cumulative incidence using the Kalbfleisch and Prentice method, considering the day 28 after the last islet infusion as the date of origin, and by treating death and delayed islet reinfusion (when a new islet infusion occurred at least one year after the last islet infusion) as competing events.

The association of PGF (defined by the Beta2-score at day 28 after the last islet infusion) with cumulative incidences of each islet transplantation outcomes were explored using Fine and Gray regression models before and after adjustment for prespecified covariates suspected or known to impact islet transplantation outcomes: age at transplantation, sex, body mass index, diabetes duration, daily insulin needs, baseline serum C-peptide level before islet transplantation, number of islet infusions, total islet mass per kg of recipient weight transplanted, total cell volume transplanted, type of recipient (IAK vs ITA), the use of various immunosuppression regimen (yes vs no): interleukin 2 receptor antagonist, TNF alpha antagonist, T-cell depleting agent, calcineurin inhibitor and m-TOR inhibitor. To handle missing data in Beta2-score components and pre-specified covariates adjustment, we used multiple imputations using the regression-switching approach (chained equations with  $m = 60$  imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion. The number of imputations was chosen to have a maximal fraction of missing information (FMI)/ $m < 1\%$  in all in the multivariable Fine and Gray regression models. The imputation procedure was performed under the missing-at-random assumption with the predictive mean-matching method for quantitative variables, logistic regression model for binary variables, and ordinal logistic regression for ordered

categorical variables. Rubin's rules were used to combine the estimates derived from multiple imputed data sets <sup>22</sup> .

The shape of relationship between PGF and each islet transplantation outcomes were investigated by after categorization of PGF by quartiles and the proportional hazard assumption was assessed by introducing a time interaction term into Fine and Gray models. The association of PGF with each islet transplantation outcome was first investigated in the overall study population and then assessed in two sensitivity analyses restricted to recipients with a single islet infusion (to avoid biases related to the multiple islet infusion), and to islet transplant alone recipients (to avoid biases related to overestimation of C-peptide levels in the IAK recipients; a sensitivity analysis restricted to IAK recipients was less relevant here because this population was more limited than that of ITA recipients in our study). Unadjusted and adjusted subHazard (sHR) ratio per 5 unit increase in PGF and for upper versus lowest PGF quartiles were derived from Fine and Gray regression models as effect sizes.

We assess the performance of PGF to predict each islet transplantation outcomes in terms of discrimination by calculating the Harrell's C-index of agreement adapted to presence of competing risk <sup>23</sup> in each imputed dataset and by reporting median (range) values across the 60 imputed datasets <sup>24</sup>. We also examined the performance of PGF in term of calibration by comparing mean predicted cumulative incidences (estimated by the univariable Fine and Gray model) to the mean observed cumulative incidences (calculated by the Kalbfleisch and Prentice method) in four risk groups determined by the quartile distributions. To address the overestimation issues in developing prognostic model <sup>25</sup>, we performed an internal validation by using bootstraps resampling method (200 resamples) to correct the C-statistic for

overoptimism. Risk prediction charts were built from the univariable model (combined estimates obtained in the 60 imputed data sets).

All statistical tests were performed at the 2-tailed  $\alpha$  level of 0.05. Data were analyzed using SAS version 9.4 [SAS Institute Inc., Cary, NC 27513, USA].

*Role of the funding source:*

There was no direct funding source for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit it for publication. The Collaborative Islet Transplant Registry is supported by Public Health Service research grants UCH DK098086 and UC4 DK114839 from the National Institutes of Health, and in the past by a supplemental grant from the Juvenile Diabetes Research Foundation International. MRR is supported in part by Public Health Service research grant R01 DK091331.



## Results

### *Patients and transplantation characteristics*

A total of 1376 patients were registered in the CTR database after having received at least one islet infusion between January 19, 1999, and July 17, 2020. We excluded from this analysis patients with other forms of diabetes than type 1 diabetes, patients who received a simultaneous islet and kidney transplantation, or kidney-after-islet transplantation, and patients with insufficient data to calculate PGF (Flow chart of the study, Figure 1). Overall, 1210 patients who were transplanted and followed for up to 5 years in 39 transplantation centers worldwide (28 in North America, 7 in Europe, 3 in Australia and 1 in Asia) were enrolled in the present study. Patients were followed up to 5 years after the last islet infusion for a total follow-up of 4670 patients.years. Among the 1210 patients of the present study, 28 deaths were reported during the follow-up. The baseline patient's characteristics, and the characteristics of transplantation are summarized in Table 1.

The mean (SD) age of the recipient was 47 (11) years with 712 (59.5%) female and a diabetes duration of 30 (11) years before transplantation. IT was performed after a median (IQR) of 7.1 (2.6-16.1) months on the waiting list, alone in 986 (82.4%) recipients or after a kidney transplantation in 211 (17.6%) recipients. Patients received a median of 2.0 (1.0-2.0) islet infusions corresponding to a median total islet mass transplanted of 11.8 (8.7-15.9) thousand islet-equivalents per kg of recipient weight, and a median total islet cell volume transplanted of 3.5 (0.0-7.5) mL.

310 (25.9%) patients received a single islet infusion and 887 (74.1%) received multiple islet infusions, including two, three or at least four infusions in 595 (67%), 241 (27%) and 51 (6%) patients, respectively.

The most frequently used immunosuppressive agents were calcineurin inhibitors, m-Tor inhibitors for maintenance and T-cell depleting agents, interleukin 2 receptor antagonists, and TNF alpha antagonists for induction immunosuppression (Table 1).

Overall, the mean value of PGF, the study exposure, estimated by the Beta2-score calculated at day 28 after the last islet infusion, was 14.3 (8.8). In participants who received a single islet infusion, the mean PGF was 9.5 (7.9) compared to patients who received multiple islet infusions where the mean PGF was 16.0 (8.5). ITA recipients had a mean PGF of 13.9 (8.6) and IAK recipients had a mean PGF of 16.2 (9.5) (Appendix, Supplementary Table S1).

Fasting C-peptide, fasting blood glucose, glycated hemoglobin, and daily exogenous insulin needs values used to measure the PGF at day 28 are reported in supplementary Table S1 before and after multiple imputations for handling of missing values.

#### *Association between primary islet graft function and IT success.*

In a survival analysis taking into account death and delayed islet reinfusion (i.e. when a new islet infusion occurred at least one year after the last islet infusion) as competing events, 19.6% (95% CI 17.2-22.2) of recipients did not reach IT success at day 28 from last islet infusion (Figure 2A). In this specific population, median PGF was 5.0 (0.6-11.8). The cumulative incidence of recipients who did not reach IT success at 1, 3 and 5 years post last islet infusion

was 39.9% (95% CI 36.7-43.1), 59.3% (95% CI 55.9-62.5) and 70.7% (95% CI 67.3-73.8), respectively.

Following adjustment on prespecified covariates and handling missing values by multiple imputations, the PGF was significantly and inversely related to unsuccessful IT with adjusted subhazard ratio of 0.77 (95% CI 0.72-0.82) per 5 units increase of Beta2-score ( $p < 0.0001$ ) (Figure 3). After categorization of PGF by quartiles, this association appeared linear, resulting in a dose-effect response (Appendix, Supplementary Table S2). Then, patients who benefited from a PGF greater than 20.1 were associated with lower incidence of unsuccessful IT at 5 years compared to patients with PGF fewer than 8, with an adjusted subhazard ratio of 0.35 (95% CI 0.26-0.45) ( $p < 0.0001$ ).

This independent and linear dose-effect association between PGF and unsuccessful IT at 5 years was further confirmed in sensitivity analyses limited to patients who received only a single islet infusion (Appendix, Supplementary Figure S1) and limited to islet transplantation alone recipients (Appendix, Supplementary Figure S2)

*Association between primary graft function and secondary outcomes:*

Similar results were observed with all three secondary study outcomes. Graft exhaustion, inadequate glucose control, and the need for exogenous insulin therapy were observed in 8.8% (95% CI 7.2-10.6), 17.8% (95% CI 15.4-20.2) and 38.2% (95% CI 35.3-41.0) of patients 28 days after the last islet infusion, respectively (Figure 2B-C-D). These patients experienced a median PGF of 0 (0-0.9), 6.7 (1.8-13.8) and 7.6 (4.3-11.5) respectively.

The cumulative incidences of graft exhaustion, inadequate glucose control, and need for exogenous insulin therapy at 5 years were 42% (95% CI 38.7-45.3), 67.6% (95% CI 64.2-70.8) and 76.5% (95% CI 73.6-79.1). Following adjustment on prespecified covariates PGF was significantly and inversely related to the cumulative incidence of graft exhaustion, inadequate glucose control, and need for exogenous insulin therapy, with adjusted subhazard ratios of 0.63 (95% CI 0.57-0.70), 0.80 (95% CI 0.76-0.85) and 0.77 (95% CI 0.73-0.81), respectively (per 5 units increase of Beta2-score;  $p < 0.0001$ ) (Figure 3). Similarly, these associations between PGF and the three secondary outcomes were all linear, resulting in a dose-effect response (Appendix, Supplementary Table S2).

The independent and linear association between PGF and the three secondary study outcomes, graft exhaustion, inadequate glucose control, and need for exogenous insulin therapy was further confirmed in sensitivity analyses limited to patients who received only a single islet infusion (Appendix, Supplementary Figure S1) and limited to islet transplantation alone recipients (Appendix, Supplementary Figure S2)

#### *Prediction of islet transplantation outcomes*

Thus, the PGF was able to predict the probability of cumulative incidence of the four IT study outcomes, i.e. unsuccessful IT, graft exhaustion, inadequate glucose control, and the need for insulin therapy during the 5-year follow-up with good accuracy.

The median (range) C-statistic values across the 60 imputed datasets of the adjusted models were 0.70 (0.69-0.71); 0.76 (0.74-0.77); 0.65 (0.64-0.66); 0.72 (0.71-0.73) respectively (after correction for over optimism bias by internal validation) (Appendix, Supplementary Table S3).

Calibrations, which reflects the extent to which the model correctly estimates whether the cumulative incidences of the four predicted IT outcomes agree with the cumulative incidences of the four observed IT outcomes, were tested by simulation exercises in the four risk groups determined by the quartile distributions. The predictive equation models were constructed after internal validation of 200 bootstraps resamples.

A prediction matrix built from the univariable models (combined estimates obtained in the 60 imputed datasets) of the risk of the cumulative incidence of the four IT study outcomes as a function of PGF values at 2 and 5 years is provided in Table 2.

Finally, the prediction models developed from the present study were integrated into a software program allowing to display cumulative incidences and median survival of the four IT study outcomes that can be predicted for an individual patient, according to the values of PGF measured simultaneously with glycated hemoglobin, daily exogenous insulin needs, body weight, fasting blood glucose and fasting C-peptide, 28 days after the last infusion. A current version of the calculator is available online (<https://lille-model.shinyapps.io/PGF-islet/>).

## Discussion

In this retrospective cohort study, we analyzed the relation between early islet graft function and the 5 years clinical outcomes of IT. The results demonstrated a linear and independent association between primary graft function, defined as the Beta2-score measured at day 28 from the last islet infusion and the 5-year IT current clinical outcome of the study including unsuccessful IT, graft exhaustion, inadequate glucose control, and the need for insulin therapy. One key asset of our study was the use of the CITR, the most comprehensive IT dataset available worldwide, which supports the generalizability of our findings. Our analysis enrolled a total of 1210 type 1 diabetes participants who were transplanted in 39 centers worldwide, using heterogenous allocation systems, patient and islet characteristics and clinical practices. This unique setting allowed us to specifically evaluate the role of early islet graft potency, independently of other factors known to impact IT outcomes, including baseline characteristics of the recipient and transplanted islets, as well as transplantation strategies and immunosuppression regimens.

The distinct impact of early graft function on the long-term survival of vascularized organs is well established. After kidney transplantation, delayed graft function is associated with poor long-term outcome, independently of immunologic factor <sup>26</sup>. In IT, the assessment of early graft function is complicated by the frequent repetition of islet infusions. In the present study, we defined PGF as islet graft function measured 28 days after the last infusion, a time sufficient to ensure the restoration of physiological blood flow to the transplanted islets through vascular sprouting <sup>27</sup>. Notably, this allows us to study multiple islet infusions as a

whole organ transplant. Several composite indexes have been proposed to measure islet graft function based on simultaneous measurements of serum C-peptide, blood glucose, and the need for exogenous insulin<sup>28</sup>. In the present study, we used the Beta2-score<sup>19</sup>, a simple and continuous endpoint derived from a single fasting blood sample measured 28 days after the last islet infusion that could be calculated in the majority of CTR participants on day 28 after the last islet infusion. This allowed us to unveil a dose-effect relation between PGF and the retention of IT success, independently of baseline patient characteristics, transplantation strategies, and immunosuppression regimens (Appendix, Supplementary Table S2). Noteworthy, the retention of IT success and other secondary outcomes were not significantly related to the overall mass of transplanted islets nor to the number of infusions received (data not shown). These results are aligned with those of a large single-center retrospective study, in which long-term graft survival was not associated to total islet mass but rather to islet graft function evaluated between 6 and 12 months<sup>12</sup>. In a prospective cohort study in which recipients deliberately received up to three islet infusions aiming to maximize initial islet graft function<sup>10</sup>, median (IQR) graft function was maintained for 10 years (IQR 8-10) among patients who experienced optimal PGF vs 6.0 years (IQR 1.9-10.0) in those with suboptimal PGF with ( $p=0.0184$ ). In a retrospective single center study, higher values of Beta2-score measured 75 days after IT were associated with longer duration of insulin independence<sup>18</sup>. Another study showed that patients who achieved and maintained insulin independence had higher Beta2-score values 7 days after IT than those who remained insulin dependent<sup>17</sup>. In line with existing evidence, the present study shows that early function of successfully engrafted islets is essential to maximize graft survival after IT, rather than the number of infusions or the total mass of islets transplanted.

Our study has several limitations. Because its design was retrospective and observational, one must remain cautious when interpreting the difference in PGF, since most participants initially received similar interventions. Moreover, a strategy favoring early repeated islet infusions for optimizing PGF could be hampered by donor pancreas availability. Second, we only evaluated efficacy outcomes and not the complications that can occur after IT, such as procedure related adverse events, alloimmunization, or change in kidney function. Thus, the clinical utility of PGF for guiding the decision of supplementary islet infusion, needs to be tested in prospective interventional trials. Furthermore, PGF could only be evaluated using data that were available for most CTR participants. One can therefore not exclude that using more sophisticated methods to evaluate islet graft function, such as dynamic tests of insulin secretory reserve<sup>2</sup>, could further refine the prediction of long-term outcomes. It is also possible that evaluating islet graft function at other time points would have been more efficient. In the present study, evaluating PGF with the Beta2-score at day 28 after last infusion allowed a prediction of good accuracy as shown by the C-statistics of the model in the four different study outcomes. Day 28 also appears as a reasonable time in clinical practice. Our analysis was not adjusted on kidney function which could have impacted Beta2-score values by increasing circulating C-peptide levels. However, our sensitivity analysis showed that the significance of PGF was maintained in patients receiving islet transplantation alone, i.e. without an associated kidney transplant. Finally, the study design did not allow us to explore the interaction between PGF and recipient characteristics or early adjuvant anti-inflammatory and/or immunosuppressive treatments, which may also have contributed to long-term outcomes of IT as recently suggested in a CTR study on the impact of donor, transplant and immunosuppression factors on transplant success<sup>29</sup>.



In conclusion, this study demonstrated in a large international cohort of IT recipients a linear and inverse independent relation between primary graft function, measured one month after the last islet infusion (Beta2-score at day 28) and the 5-year cumulative incidence of unfavorable IT outcomes. This distinct association between early graft potency and long-term outcomes has important clinical implications. First, PGF could be used as an early and reliable surrogate endpoint of IT success in future clinical trials. PGF could also guide current clinical practice, by helping to individualize the decision to repeat islet infusions, independently of a predefined islet mass threshold, or the achievement of clinical outcomes such as insulin independence or disappearance of severe hypoglycemia episodes. Overall, our results indicate that, to improve the outcome of current strategies for beta-cell replacement, more attention should be directed to evaluate and optimize early islet potency, i.e. by enhancing the viability and function of transplanted islets or other insulin-secreting cells.

## **Declaration of interests**

There was no direct funding source for the present study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit it for publication.

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## **Contributors**

All authors had full access to all the data in the study and accept responsibility to submit for publication

MC, MM, JKC, MCV, LP, MR and FP contributed substantially to the conception and design of the study, the acquisition of data, or the analysis and interpretation.

ED JL CB EP FB conducted the data analysis.

MC, MM, JKC, MCV, LP, MR and FP drafted the article.

MC, ED, CB, MM, EP, FB, JKC, MCV, LP, MR, JL and FP reviewed/edited the manuscript.

All authors contributed to the interpretation of data and critical revision of the article.

FP is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

All authors read and approved the final manuscript.

## **Data sharing**

The data underlying the results presented in this article are not publicly available. Deidentified individual participant data, as well as a data dictionary, can be made available by the CITR to investigation centers that provide a methodologically robust study design and agree to report results from the cohort without stratifying results by investigating center.

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**Figures and Tables titles:**

**Figure 1:** Flow Chart of the Study

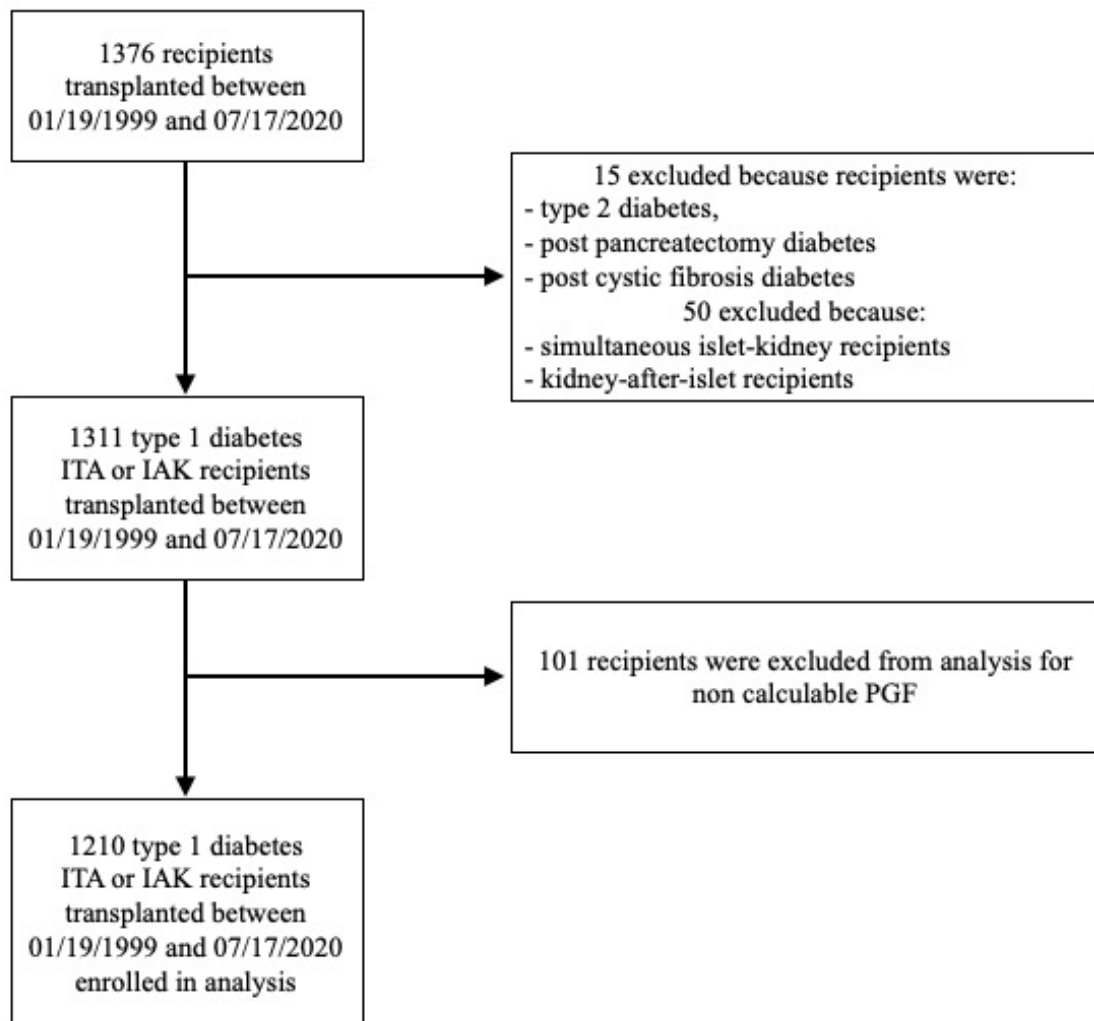
**Figure 2:** 5-year cumulative incidence of unfavorable outcomes of islet transplantation: unsuccessful IT (Figure 2A) graft exhaustion (Figure 2B), inadequate glucose control (Figure 2C), and need for insulin therapy (Figure 2D).

**Figure 3:** Association of primary graft function and 5-year cumulative incidence of unfavorable islet transplantation outcomes in the whole cohort.

**Table 1:** Patient and transplantation characteristics in the 1210 recipients of the cohort

**Table 2:** 2-year and 5-year prediction risk matrix of islet transplantation unfavorable outcomes according to PGF values measured 28 days after the last islet transplantation with the Beta2-score.

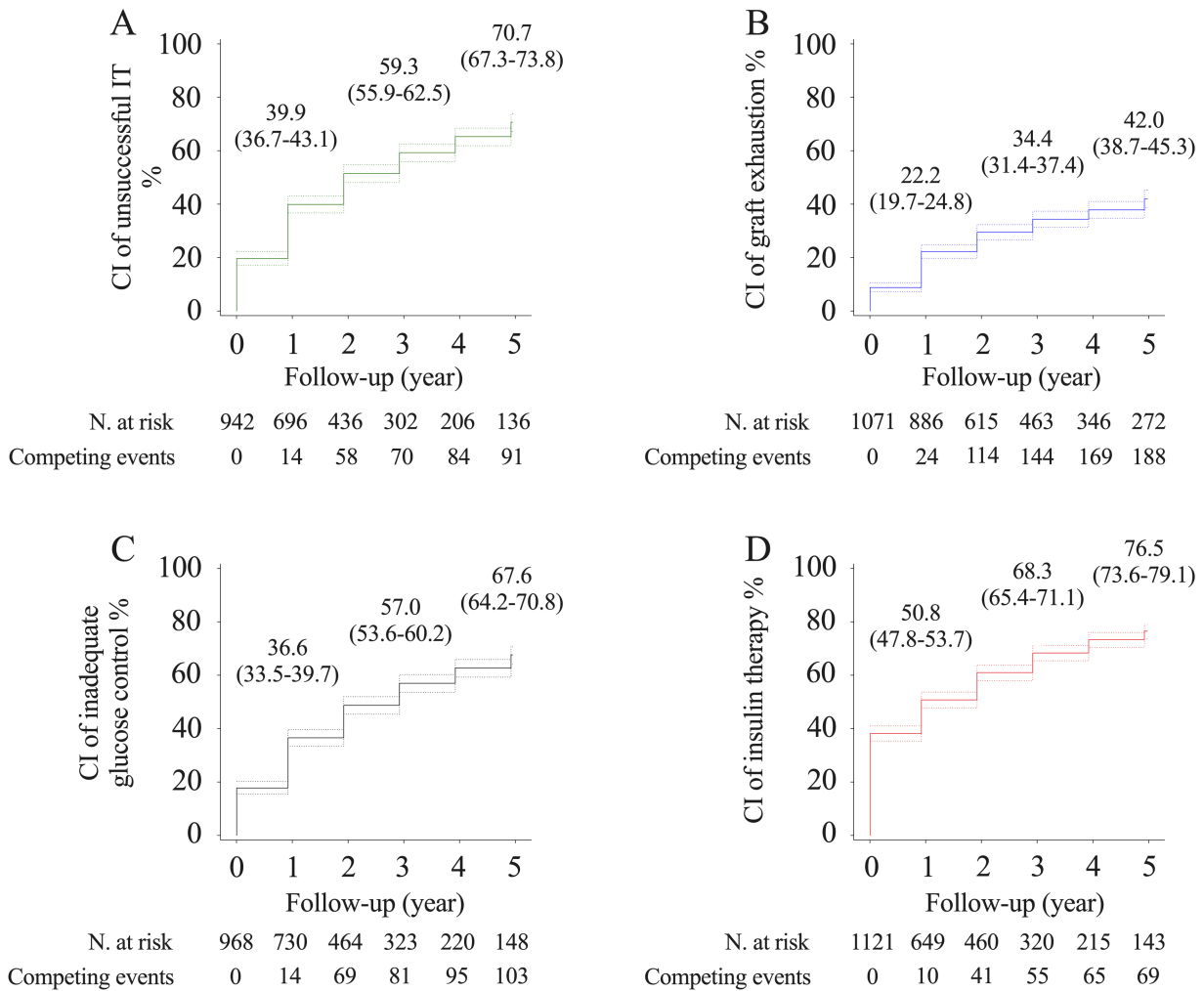
**Figure 1:** Flow Chart of the Study



IAK= Islet-After-Kidney transplantation recipient; ITA= Islet Transplantation Alone recipient;

PGF=Primary Graft Function.

**Figure 2:** 5-year cumulative incidence of unfavorable outcomes of islet transplantation: unsuccessful IT (Figure 2A) graft exhaustion (Figure 2B), inadequate glucose control (Figure 2C), and need for insulin therapy (Figure 2D).



Cumulative incidence curves are shown as solid lines and 95% CI confidence intervals as dashed lines for the four outcomes: unsuccessful IT (green), graft exhaustion (blue), inadequate glucose control (black), and the need for insulin therapy (red). Estimates with 95% CIs are reported at 1-, 3- and 5-years following IT for the four outcomes. The number of

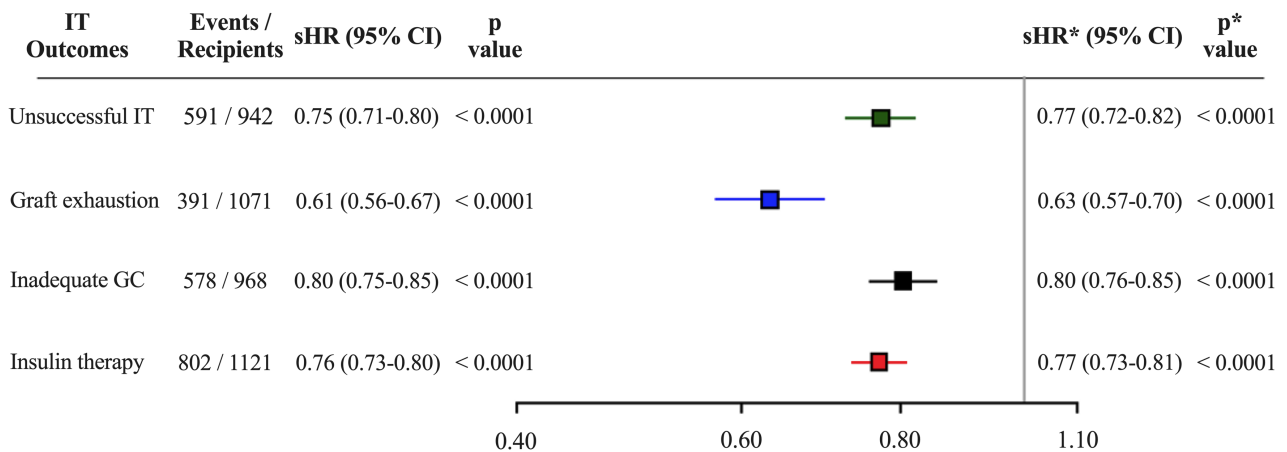
patients at risk and the number of competitive events are plotted for each time point below each survival curve.

For each islet transplantation outcomes (unsuccessful IT, graft exhaustion, inadequate glucose control and the need for insulin therapy), we estimated the cumulative incidence using the Kalbfleisch and Prentice method, considering the day 28 after the last islet infusion as the date of origin, and by treating death and delayed islet reinfusion (when a new islet infusion occurred at least one year after the last islet infusion) as competing events. To handle missing data in Beta2-score components and pre-specified covariates adjustment, we used multiple imputations using the regression-switching approach (chained equations with  $m = 60$  imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion.

N.= Number; CI= Cumulative Incidence; IT= Islet Transplantation.



**Figure 3:** Association of primary graft function and 5-year cumulative incidence of unfavorable islet transplantation outcomes in the whole cohort.



Association of PGF (defined by the Beta2-score at day 28 after the last islet infusion) with cumulative incidence of the four unfavorable islet transplantation outcomes: unsuccessful IT (green), graft exhaustion (blue), inadequate glucose control (black), and the need for insulin therapy (red) are represented with Forest plot. This association was explored using Fine and Gray regression models before and after adjustment on prespecified covariates included all variables suspected or known to impact islet transplantation outcomes (\*): recipient age, sex, body mass index, diabetes duration, daily insulin needs, baseline C-peptide level before islet transplantation, number of islet infusions, total islet mass transplanted per kg of recipient weight, total cell volume transplanted, type of recipient (ITA or IAK), and the use of specific immunosuppression agents (interleukin 2 receptor antagonist, TNF alpha antagonist, T-cell depleting agent, calcineurin inhibitor and M-Tor inhibitor). SubHazard ratio (sHR) was calculated for 5 units increase of primary graft function. SHs, 95%CI, and p-values are

calculated after handling missing values by multiple imputations using the regression-switching approach (chained equations with  $m = 60$  imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion. SHs and 95% CI are represented in a logarithmic scale.

IT= Islet Transplantation; sHR= subHazard Ratio; CI= Confidence Interval; Inadequate GC= Inadequate Glucose Control

**Table 1:** Patient and transplantation characteristics in the 1210 recipients of the cohort

	N	Values
<b>Patient characteristics at baseline</b>		
Female, n (%)	1196	712 (59.5%)
Age, years	1197	47 ± 10
Race, n (%)	884	
White		885 (97.9%)
Black		9 (1.0%)
Asian		3 (0.3%)
Other		7 (0.8%)
ABO blood type, n (%)	1077	
O group		444 (41.2%)
A group		470 (43.6%)
Other groups		163 (15.2%)
Body Mass Index, kg/m <sup>2</sup>	810	23.7 ± 3.0
Duration of Type 1 diabetes, years	1004	30 ± 11
Daily exogenous insulin needs, IU/kg/day	938	0.6 ± 0.2
HbA1c, %	1005	7.8 (7.0-8.8)
Fasting blood glucose, mg/dL	819	157 (103-222)
Fasting C-peptide, ng/mL	911	0.00 (0.00-0.09)
Patients with severe hypoglycemia episodes	1024	731 (71.4%)
<b>Transplantation characteristics</b>		
ITA / IAK recipients, n (%)	1197	986 (82.4%) / 211 (17.6%)
Duration on islet transplantation waiting list, months	838	7.1 (2.6-16.1)
Number of pancreas isolated per recipient	1210	2.0 (1.0-2.0)
Number of infusions per recipient	1210	2.0 (1.0-2.0)
Recipients with a single islet infusion, n (%)	1197	310 (25.9%)
Recipients with multiple islet infusions, n (%)	1197	887 (74.1%)
Multiple islet infusion recipients with 2 ; 3 ; ≥ 4 infusions, n (%)		595 (67%) ; 241 (27%) ; 51 (6%)
Time between first and last infusion, months		3.9 (0.0-10.8)
Recipients with all infusions in ≤ 3 ; ≤ 6 ; ≤ 12 months, n (%)		532 (44%) ; 733 (61%) ; 915 (76%)
Total islet mass transplanted, IEQ x1000	928	798 (571-1074)
Total islet mass transplanted, IEQ/kg of recipient weight x1000	851	11.8 (8.7-15.9)
in single islet infusion recipients		7.0 (5.4-9.4)
in multiple islet infusion recipients		13.3 (10.7-17.6)
Patients transplanted with < 5 ; 5 to <10 ; 10 to <15 ; ≥15 x 1000 IEQ/kg of recipient weight, n (%)	851	49 (6%) ; 229 (27%) ; 315 (37%) ; 258 (30%)
Total islet cell volume transplanted, mL	1197	3.5 (0.0-7.5)
Immunosuppression regimens received, n (%)	1148	

T-cell depleting agent	525 (45.7%)
Interleukin 2 receptor antagonist	519 (45.2%)
TNF alpha antagonist therapy	425 (37.0%)
Calcineurin inhibitor	1096 (95.5%)
m-TOR inhibitor	619 (53.9%)

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Data are shown as number (percentage), mean  $\pm$  standard deviation or median (25<sup>th</sup> to 75<sup>th</sup> percentile).

n (%)= Number (percentage); IEQ=islet-equivalents is a unit for counting islets with a standardized diameter of 150 micrometers; HbA1c= glycated hemoglobin; ITA= Islet Transplant Alone recipient; IAK= Islet-After-Kidney recipient; TNF= Tumor Necrosis Factor; m-TOR= mammalian target of rapamycin

**Table 2:** 2-year and 5-year prediction risk matrix of islet transplantation unfavorable outcomes according to PGF values measured 28 days after the last islet transplantation with the Beta2-score.

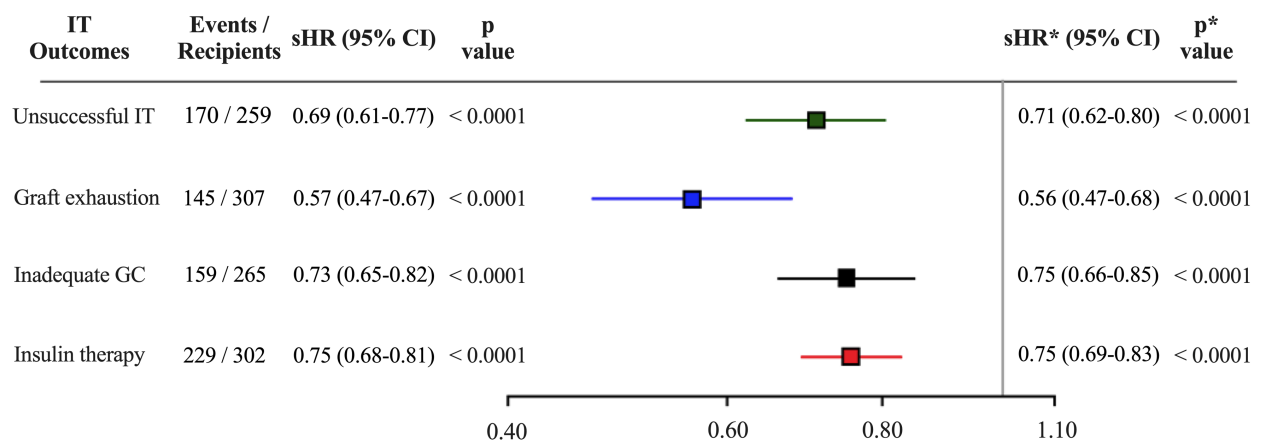
PGF	Unsuccessful IT		Graft exhaustion		Inadequate GC		Insulin therapy	
	2-year	5-year	2-year	5-year	2-year	5-year	2-year	5-year
0	77	94	69	89	68	88	83	95
5	67	88	51	74	60	82	74	90
10	56	80	36	56	52	75	64	83
15	47	70	24	40	44	67	54	74
20	38	59	15	27	37	58	45	64
25	30	49	10	17	31	50	36	54
30	24	40	6	11	26	43	29	45
35	18	32	4	7	21	36	23	37
40	14	25	2	4	17	30	18	29

Values are the predicted probabilities (expressed in %) of cumulative incidence of islet transplantation unfavorable outcomes according to PGF value.

PGF= Primary Graft Function; Inadequate GC= Inadequate Glucose Control

**Supplementary Appendix**

**Supplementary Figure S1: Sensitivity analysis of the association of primary graft function and 5-year cumulative incidence of unfavorable islet transplantation outcomes restricted to recipients with a single islet infusion**

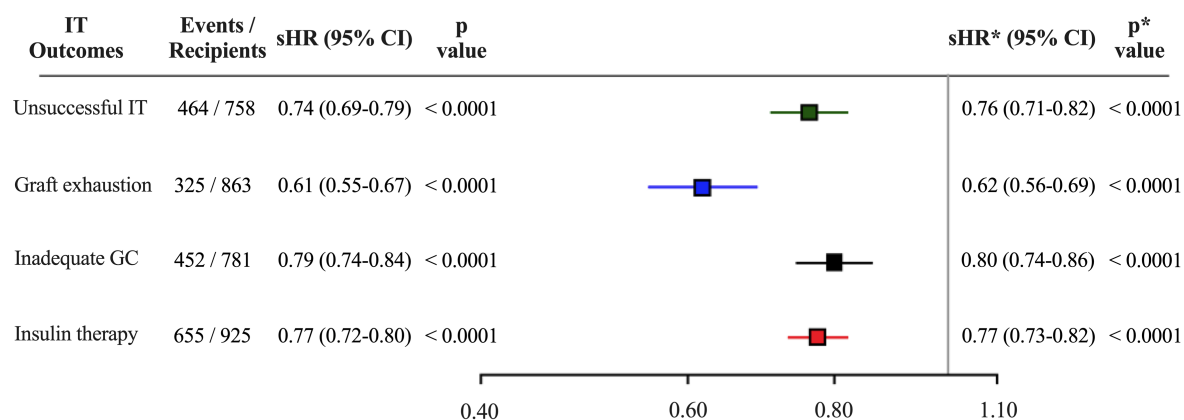


Association of PGF (defined by the Beta2-score at day 28 after the last islet infusion) with cumulative incidence of the four unfavorable islet transplantation outcomes: unsuccessful IT (green), graft exhaustion (blue), inadequate glucose control (black), and the need for insulin therapy (red) are represented with Forest plot. This association was explored using Fine and Gray regression models before and after adjustment on prespecified covariates included all variables suspected or known to impact islet transplantation outcomes (\*): recipient age, sex, body mass index, diabetes duration, daily insulin needs, baseline C-peptide level before islet transplantation, number of islet infusions, total islet mass transplanted per kg of recipient weight, total cell volume transplanted, type of recipient (ITA or IAK), and the use of specific immunosuppression agents (interleukin 2 receptor antagonist, TNF alpha antagonist, T-cell

depleting agent, calcineurin inhibitor and m-TOR inhibitor). SubHazard ratio (sHR) was calculated for 5 units increase of primary graft function. SHs, 95%CI, and p-values are calculated after handling missing values by multiple imputations using the regression-switching approach (chained equations with m = 60 imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion. SHs and 95% CI are represented in a logarithmic scale.

IT= Islet Transplantation; sHR= subHazard Ratio; CI= Confidence Interval; Inadequate GC= Inadequate Glucose Control

**Supplementary Figure S2: Sensitivity analysis of association of primary graft function and 5-year cumulative incidence of unfavorable islet transplantation outcomes restricted to Islet Transplantation Alone (ITA) recipients**



Association of PGF (defined by the Beta2-score at day 28 after the last islet infusion) with cumulative incidence of the four unfavorable islet transplantation outcomes: unsuccessful IT (green), graft exhaustion (blue), inadequate glucose control (black), and the need for insulin therapy (red) are represented with Forest plot. This association was explored using Fine and Gray regression models before and after adjustment on prespecified covariates included all variables suspected or known to impact islet transplantation outcomes (\*): recipient age, sex, body mass index, diabetes duration, daily insulin needs, baseline C-peptide level before islet transplantation, number of islet infusions, total islet mass transplanted per kg of recipient weight, total cell volume transplanted, type of recipient (ITA or IAK), and the use of specific immunosuppression agents (interleukin 2 receptor antagonist, TNF alpha antagonist, T-cell depleting agent, calcineurin inhibitor and m-TOR inhibitor). SubHazard ratio (sHR) was calculated for 5 units increase of primary graft function. SHs, 95%CI, and p-values are



calculated after handling missing values by multiple imputations using the regression-switching approach (chained equations with  $m = 60$  imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion. SHs and 95% CI are represented in a logarithmic scale.

IT= Islet Transplantation; sHR= subHazard Ratio; CI= Confidence Interval; Inadequate GC= Inadequate Glucose Control

**Supplementary Table S1:** Description of the PGF and its component values before and after multiple imputation

	N	Before handling missing values	After handling missing values (m=60)
<b>Components of the Primary Graft Function</b>			
Fasting C-peptide, ng/mL	911	1.38 ± 1.02	1.38 ± 1.02
Daily exogenous insulin needs, IU/kg/day	460	0.10 (0.00-0.26)	0.10 (0.00-0.27)
Fasting blood glucose, mg/dL	826	108 (95-127)	110 (97-130)
HbA1c, %	797	6.43 ± 1.00	6.43 ± 0.98
<b>Primary Graft Function</b>	318	13.8 ± 7.9	14.3 ± 8.8
PGF < 10; 10 to < 20 ; 20 to < 30; ≥ 30		100 (31%); 150 (47%) ; 63 (20%); 5 (2%)	388 (32%); 515 (43%) ; 268 (22%); 39 (3%)
PGF in single islet recipients	108	10.0 ± 7.1	9.5 ± 7.9
PGF in multiple islet recipients	210	15.8 ± 7.6	16.0 ± 8.5
PGF in ITA recipients	251	12.9 ± 7.6	13.9 ± 8.6
PGF in IAK recipients	64	17.4 ± 8.2	16.2 ± 9.5

Data are shown as number (percentage), mean ± standard deviation or median (25<sup>th</sup> to 75<sup>th</sup> percentile). Data are presented before and after handling missing values by multiple imputations using the regression-switching approach (chained equations with m = 60 imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion.

**Supplementary Table S2: Association of primary graft function quartile categories and 5-year cumulative incidence of unfavorable islet transplantation outcomes in the whole cohort**

IT Outcomes	PGF quartiles	Events / Recipients	Unadjusted		Adjusted	
			sHR (95%CI)	p value	sHR* (95%CI)	p* value
Unsuccessful IT						
	< 8.0	177 / 219	1.00 (ref.)	-	1.00 (ref.)	-
	8.0-14.2	153 / 238	0.51 (0.40-0.63)	< 0.0001	0.56 (0.44-0.70)	< 0.0001
	14.2-20.1	136 / 242	0.38 (0.30-0.49)	< 0.0001	0.44 (0.33-0.56)	< 0.0001
	> 20.1	125 / 243	0.32 (0.25-0.40)	< 0.0001	0.35 (0.26-0.45)	< 0.0001
Graft exhaustion						
	< 8.0	165 / 258	1.00 (ref.)	-	1.00 (ref.)	-
	8.0-14.2	96 / 264	0.36 (0.27-0.46)	< 0.0001	0.38 (0.28-0.49)	< 0.0001
	14.2-20.1	71 / 273	0.22 (0.16-0.30)	< 0.0001	0.25 (0.18-0.35)	< 0.0001
	> 20.1	58 / 276	0.17 (0.12-0.23)	< 0.0001	0.19 (0.13-0.27)	< 0.0001
Inadequate GC						
	< 8.0	167 / 230	1.00 (ref.)	-	1.00 (ref.)	-
	8.0-14.2	151 / 245	0.59 (0.47-0.74)	< 0.0001	0.64 (0.50-0.80)	< 0.0001
	14.2-20.1	136 / 247	0.46 (0.36-0.58)	< 0.0001	0.49 (0.37-0.63)	< 0.0001
	> 20.1	124 / 246	0.38 (0.30-0.47)	< 0.0001	0.39 (0.29-0.50)	< 0.0001
Insulin therapy						
	< 8.0	255 / 280	1.00 (ref.)	-	1.00 (ref.)	-
	8.0-14.2	211 / 278	0.61 (0.50-0.73)	< 0.0001	0.65 (0.53-0.78)	< 0.0001
	14.2-20.1	179 / 280	0.40 (0.33-0.48)	< 0.0001	0.42 (0.34-0.52)	< 0.0001
	> 20.1	156 / 283	0.31 (0.25-0.37)	< 0.0001	0.32 (0.25-0.40)	< 0.0001

Association of PGF (defined by the Beta2-score at day 28 after the last islet infusion) described in four risk groups according to quartile categories with cumulative incidence of the four unfavorable islet transplantation outcomes. This association was explored using Fine and Gray regression models before and after adjustment on prespecified covariates included all variables suspected or known to impact islet transplantation outcomes (\*): recipient age, sex,

body mass index, diabetes duration, daily insulin needs, baseline C-peptide level before islet transplantation, number of islet infusions, total islet mass transplanted per kg of recipient weight, total cell volume transplanted, type of recipient (ITA or IAK), and the use of specific immunosuppression agents (interleukin 2 receptor antagonist, TNF alpha antagonist, T-cell depleting agent, calcineurin inhibitor and m-TOR inhibitor). SubHazard ratio (sHR) was calculated with the first quartile (PGF < 8) as reference. SHs, 95%CI, and p-values are calculated after handling missing values by multiple imputations using the regression-switching approach (chained equations with m = 60 imputations) using all patients and transplantation's characteristics and Beta2-score components at day 28 after the last islet infusion.

PGF= Primary Graft Function; IT= Islet Transplantation; sHR= subHazard Ratio; CI= Confidence Interval; Inadequate GC= Inadequate Glucose Control

**Supplementary Table S3:** Discrimination and calibration assessment of primary graft function measured at day 28 following the last islet infusion to predict unfavorable islet transplantation outcomes.

	2-year		5-year	
	Observed	Predicted	Observed	Predicted
<b>Unsuccessful islet transplantation</b>				
<b>PGF quartiles</b>				
<8.0	0.789	0.688	0.882	0.888
8.0-14.2	0.555	0.538	0.721	0.769
14.2-20.1	0.439	0.43	0.641	0.646
>20.1	0.314	0.295	0.608	0.486
<b>C-statistics, median (range)*</b>	<i>0.70 (0.69 to 0.71)</i>			
<b>Graft exhaustion</b>				
<b>PGF quartiles</b>				
<8.0	0.632	0.547	0.713	0.755
8.0-14.2	0.295	0.324	0.429	0.508
14.2-20.1	0.176	0.204	0.317	0.334
>20.1	0.107	0.097	0.255	0.17
<b>C-statistics, median (range)*</b>	<i>0.76 (0.74 to 0.77)</i>			
<b>Inadequate glucose control</b>				
<b>PGF quartiles</b>				
<8.0	0.715	0.632	0.827	0.829
8.0-14.2	0.527	0.497	0.694	0.723
14.2-20.1	0.434	0.415	0.622	0.627
>20.1	0.303	0.313	0.582	0.501
<b>C-statistics, median (range)*</b>	<i>0.65 (0.64 to 0.66)</i>			
<b>Need for insulin therapy</b>				
<b>PGF quartiles</b>				
<8.0	0.875	0.773	0.941	0.913
8.0-14.2	0.682	0.608	0.809	0.804
14.2-20.1	0.506	0.493	0.699	0.698
>20.1	0.383	0.35	0.614	0.537
<b>C-statistics, median (range)*</b>	<i>0.72 (0.71 to 0.73)</i>			

Observed and predicted probabilities of unfavorable islet transplantation outcomes at 2 and 5 years are presented in the table by four risk groups according to PGF quartile categories. Values labeled “observed” were calculated using Kalbfleisch and Prentice method and values labeled “predicted” were calculated as the mean predicted probabilities by the Fine and Gray regression models. Observed and predicted probabilities were calculated after handling missing values by multiple imputations using the regression-switching approach (chained equations with  $m = 60$  imputations) using all patients and transplantation’s characteristics and Beta2-score components at day 28 after the last islet infusion.

\* median and range values of C-statistics corrected for over optimism bias obtained in the 60 imputed datasets by internal validation using bootstrap resampling method (200 resamples)

PGF= Primary Graft Function

**Supplementary Appendix: Complete list of co-authors and institutions from the Collaborative Islet Transplant Registry investigators study group**

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## Part 3: Discussion and Perspectives

## 3.1 Findings

These clinical research studies have allowed us to explore the concept of PGF described in a previous study<sup>72</sup> and initially measured with the Beta-score in 14 ITA recipients and confirm its impact on IT outcomes.

We firstly explored on the Lille single-center cohort the relationship between PGF and the transplantation outcomes at 5 years on ITA and IAK recipients and secondly, we investigated the impact of PGF on the long-term success of IT (10 years prospective cohort) with multivariable analysis on data from donors and transplantation characteristic in a population who benefited from the Edmonton immunosuppression.

Then, in collaboration with other colleagues, we participated in the refinement of the definition of the  $\beta$ -cell replacement success, in particular by applying the measure of continuous glucose monitoring. This definition was, then, used for the last study of our work. Finally, our last work was concluded on a clinical study exploring the largest population of islet transplant recipients in the Collaborative Islet Transplant Registry and using the refined Igls 2.0 success definition as primary outcome.

In this study we investigated the relationship between primary islet graft function (defined as Beta2-score measured on day 28 of the last islet infusion) and four current clinical outcomes of IT at a 5-year follow-up.

Beta2-score measured on day 28 was intentionally chosen because a planned time point is scheduled at day 28 in the registry and because Beta2-score seemed to be more accurate than other indexes and easier to use with a single fasting blood sample to measure PGF.

The strength of our study was the use of the CITR international cohort of allogeneic islet recipients and our analysis included a total of 1210 T1D transplanted recipients in 39 centers

worldwide, using heterogeneous donor graft allocation systems, islet graft and recipient characteristics, and various clinical transplantation and immunosuppression strategies.

We then designed a robust statistical methodology to validate the predictive role of PGF at 5 years on transplantation success, and then to generalize our results into a clinical perspective that will help the clinician in the decision to perform a new islet infusion or not after a previous infusion.

As we have shown, the association between PGF and IT success was linear, in a dose-response manner, demonstrating that increase in PGF is associated with sustained IT success.

We also demonstrated thanks to this large registry the independent relationship between PGF and IT success from other known predictors impacting IT and used to adjust the strength of the relation between PGF and the 5-year outcomes.

## 3.2 Limitations

The primary islet graft function concept has therefore some limitations.

Indeed, the primary islet graft function in the context of multiple donors is difficult to capture.

In kidney transplantation, it is possible to easily evaluate whether the patient has urinated or not the day after the transplantation, or even more precisely by measuring the glomerular filtration rate of the kidney transplant.

Islet transplantation, unlike solid organ transplantation, is currently a sequential cell transplantation of multiple transplants from different donors. This means that the graft is composed of several clusters of free islet cells in the liver sinusoidal capillaries and will require from 1 to 4 weeks to be correctly re-vascularized following infusion in the portal vein.

In IT, the primary islet graft function is therefore the result of interactions between different entities that impact islet functional mass such as donor characteristics, islet cell isolation and culture processes, quality of the recipient and its auto and alloimmune conditions, transplantation and immunosuppression strategies.

Thus, primary islet graft function is a global and composite metric of early islet potency and engraftment and reflects at the time of its measurement factors that cannot always be controlled or appreciated. Finally, PGF is the overall early potency of islet cells transplanted from multiple donors. This composite index accounts for multiple donor factors and is ultimately a proxy for the whole islet graft treated as a “single organ”, which we recognize is a potential source of bias.

The Transplant Estimated Function developed by Piemonti and colleagues <sup>64</sup> is a different approach because it estimates the daily insulin secretion and therefore the secretory capacity of the graft, has similar performances to the Beta-score and has the benefit of being

normalized to the number of transplanted islets. However, in the registry, we mostly did not have the data between the different islet infusions to calculate it.

The method used to measure primary graft function in the CTR study was the Beta2-score, a composite continuous (0 for no beta-cell function) index requiring a fasting blood sample and calculated with values of fasting C-peptide and blood glucose, glycated hemoglobin and daily exogenous insulin needs per kg of recipient weight.

Several composite indexes have been proposed to measure islet graft function based on measurements of serum C-peptide and blood glucose in fasting or stimulated conditions and based on exogenous insulin requirements<sup>65,64,74,66</sup>.

In this study, we chose to use the Beta2-score, because it is a validated index, easy to use and does not require measurement after stimulation by a standardized meal test like the Beta-score. On the other hand, Forbes and colleagues showed that Beta2-score was more accurate than Beta-score in the discrimination of glucose intolerance and insulin independence, which is partly related to its continuous characteristic as opposed to Beta-score as a categorical variable<sup>66</sup>. Lam and colleagues showed that the Beta2-score measure of primary graft function was more accurate than the Secretary Unit of Islet Transplant Objects (SUITO) index, the homeostasis model assessment index of beta-cell function (HOMA2-B%), the C-peptide/glucose ratio, and the Transplant estimated function (TEF) index to discriminate insulin independence in patients.

Therefore, we cannot exclude that more refined approaches to estimate islet graft functional mass, such as dynamic testing of endogenous insulin resources, could improve the accuracy of predicting long-term outcomes<sup>1</sup>.

We originally estimated PGF one month after the last islet infusion as reported in the first two articles of our work<sup>6,73</sup> and at day 28 from the last infusion in the CTR study because this corresponded to an expected registry time point.

However, assessment of islet graft function at other time points could be more accurate.

Lam and colleagues reported that primary graft function assessed 7 days after islet infusion was related to transplantation success at 5 years in an univariate analysis of the Edmonton cohort<sup>75</sup>. However, they also clearly showed that the Beta2-score still increased until 4 to 6 weeks after islet infusion.

In the registry, we did not have available visit at 6 weeks and we believe that day 28 seems to be a reasonable time in clinical practice to assess the functional mass of engrafted islets. It also seems to be an appropriate time to ensure the restoration of physiological blood flow to the transplanted islets, as previously suggested<sup>76,77</sup>.

In this study, we performed a multivariate analysis to explore the relationship between PGF and transplantation success in order to adjust the strength of the relation between PGF and IT success on known or suspected confounding factors impacting transplantation outcomes.

The validated prediction model, in contrast, was univariate and based only on PGF to predict IT success because PGF explained alone IT outcomes with good accuracy as shown by the median (range) C-statistic values of 0.70 (0.69-0.71); 0.76 (0.74-0.77); 0.65 (0.64-0.66); 0.72 (0.71-0.73) for prediction of the cumulative incidence of unsuccessful IT, graft exhaustion, inadequate glucose control, and the need for insulin therapy, respectively.

Some factors also impacted transplantation outcomes, and were not reported in the final manuscript in agreement with the methodological approach.

Indeed, in univariate analysis, m-TOR maintenance immunosuppression was associated with sustained insulin independence and the use of TNF alpha antagonist was associated with

sustained graft function after IT. End to note, the positive impact of anti-TNF potentiated induction has been previously reported in the CITR registry <sup>59,78</sup>.

Higher insulin requirements before transplantation was associated with unsuccessful IT, inadequate glucose control and with the need for insulin therapy after IT. Higher recipient body mass index was associated with inadequate glucose control after IT. These factors were previously suspected to impact long-term success of IT <sup>6,72,73</sup>.

In our study, the number of islet infusions and the total islet mass transplanted had no impact on the long-term islet graft function, which was in agreement with the results on the Lille cohort <sup>6,73</sup> and also recently reported by the Edmonton group <sup>53</sup>.

However, and this is the most common and frequent approach in IT, we repeat islet infusion to intuitively increase the primary graft function <sup>4</sup>.

This strategy is therefore not always necessary as Hering and colleagues demonstrated that insulin independence could be achieved with single-donor transplants by careful selection of islet graft and recipient <sup>21</sup>.

On the other hand, repeated islet infusion exposes to the lack of organ donors, to the risk of alloimmunization, even if the impact of donor specific HLA antibodies on the transplantation outcome is controversial <sup>54,55,79</sup>. Finally, the repetition of islet infusion exposes to the risk of complications. Caiazzo and colleagues reported a significant lower primary graft function when patients had procedure-related complications following IT and demonstrated its specific impact on the long-term graft survival <sup>50</sup>.

Repeated islet infusion represents therefore ethical, logistical, medical and financial issues.

### 3.3 Perspectives and clinical implications

First, this PhD study showed that primary graft function measured at one month after islet infusion was an accurate predictor of long-term IT outcomes. And our research aimed at exploring, validating and predicting the impact of PGF on clinical IT outcomes at 5 years.

This study also highlighted that in order to improve IT outcomes, it is crucial to improve PGF, which reflects the quality of the islet graft. Therefore, PGF in clinical trials could be an early surrogate marker of transplant success. And future clinical trials comparing strategies to improve islet success could use PGF calculated at day 28 as an early primary endpoint.

Thus, there are different approaches to improve islet graft quality and they could be assessed by measuring PGF.

Improving PGF by selecting donors or isolated islets on qualitative parameters is currently not very realistic, because of the lack of donors.

However, if specific biomarkers have been related with clinical transplantation outcomes and could assess early islet potency, their values are not available before the decision of transplanting an islet preparation in a candidate recipient<sup>77,80-82</sup>. For this reason, pancreas donors are mostly selected on quantitative criteria, i.e. their potential to deliver a large enough islet mass following isolation as shown with the use of the North American Islet Donor Score (NAIDS)<sup>83</sup> or by using specific tests in the donor<sup>84</sup>.

In the CITR cohort, we have explored the determinants of primary islet graft function in patients who received a single islet infusion to analyze factors that impacted PGF value (data not reported in the manuscript). Age of the donor, islet culture before transplantation, islet mass transplanted, immunosuppression, type of recipient (ITA or IAK), pre-transplant glycated



hemoglobin level and the recipient's autoimmune status are key factors impacting the Beta2-score value measured at 28 days after islet infusion.

As islet processing strategies prior to transplantation could impact islet quality, viability and thus primary islet graft function, promising tools for pancreas organ management, such as hypothermic perfusion following procurement and before isolation, are investigated<sup>85,86</sup>.

These innovative enhancements could benefit in improving islet graft quality, at least in marginal donors. However, these highlights need to be studied more extensively on pancreas organs, even if they provided demonstrated benefits in kidney and liver transplantation in humans<sup>87</sup>.

Improving the engraftment of islet cells and their early potency is probably one of the most promising paths to increase primary islet graft function.

Thus, novel anticoagulation strategies associated with intraportal IT<sup>88</sup> or the identification of alternative transplant sites<sup>89-91</sup> potentiated by immune-protective strategies are a constant source of research in the field of IT.

To date, intraportal transplantation with heparin anticoagulation remains the approach that has achieved the best clinical outcomes when compared to extra hepatic transplantation as shown in a recent comparative cohort study<sup>92</sup>.

On the other hand, our study was able to demonstrate that the outcome of IT could be accurately predicted by calculating the PGF 28 days after islet infusion. A clinical application of our study is to help the clinician to decide whether or not to repeat an islet infusion, by measuring the PGF, 28 days after the first infusion. If the predicted outcomes are satisfactory, the transplantation program could be ended, but if the predicted outcomes are judged inadequate for the needs of a given patient, the clinician could decide to put the patient back on the waiting list for a new IT. A predicted risk calculator has been developed and is available

online to help clinicians in this decision-making process (<https://lille-model.shinyapps.io/PGF-islet/>)

The preliminary "proof of concept" report of an ongoing multi-center open-label clinical trial phase 1/2, in several North American expert centers, has shown that pluripotent stem cells derived from endodermal cells and transplanted subcutaneously under immunosuppressive regimen using a macro-encapsulation device in 17 patients with T1D were able to survive and differentiate into insulin-secreting cells in 63% of patients<sup>93</sup>. This trial was not able to detect circulating C-peptide in the recipient. Another open-label phase 1/2 study of subcutaneous transplantation of pancreatic endodermal stem cells into macro-encapsulation devices, combined with an immunosuppressive regimen reported increased fasting C-peptide levels and increased C-peptide levels after stimulating with standardized meal challenge at one year in 15 patients<sup>94</sup>. Finally, the Vertex firm recently reported at the American Diabetes Association 2022 Annual Meeting the first patient treated with an intraportal transplantation of fully differentiated stem cell-derived pancreatic islet cells (VX-880™). At day 90 post-transplantation a large increase in fasting and stimulated C-peptide and a significant improvement in glycemic control and exogenous insulin requirements were observed<sup>95</sup>.

This three recent reports of ongoing clinical studies using insulin-secreting stem cells represent a breakthrough in our field and could dramatically shift the management of type 1 and type 2 diabetes in the near future.

Indeed, these pancreatic islet-like tissues derived from stem cells are an unlimited source of cells. The measurement of PGF could allow to identify in future trials the required and adequate dose of transplanted stem cells to reach a sustainable metabolic success in diabetic patients.

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## Part 5: Abstract

## Abstract

Pancreatic islet transplantation (IT) is an established  $\beta$ -cell replacement therapy for the treatment of severe type 1 Diabetes (T1D). The allogeneic human islet isolated from a pancreas procured from a brain-dead donor and transplanted under immunosuppression in the liver allows restoration of endogenous insulin secretion which decreases severe hypoglycemic events and maintains optimal glucose balance and quality of life.

A recent study reported patients with prolonged islet graft survival and sustained metabolic benefits for at least 20 years.

Unfortunately, Half of the patients will experience islet graft failure within the first 5 years after IT, as reported by the international Collaborative Islet Transplant Registry (CITR).

This failure is multifactorial and is due to the characteristics of the donor, recipient, isolation process, cell culture, transplantation and immunosuppressive.

Our work focused on the optimization of IT and especially on the predictive role of the primary islet graft function (PGF).

We firstly explored on the Lille cohort (2003-2012, 28 adult T1D patients), the relationship between PGF and 5-year IT outcomes on islet transplant alone (14 ITA) and islet-after-kidney transplantation (14 IAK) recipients.

PGF, measured with the Beta-score one month after the last islet infusion was optimal in 18 patients (64%) (Beta-score  $\geq 7$ ) and suboptimal in 10 patients (36%) (Beta-score  $< 7$ ).

82%(95% CI, 62-92) of patients sustained graft survival and 39%(22-57) were insulin-independent at 5 years. Optimal PGF recipients had better 5-year graft survival rate than suboptimal PGF recipients with 100% vs 50%(18-75), respectively (P  $< 0.001$ ).

56%(31-75) vs 10%(1-36), ( $P < 0.001$ ) were insulin-independent at 5 years, respectively. Patients with suboptimal PGF had a 6-fold higher risk of insulin reintroduction, and a 13-fold higher risk of graft failure at 5 years.

28%(13-45) of patients remained insulin independent and graft function was sustained in 78%(57-89) at 10 years. We then confirmed that optimal PGF confirmed to be associated with sustained IT success in the long-term. Type of recipient (ITA/IAK) did not impact success.

In the second part of our work, we participated in the refinement of the definition of the  $\beta$ -cell replacement success, by applying the measure of continuous glucose monitoring (CGM). Data were collected for 10 years in 39 ITA and 16 IAK T1D recipients and compared to the four categories of success at any time points.

Following IT, the median (IQR) time spent in the glucose range (70-180 mg/dL) was gradually and significantly improved and the time spent below the range (HYPO  $<70$  mg/dL) was significantly and gradually decreased in patients with failure, marginal, good and optimal categories of success, respectively, and allowed us to refine the definition of success by including CGMS measures.

Finally, our last work explored the largest population of islet transplant recipients in the CITR. PGF confirmed to be related to the 5-year IT success and this association was linear, resulting in a dosage-effect response independently from the number of infusions, total islet mass transplanted, and immunosuppression regimen in this large observational cohort study of 1210 patients. PGF measure, 28 days after the last infusion predicted IT outcome with good accuracy.

In conclusion, PGF could guide current clinical practice by informing one month after the last infusion, the decision to repeat a new islet infusion or not and could serve as an early surrogate endpoint in future trials.

Recent ongoing clinical studies reported the first patient treated with intraportal transplantation of pancreatic islet-like cells derived from stem cells and observed significant improvement in glycemic control and decrease in exogenous insulin requirements.

PGF could also allow to identify in future trials the required and optimal dose of transplanted stem cells to reach a sustainable metabolic success in diabetic patients.

## Résumé

La transplantation d'îlots de Langerhans (TIL) allogénique intrahépatique, issus des cellules d'un pancréas de donneur cadavérique est une thérapie validée de remplacement des cellules  $\beta$  du diabète de type 1 (DT1) sévère et permet de restaurer la sécrétion endogène d'insuline, diminuent les hypoglycémies sévères et maintiennent un équilibre glycémique optimale.

Une étude récente montre des bénéfices métaboliques durables ( $\geq 20$  ans) chez les patients greffés avec un greffon fonctionnel.

Cependant, dans les 5 premières années, la moitié des patients connaissent une perte du greffon comme rapporté dans le registre international (CITR).

Cet échec est multifactoriel et dépend des caractéristiques du donneur, du receveur, de l'isolement, de la transplantation et de l'immunosuppression.

Notre travail s'est concentré sur l'optimisation de la TIL et sur le rôle prédictif de la fonction primaire du greffon d'îlots (PGF).

Nous avons exploré sur la cohorte lilloise, la relation entre le PGF et les résultats de la TIL à 5 ans chez les receveurs de greffe d'îlots seuls (14 ITA) et de greffe d'îlots après rein (14 IAK).

La PGF, mesuré par le Beta-score un mois après la dernière infusion était optimale chez 18 patients (64%) (Beta-score  $\geq 7$ ) et suboptimale chez 10 patients (36%) (Beta-score  $< 7$ ).

82 % (IC95 %, 62-92) des patients ont bénéficié d'une survie du greffon et 39% (22-57) étaient insulino-indépendants à 5 ans. Les patients avec une PGF optimale avaient une survie prolongée du greffon à 5 ans par rapport à ceux présentant une PGF sous-optimale : 100 % vs 50 % (18-75), respectivement (P  $< 0,001$ ).

56%(31-75) vs 10%(1-36) de ces patients ( $P < 0,001$ ) étaient insulino-indépendants à 5 ans, respectivement. Les patients avec une PGF sous-optimale avaient un risque 6 fois plus élevé de reprise de l'insuline, et 13 fois plus élevé de perte du greffon à 5 ans.

28%(13-45) des patients étaient insulino-indépendants et 78%(57-89) avaient un greffon fonctionnel à 10 ans. La PGF optimale était associée au succès durable de la TIL à 10 ans. Le type de receveur (ITA/IAK) n'avait pas d'impact sur le succès.

Ensuite, nous avons participé au raffinement de la définition du succès des stratégies de remplacement des cellules  $\beta$ , en appliquant les données de la surveillance continue du glucose (CGM) collectées pendant 10 ans chez 39 patients ITA et 16 IAK diabétiques et comparées aux quatre catégories de succès.

Après la TIL, le temps médian (IQR) passé dans l'intervalle glycémique recommandé (70-180 mg/dL) s'est progressivement et significativement amélioré et le temps passé en dessous de cette limite (HYPO  $<70$  mg/dL) a significativement diminué, et ce graduellement dans les catégories croissantes de succès de la greffe nous permettant d'affiner la définition du succès en incluant les mesures du CGMS.

Enfin, nous avons exploré la plus grande population de transplantés d'îlots dans le registre international du CTR chez 1210 patients. La PGF a confirmé être liée au succès de la TIL à 5 ans et cette association était linéaire, avec une réponse dose-dépendante et indépendamment du nombre d'infusions, de la masse totale d'îlots transplantés et du régime d'immunosuppression. La PGF, mesurée 28 jours après la dernière infusion, permettait de prédire le résultat de la TIL avec une bonne précision.

En conclusion, la PGF pourrait influencer la pratique clinique actuelle en informant, un mois après la dernière infusion d'îlots, la décision de répéter ou non une nouvelle infusion et pourrait servir de critère de jugement précoce dans les essais futurs.

Des études cliniques récentes en cours ont rapporté le premier patient traité par transplantation intraportale de « d'îlots » pancréatiques dérivés de cellules souches et ont montré une amélioration significative du contrôle glycémique.

La PGF pourrait ainsi permettre d'identifier la dose nécessaire et optimale de cellules souches à transplanter pour atteindre un succès métabolique durable chez les patients diabétiques.