

Chapter V.1

Islet Transplantation in Type 1 Diabetes: Ongoing Challenges, Refined Procedures, and Long-Term Outcome

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■ Abstract

Remarkable progress has been made in islet transplantation over a span of 40 years. Once just an experimental curiosity in mice, this therapy has moved forward, and can now provide robust therapy for highly selected patients with type 1 diabetes (T1D), refractory to stabilization by other means. This progress could not have occurred without extensive dynamic international collaboration. Currently, 1,085 patients have undergone islet transplantation at 40 international sites since the Edmonton Protocol was reported in 2000 (752 allografts, 333 autografts), according to the Collaborative Islet Transplant Registry. The long-term results of islet transplantation in selected centers now match registry data of pancreas-alone transplantation, with 6 sites reporting five-year insulin independence rates $\geq 50\%$. Islet transplantation has been criticized for the use of multiple donor pancreas organs, but progress has also occurred in single-donor success, with 10 sites reporting increased single-donor en-

graftment. The next wave of innovative clinical trial interventions will address instant blood-mediated inflammatory reaction (IBMIR), apoptosis, and inflammation, and will translate into further marked improvements in single-donor success. Effective control of auto- and alloimmunity is the key to long-term islet function, and high-resolution cellular and antibody-based assays will add considerable precision to this process. Advances in immunosuppression, with new antibody-based targeting of costimulatory blockade and other T-B cellular signaling, will have further profound impact on the safety record of immunotherapy. Clinical trials will move forward shortly to test out new human stem cell derived islets, and in parallel trials will move forward, testing pig islets for compatibility in patients. Induction of immunological tolerance to self-islet antigens and to allografts is a difficult challenge, but potentially within our grasp.

Keywords: diabetes • islet • transplantation • immunosuppression • allograft

1. Overview perspective

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of beta-cells within the pancreas, resulting in absolute deficiency of insulin. Hyperglycemia, ketoacidosis, and dehydration are the immediate consequences if left untreated. Subcutaneous bolus injection of insulin has been the definitive, life-sustaining therapy since 1922, following its discovery by Nobel Laureates Banting, McCleod, and co-workers Best and Collip. Standard or intensive insulin therapy remains the gold-standard therapy

for the majority of patients with T1D. Tight glycemic control lessens the risk of secondary diabetic complications, but substantially increases risk of troublesome and occasional life-threatening hypoglycemia. For a minority of patients, a life troubled by frequent hypoglycemia, and fear of the 'dead-in-bed' syndrome or of progressive complications, is not tolerable, and alternative strategies become increasingly attractive [3-6]. Whole pancreas transplantation remains a realistic option for some patients, especially in the setting of renal failure where a combined kidney-pancreas can be life-changing, restoring euglycemia with considerable

reserve. However, this approach requires major surgical intervention with attendant risk of complications and occasional mortality.

A far more elegant means to restore endogenous, physiologic insulin secretion was conceived and tested in 1893, almost 30 years before the discovery of insulin, when Williams and Harsant from Bristol, England, transplanted sheep pancreatic fragments subcutaneously under chloroform anesthesia in a desperate attempt to save a 15 year old boy dying of ketoacidosis [7]. The boy succumbed on the third day after the xenograft failed.

The concept of cellular replacement therapy for T1D lay dormant for 80 years until 1972, when Ballinger and Lacy cured chemical diabetes in rats by islet transplantation [8, 9]. Intensive, collaborative research over the next 40 years has moved islet transplantation forward as a viable option for highly selected T1D patients with 'brittle' control. Essential steps on this recent journey include the Ricordi Chamber for large-scale processing of the human pancreas [10], density-gradient islet purification on cell apheresis systems [11], and optimized processing and culture conditions in clinical good manufacturing practice (cGMP) facilities [12].

Lacy's team carried out the first successful clinical islet transplant in 1989. Insulin independence lasted for one month, but the cells were rejected due to inadequate immunosuppression [13]. More success was obtained with islet autotransplantation after surgical pancreatectomy for chronic pancreatitis. No immunosuppression was needed, and autoimmunity was not an issue, which explained the higher rates of insulin independence [14]. Tzakis *et al.* performed abdominal extirpation with combined abdominal liver and islet transplantation in 1990, and half the cohort attained insulin independence before dying from recurrence of abdominal malignancy [15, 16].

The concept of islet replacement clearly worked and was enticing, but the added challenge of autoimmune T1D seemed to create an insurmountable barrier. Indeed, 447 attempts to treat T1D with islet transplantation were made between 1974 and 2000, but the results were dismal, with less than 10% maintaining insulin independence and only 28% having detectable C-peptide by one year [17]. The introduction of the Edmonton Protocol in 2000 was therefore seen as a milestone success, as 100% of the first 7 patients treated achieved and maintained insulin independence at one year [18]. An essential component was the use of corticosteroid-free immunosuppression with anti-CD25 monoclonal antibody (mAb) induction and maintenance tacrolimus and sirolimus. Of

Abbreviations:

- ATG – antithymocyte globulin
- BK – Brennan-Krohn
- BLA – Biological License Application
- BMI – body mass index
- cGMP – clinical good manufacturing practice
- CMRL – chemically-defined basal culture medium
- CMV – cytomegalovirus
- CNI – calcineurin inhibitor
- CIT – Clinical Islet Transplant Consortium
- CITR – Collaborative Islet Transplant Registry
- CT – computed tomography
- DCCT – Diabetes Control and Complications Trial
- DCD – deceased cardiac death
- ECD - expanded criteria donors
- FDA – Food and Drug Administration
- GADA – glutamic acid decarboxylase
- GLP-1 – glucagons-like peptide 1
- HbA1c – glycosylated hemoglobin
- HIV – human immunodeficiency virus
- HLA – human leukocyte antigen
- HTK – histidine-tryptophan-ketoglutarate
- HYPO score – composite hypoglycemic score
- IA2A – insulinoma-associated protein 2A
- IAK – islet after kidney
- IBMR – instant blood-mediated inflammatory reaction
- IEQ – islet equivalent
- IL – interleukin
- ITA – islet transplantation alone
- LFA1 – lymphocyte function-associated antigen 1
- LI – lability index
- mAb – monoclonal antibody
- MHC – major histocompatibility complex
- MMF – mycophenolate mofetil
- MTF - liberase mammalian tissue-free
- NDD – neurological determination of death
- NOD – non-obese diabetic
- PRA – panel reactive antibody
- RCT – randomized controlled trial
- SCD – standard criteria donors
- SRL – sirolimus
- T1D – type 1 diabetes
- Tac – tacrolimus
- TNF – tumor necrosis factor
- UW – University of Wisconsin

equal importance was the delivery of a sufficiently large islet transplant mass (>10,000 islet equivalents (IEQ)/kg recipient body weight) prepared from two or more donors.

Clinical islet transplantation has since transitioned over the past 13 years from rare, experimental curiosity to routine treatment, providing robust glycemic control for a small subset of 'brittle' T1D patients refractory to stabilization by other means. Since 2000, 1,085 patients have undergone islet transplantation at 40 international sites (752 allografts, 333 autografts), according to the Collaborative Islet Transplant Registry (CITR) [19]. The most active clinical center (University of

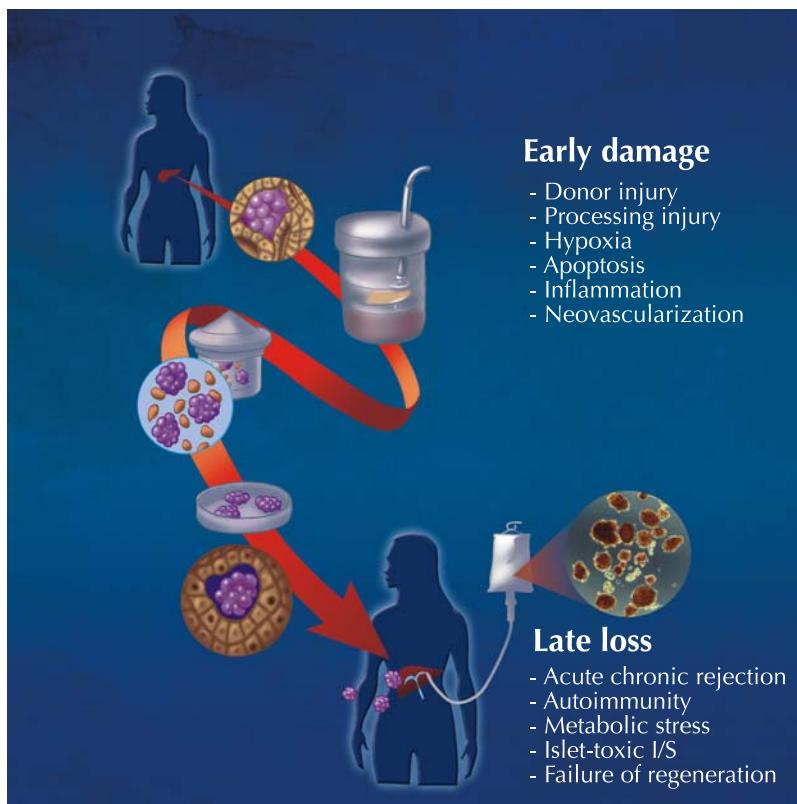


Figure 1. Illustration of the steps involved in pancreatic digestion, including islet isolation and intraportal transplantation within the liver. Inset photomicrograph shows human islets stained with dithizone red dye, indicative of a highly pure preparation. The lower inset labels indicate the challenges involved with early islet damage post-transplant, and the factors leading to late islet graft loss; both of which must be addressed to maintain excellent long-term graft function.

Alberta) performed a total of 61 islet transplants in 2012 alone, and has done a cumulative total of 382 transplants in 183 recipients to the present day. At least in expert hands, islet transplantation may be considered among the safest of all transplantation procedures compared with solid organs. In several countries, including Canada, Australia, the United Kingdom, France, Switzerland, Norway, Sweden, and other parts of Europe, islet transplantation is funded as 'non-research' standard clinical care. Major trials are underway in the US, funded by the National Institutes of Health through the Clinical Islet Transplant Consortium (CIT), designed to generate sufficient data for a Food and Drug Administration's (FDA) Biological License Application (BLA). Two registration trials (CIT-06, islet-after-kidney, and CIT-07, islet-alone) will deliver data in 2015 that will likely fa-

cilitate insurance reimbursement through Medicare, Medicaid, and other third-party payers. Funding for islet processing and clinical care has been a major rate-limiting step in the US, and reimbursement will certainly lead to a more sustained and expanded clinical activity.

This review highlights the recent progress in islet isolation and clinical transplantation that now justifies further expansion of islet transplantation. An adequately powered, head-to-head randomized, controlled trial (RCT) of islet transplantation with intensive insulin and insulin pumps is much needed, but may be difficult to fund. In the longer term, a limited human islet supply and the need for intense immunosuppression are seen as the two drawbacks that prevent broader application of islet transplantation in T1D [20, 21]. Improvements in single-donor transplant engraftment, techniques to expand islet mass *in vitro*, and alternative cellular therapeutics, including human stem cell-derived islets or a xenogeneic pig source, will be required to bridge the gap in supply. To minimize the risk from immunosuppression, im-

munological tolerance is desirable, but will be challenging to establish in the presence of dual allo- and autoimmunity. Despite the perceived and known risks of current immunosuppression, risks are likely equivalent or perhaps lower than the risks of poorly controlled T1D and accelerated complications, but a matched RCT is needed to determine this with clarity. Transplanting early in the course of T1D could further improve the protective benefit, but justification for inclusion of children will require additional advances in immunosuppression and monitoring [22]. The risk-benefit equation would be improved considerably if we had effective, predictive immune biomarkers for autoimmunity and alloreactivity. Progress in peripheral blood T cell and antibody-based assays of auto-and alloreactivity may change this

equation, permitting more precise titration of therapy to those with higher immunologic risk, while considerably lowering risk of infection or malignancy for those at less risk [23-25].

Successful islet transplantation begins with the selection and care of the multiorgan donor. Protection of the pancreas during transportation with minimization of cold ischemic injury is important for islet viability. The sequential steps involved in islet processing, purification, and culture are critical to the quality of the final islet product (**Figure 1**). Detailed clinical protocols are required for safe cellular transplantation, and for effective control of inflammation, auto- and alloimmunity, if the islet graft function is to be sustained long-term, while minimizing recipient risk. What follows is a step-by-step overview of the critical steps, and is based on a remarkable collaborative consensus between international investigators.

2. Donor selection, pancreas procurement, islet isolation and culture

While careful donor selection is imperative to avoid transmissible infection or malignancy, expensive processing of organs is unlikely appropriate to yield potent islets. Multivariate analyses suggest an optimal donor age >20, high donor BMI (provided HbA1c <6.0%), normoglycemia, no hypotension or cardiac arrest, and minimal inotropic support as key to successful islet isolation [26-34]. Donor selection is especially critical for the success of single-donor islet engraftment; donor age <50 and BMI >27 kg/m² were important components of the Minnesota series [35, 36]. Recent refinement in isolation techniques by the San Francisco group, a collaborative member of CIT, has resulted in marked improvement in islet yield from younger donors. Combined with improved single-donor success rates, these techniques may have an important impact on future allocations of pancreas organs for whole pancreas vs. islet transplantation [33, 37-40].

The majority of donors fall into the categories standard criteria donors (SCD) or expanded criteria donors (ECD), where the heart is beating, but there is neurological determination of brain death (NDD). To address the donor shortage, increasing use of deceased cardiac death (DCD) donors are being used, where circulation has ceased and organs are subject to warm ischemic injury. DCD donors have been used successfully for islet transplantation, but mandate stringent selection criteria (controlled setting, warm ischemia < 30 min, cold

ischemia < 4 hours) to preserve islet potency [41, 42].

The pancreas is provided by meticulous surgery with preservation of capsular integrity. This is important for subsequent enzymatic delivery. Handling is minimized to avoid pancreatitis and local injury prior to aortic cross-clamping [43]. The pancreas is cooled rapidly with intracellular preservation solutions and topical ice, then transported to the cGMP islet isolation center. Both solutions, University of Wisconsin (UW) and histidine-tryptophan-ketoglutarate (HTK), provide equivalent protection of the pancreas during transport for islet isolation, although HTK may be inferior to UW for whole pancreas transplantation preservation [44-46]. Cold ischemic times are ideally kept to below 6-8 hours where possible.

In the cGMP facility, the pancreas is trimmed, the duodenum and spleen removed, and the pancreatic duct is cannulated. Enzyme is first delivered at 4-10°C for 10 minutes, then warmed for 4 minutes to 37°C to deliver active collagenase enzyme to the islet-acinar interface [47, 48]. The process is designed to reduce a 70-100g pancreas to extract the 1-2% containing islets in as lower purity and volume as possible. A minimal islet mass of 5,000 IEQ/kg is generally required for each transplant, and > 6,000-7,000 IEQ/kg for single-donor success. An inability to extract high-yield, high-potency human islets was a formidable challenge in the early 1980's, and precluded success in early clinical trials. Ricordi's automated method revolutionized the process. It involves the sectioning of the distended pancreas into several large pieces, and transfer to a chamber containing steel marbles and a 500 µm mesh screen [10]. The enzymatic solution recirculates at 37°C for as long as it takes (typically 15-30 minutes) to free islets from their surrounding matrix. Serial samples are stained with dithizone to determine the optimal time-point to dilute and cool the process [49, 50].

A major key factor to successful islet isolation is the quality, specificity, and stability of the collagenase enzymatic blend used. Liberase HI (Roche Diagnostic Pharmaceuticals, Indianapolis, USA) was a major advance over previous products, but was discontinued based on potential but infinitesimally small concern of transmission of Creutzfeld-Jacob related prion disease [51]. Alternative, and more optimal blends have been developed, including Serva neutral protease NB1 (Serva Electrophoresis GmbH, Heidelberg, Germany) [33, 52, 53]. Kin *et al.* found that separation of NB-1 and neutral protease components, with ductal de-

livery of NB-1 and neutral protease added subsequently during recirculation, led to less enzymatic degradation and more potent yields [54]. A Liberase Mammalian Tissue Free blend (MTF, Roche Diagnostics Inc., USA) has since been released, and has optimal digestion kinetics similar to NB-1 [33]. A further enzymatic blend called Vitacyte HA (Vitacyte LLC, Indianapolis, USA), has been blended with controlled component activity for less class II degradation, and is being further evaluated presently [55].

After digestion and serial wash steps, the digest is purified using density-gradient separation on refrigerated COBE2991 cell processors [56, 57]. The duration of centrifugation is 10 minutes. Discontinuous Ficoll gradients were used previously, but Hering *et al.* introduced continuous iodixanol gradients (Optiprep, Axis-Shield, Oslo, Norway) to increase efficiency, and to reduce inflammation [35, 58]. The combination of iodixanol with University of Wisconsin preservation solution increases the differential density between exocrine and islet tissue, further supporting the enrichment process [59]. Repeating the purification run as a 'rescue gradient' may additionally augment islet recovery [60-62].

After further wash and recombination steps, this islet preparation is maintained in culture for 24-72 hours before release and clinical transplantation. The culture step improves purification as contaminating exocrine tissue survives poorly in culture. 10-20% of the islet mass is lost during culture, but a reduced state of inflammation in the final product reduces early innate and adaptive immune events, and those marginal islets likely would not engraft in the recipient. Islet culture at 24°C was previously shown to reduce MHC antigen expression [63]. Addition of insulin, transferrin, zinc, selenium, and pyruvate to CMRL-based culture media (Miami Media) supplemented by nicotinamide [64], and recently modified by the Lille group, further optimizes islet survival in culture [65-67]. The culture period also provides an important opportunity to transfer the islet recipient to the transplantation center, to condition, administer T cell-depletional agents, and give adjunctive anti-inflammatory agents, while avoiding exposure of newly transplanted islets to an injurious cytokine storm [31, 68-73]. If islets are to be shipped from a remote cGMP facility to a distant clinical transplantation site, the obligate culture is helpful, concentrating skill and expertise locally and minimizing costs associated with isolation [74-76].

Before transplantation, the final islet preparation must meet all 'product release criteria', which include:

1. Sterility (absence of bacteria on gram stain, low endotoxin content <5 EU/kg), with final *post hoc* cultures available by 14 days
2. Potency (static insulin release stimulation index >1.0)
3. Volume (packed volume ≤5.0 cc or settled volume ≤7.5 cc)
4. Purity (≥30% based on dithizone staining)
5. Viability (≥70% on membrane integrity dye exclusion staining with fluorescein diacetate/propidium iodide or Syto green)
6. Minimal islet mass (≥5,000 IEQ/kg for routine initial transplants, ≥6,000 IEQ/kg for single-donor protocols, and ≥4,000 IEQ/kg for retransplants)
7. Compatibility (identical or compatible ABO blood, negative cytotoxic cross-match if panel reactive antibody (PRA) >10-15%).

The product release criteria are minimal criteria needed to transplant, but the FDA has emphasized a need to develop predictive islet potency assays to correlate with clinical efficacy. Alternative options include high-throughput kinetic flux imaging for beta-cell potency [77], laser scanning cytometry for cellular composition and mitochondrial apoptosis [78], and oxygen consumption rates, as developed by Papas and coworkers. These options strongly predict clinical potency and correlate closely with the bio-assay of reversal of diabetes in immunodeficient mice using small aliquots of the final product [79].

3. Clinical islet transplantation

3.1 Selection of patients

The indications for islet transplantation are summarized in **Table 1**. Islet transplant alone (ITA) refers to C-peptide-negative T1D patients with a sufficient duration (>5 years) to justify that all reasonable attempts have been made to correct refractory poor glycemic control by all other means. This requires an independent endocrinologist or diabetologist to optimize intensive insulin management and to consider insulin pump therapy and frequent glycemic monitoring, possibly including the use of a continuous glucose monitor and alarm [80]. Objective scoring of severity of hypoglycemia by questionnaire (Clark Score) [81], re-

Table 1. Indications for islet transplantation

Kind of transplantation	Indications
Islet transplantation alone (ITA)	Type 1 diabetes, duration > 5 years Age > 18 years, weight < 90 kg, insulin requirement < 1.0 U/kg/day Absence of malignancy or untreated infection Ability to comply with immunosuppression and close follow-up Refractory hypoglycemia or lability despite: 1. Optimal intensive insulin or insulin pump with appropriate monitoring 2. Supervision by a diabetologist or endocrinologist 3. Increased hypoglycemic risk, evidenced by at least one of the following criteria: i) Clarke score ≥ 4 ii) HYPO score ≥ 1000 iii) Lability index (LI) ≥ 400 iv) Combined HYPO ≥ 400 and LI ≥ 300
Islet after kidney (IAK) transplantation	Type 1 diabetes, successful prior renal allograft Tolerating maintenance immunosuppression Prednisone ≤ 5 mg/day Absence of BK virus, or other active opportunistic infection Non-sensitized (PRA < 20%)

Legend: The Clarke method [81] comprises eight questions characterizing the participant's exposure to episodes of moderate and severe hypoglycemia (0 = no hypoglycemia, ≥4 = hypoglycemia unawareness). The HYPO score is a composite measure of the severity of the problem based on 4 weeks of records and a year historical review of the number of episodes of severe hypoglycaemia [36]. Once measures in the general diabetes population, the HYPO score median was 143, 25th to 75th interquartile range 46-423, and the 90th centile 1047. The lability index (LI) is calculated based on changes in glucose levels over time, using 4 weeks of glucose records, and compared with a clinical assessment of glycemic lability [36]. BK – Brennan-Krohn, PRA – panel reactive antibody.

view of glycemic records and symptoms (Ryan HYPO score), and assessment of glycemic excursions (Lability Index) have been helpful in screening for potential candidates [36].

By contrast, selection of islet after kidney (IAK) patients is more straightforward, as the decision to initiate immunosuppression and accept that risk has already been pre-empted by the prior kidney transplant. If prednisone is part of the regimen, the dose should be minimized to ≤5 mg per day. Screening for Brennan-Krohn (BK) virus, named after the first kidney patient to have polyoma virus isolated from urine in 1971, is especially important where depletional T cell induction is given for the subsequent islet transplantation, as this can markedly elevate the risk of BK nephropathy [82, 83].

3.2 Intraportal access

The portal vein may be accessed by a non-invasive percutaneous transhepatic route or by an open surgical approach. The percutaneous route is

preferred, and can be accomplished safely in centers with experienced interventional radiological expertise. At the University of Alberta, 98% of the 382 procedures have been done by the percutaneous approach, with fewer than 2% requiring open surgical access. The ability to carry out islet transplantation without major surgery is the advantage of islets over whole pancreas transplantation, and makes it one of the safest and most attractive procedures in transplantation medicine.

The percutaneous approach was first described by Weimar *et al.* who combined CT and fluoroscopy [84]. The Edmonton group used combined ultrasound and fluoroscopy for their initial seven-patient study [18]. The use of preliminary color duplex ultrasonography reduces the number of capsular punctures and shortens procedural time [85, 86]. Patients should have a normal liver parenchyma without cirrhosis or portal hypertension, and without large right-sided hemangioma. Anti-platelet agents, direct thrombin, or Xa inhibitors should be discontinued within 7-14 days of the procedure if safe to do so, or delisted if not safe.

Patients with known thrombophilia (protein C, S, anti-thrombin III, factor V Leiden deficiency) should not undergo percutaneous intraportal islet transplantation [87]. After brief skin infiltration with local anesthesia, and with ultrasonic guidance, a 22-gauge Chiba needle is advanced intraparenchymally until a peripheral branch of the right portal tree is identified [88-93]. An 18-gauge guidewire is then threaded in to the main portal vein and exchanged for a **4-5 French angio catheter** (e.g. NEFF, Cook Canada, Stouffville, Ontario, Canada), with the tip positioned just above the portomesenteric confluence. A portal venogram confirms the position, and a baseline portal pressure measurement is obtained.

The final islet product, suspended in 250 cc of transplant media in an infusion bag, is loaded with heparin (70 units/kg recipient weight) and infused under gravity [94]. If baseline portal pressure >20 mmHg, or if portal pressure rises >22 mmHg during infusion, no further islets are given until the pressure normalizes, to avoid precipitating portal thrombosis. After completion of the transplant and rinse solution, the catheter tract is ablated with Avitene paste (Medchem Products, Woburn, Massachusetts, USA) or D-STAT (Vascular Solutions, Minneapolis, MN, USA) [95, 96]. If 1 g Avitene powder is mixed with 3 cc saline and 3 cc contrast media, deployment can be followed by fluoroscopy, and the goal is to generate a track of ≥ 4 cm in length. This approach almost completely eliminates the risk of bleeding following percutaneous access [95, 97]. A therapeutic heparin infusion is initiated in the radiology suite at 3 units/kg/hr, then titrated to maintain a partial thromboplastin time of 60-80 seconds, and continued for 48 hours. Low molecular weight heparin is continued for 7 days (enoxaparin 30 mg s.c. twice daily) together with enteric-coated aspirin 81 mg for 14 days. This approach, combined with low-volume islet products and frequent portal pressure monitoring during infusion reduces the risk of portal thrombosis, and may facilitate single-donor islet engraftment by reducing activation of the instant blood-mediated inflammatory reaction (IBMIR) [98, 99]. Partial-branch venous occlusion of a peripheral, anterior or posterior segmental branch may occasionally occur, and carries extremely low risk of further propagation if managed with heparin/coumadin and followed with Doppler ultrasound. Complete occlusion of the entire portal tree would be the most concerning complication, but fortunately is exceedingly rare (**0%, 0/382 transplants in Edmonton since 2000**) [100].

4. Risks with islet transplantation

Islet transplantation is considered the safest of all organ transplants when conducted in experienced centers, as invasive surgery is not required, and patients are not in a debilitated state requiring life-support at the time of transplantation. Thus, recovery is swift, and risk of hospital-acquired infections is extremely low. Immunosuppressive care, monitoring, and prophylaxis remain almost identical to other solid organ transplants. Nonetheless there are clear procedural risks of intraperitoneal bleeding, portal thrombosis, and injury to the gallbladder; the latter is avoidable if ultrasound guidance is used routinely. Inadvertent intraparenchymal cholangiography is anticipated with the 22 gauge seeker Chiba needle approach, and does not lead to bile leak [101]. As outlined above, the risks of portal thrombosis or bleeding are almost completely avoidable if the catheter tract is ablated effectively and therapeutic heparin is administered [102]. The commonest complication is mild pain or discomfort either at the catheter insertion site, or as referred pain to the right shoulder tip, is transient, occurs half of patients, is easily controlled by standard analgesic medications, and generally resolves fully in 24-48 hours. While we have encountered clinically insignificant peripheral portal branch vein occlusions unilaterally in 3.7% of the 382 islet transplants in T1D at the University of Alberta, there has been a 0% risk of complete portal occlusion. None of our patients have ever manifested signs of portal hypertension with up to 14 years of follow-up [102, 103].

Transient mild elevation in liver function has been described previously in half of the subjects, but normalizes completely by one month [104]. A 5-fold transaminase rise was observed in 27%, but also resolved within one month. Interestingly, with improved collagenase enzymes, routine adoption of islet culture protocols, and use of anti-inflammatory agents at induction, the incidence and severity of observed transaminitis has diminished markedly in our more recent experience.

Hepatic steatosis has been observed in up to 20% of the cases on ultrasound and magnetic resonance liver imaging, and confirmed by biopsy on rare occasion [105-109]. The fat is macrovesicular but focal, and reflective of high local insulin release from functioning islets. These changes are reversible, have yet to be associated with sequelae of non-alcoholic steatohepatitis, and are perceived as inconsequential.

Potential risk of hepatocellular adenoma and carcinoma has been raised by Dombrowski *et al.* in rats receiving low islet mass after treatment with streptozotocin, and similar changes were observed in spontaneously diabetic BB/Pfd rats [110, 111]. Fortunately, this observation appears to be unique to the rat. Of over 1,500 auto- and allo-islet transplantations carried out in humans with up to 35 years of follow-up, there has yet to be a case report of this complication in patients [21, 112-114].

The risk of transmissible infection from contaminated donor islets has been exceedingly low to date. While the risk may be low, it is well described in solid organ transplantation, with transmission of hepatitis C, human immunodeficiency virus, fatal lymphocytic choriomeningitis, and rabies [115-118]. A fatal case of untreated West Nile Virus encephalitis has been described in one islet recipient who received two islet transplants three years previously, but this was not of donor origin [119]. Careful screening of donors with avoidance of high-risk donors for non-life-threatening transplants is required in all cases. The period of islet culture can provide additional time to complete nuclear acid testing for HIV, hepatitis B and C, with a plan to wait for negative results before proceeding with transplantation [120].

The risk of death following islet transplantation has been exceedingly rare. Our actuarial survival rate at the University of Alberta is 97% with 14 years of follow-up (6/183 subjects). Specifically, we have encountered no deaths as a direct or indirect consequence of immunosuppression, to the best of our knowledge. Of 6 deaths, 4 were cardiovascular from diabetic microangiopathy, coronary occlusion, or arrhythmia, 1 from fatal hypoglycemia after a failed islet transplant and return to insulin, and 1 inadvertent overdose of methadone [121]. A mortality rate of 1.3% (18 cases including the 6 from Edmonton) has been reported in the latest CITR report. Of these, 3 were possibly linked and only 1 definitely related to islet transplantation or immunosuppression [19]. Conversely, over a similar time period, several cases of death from severe hypoglycemia have been noted in patients on waiting lists for islet transplantation who never received therapy. This suggests risk equipoise between islet transplantation and poorly controlled T1D, and would further support the need for a randomized controlled trial to quantify the risk-benefit balance more precisely.

In terms of malignancy, the CITR registry highlights 13 patients with treatable skin basal or squamous cell carcinoma, with an overall rate of

2.3% [19]. These are likely resultant from chronic immunosuppression. Interestingly, the drug sirolimus may be protective in this regard [122-131].

The risk of HLA sensitization has been raised previously by our group, but only in a small subset of patients with failed islet transplants who became C-peptide-negative and were withdrawn from immunosuppression, with an overall risk of 16% [132]. Our more recent experience with potent T cell-depletional induction with alemtuzumab has been associated with exceedingly low rates of PRA sensitization. The Geneva group also reported low rates of HLA sensitization (10.8% risk) in islet after kidney recipients [133].

5. Immunosuppression for islet transplantation

Immunosuppression for clinical islet transplantation must consistently suppress both auto- and alloreactivity, and for this to be achieved, threshold therapeutic drug levels and dosing must be sustained at all times. In contrast to all other solid organ transplants, this small-volume endocrine graft is widely dispersed throughout the liver, and the only surrogate marker of dysfunction, hyperglycemia, is usually an end-stage response to irreversible graft injury. Only on rare occasion, islet grafts have been rescued from acute cellular or humoral rejection by interventional treatment with corticosteroids or rituximab and intravenous immunoglobulin [134, 135].

Recurrent autoimmunity in T1D was incorrectly assumed to be readily controlled by standard immunosuppression, perhaps driven by preliminary encouraging data from the early cyclosporine intervention trials in new-onset T1D [136]. Another evidence for this assumption was provided by the observation that recurrent autoimmunity led to rapid islet destruction in an identical twin recipient of a segmental pancreas transplant in the absence of immunosuppression [137], while pancreas allografts functioned well in thousands of other recipients with T1D receiving immunosuppression [138]. Burke *et al.* have clearly documented several cases of recurrent autoimmunity arising in whole pancreas transplantations, despite maintenance of therapeutic levels of immunosuppression [139]. Rossini *et al.* demonstrated evidence of recurrent autoimmunity after intraportal islet transplantation under the Edmonton Protocol, with liver biopsies showing surviving islets, but with specific destruction of beta-cells [140].

Finding immunosuppressive cocktails that more effectively suppress autoimmunity in humans is especially challenging as the only experimental model, the non-obese diabetic (NOD) mouse, has a flawed immune system, and consistently fails to reflect clinically relevant response [141, 142]. Fortunately, advances by Roep and the Leiden group in peripheral blood monitoring of T cell autoreactivity against insulinoma-associated protein 2 (IA2A) and glutamic acid decarboxylase (GADA), with strong correlation with islet outcome, suggest that such tools may help guide future specific therapeutic suppression of autoimmunity [23, 143].

The added challenge is that most of the immunosuppressive agents in common use in transplantation are directly toxic to beta-cells, and this is compounded when islets are transplanted intraportally and exposed to high local drug levels [144-146]. Using lineage tracing techniques, Nir *et al.* demonstrated that both tacrolimus and sirolimus, drugs used in the Edmonton Protocol, are potent inhibitors of beta-cell regeneration in mice [147].

5.1 Induction of immunosuppression

T cell-depleotional induction strategies are being used increasingly in islet transplantation as long-term results appear more durable with this approach [21, 148, 149]. Previous use of the anti-IL2R monoclonal antibodies (mAb) daclizumab and basiliximab were well tolerated without side effects in the Edmonton Protocol, but probably add little additional allograft protection, and minimal if any protection against T1D autoimmunity. Large-scale clinical trials of daclizumab and mycophenolate mofetil (MMF) in new-onset T1D failed to preserve beta-cell function or prolong the honeymoon period [150]. Hering *et al.* promoted the use of T cell-depleotional or modulatory agents for their single-donor islet transplant series, and a large number of preclinical studies support their use as a means to facilitate autoimmune regulation and tolerance [35, 151-158]. Bellin *et al.* analyzing the Minnesota and CITR islet data, found that T cell-depleotional induction, especially in combination with tumor necrosis factor alpha (**TNFalpha**) blockade, provided the most durable 50% insulin independence rates at five years post-transplant [148].

Thymoglobulin (rabbit ATG) is given as a cumulative dose of 6 mg/kg by peripheral intravenous infusion over 2-3 days, and at least 2 mg/kg is infused prior to islet infusion [35]. In Edmonton,

we currently favor T cell-depleotional induction with alemtuzumab, 30 mg by peripheral i.v. over 3 hours, based on superior potency, tolerability, sustained effect, and cost. To minimize dose-related side effects, acetaminophen 650 mg p.o., diphenhydramine 50 mg i.v. and solumedrol 250 mg i.v. are given 30 minutes prior to alemtuzumab. Alemtuzumab was first tested in renal transplantation by Calne *et al.* in a *prope-tolerance* approach together with low-dose cyclosporine [159-161]. Alemtuzumab generally requires maintenance therapy with calcineurin inhibitors. Maintenance monotherapy sirolimus is associated with high rates of rejection in kidney, and in our previous experience in islet transplantation [162-165]. Therefore, we recommend tacrolimus (target trough 10 ng/ml for 3 months, then 8-10 ng/ml) together with MMF up to 2 g per day in divided dose as tolerated thereafter, as this has provided superior insulin independence rates at 5 years.

We have observed increased rates of late CMV transmission and reactivation with the use of either thymoglobulin or alemtuzumab T-depleotional induction, despite the use of early CMV prophylaxis, but these have been largely sub-clinical and without sequelae [166-168]. Valganciclovir is recommended for all subjects receiving T-depleotional therapies, irrespective of donor and recipient mismatch status, given at 450 mg daily for 14 days, then increased to 900 mg daily for 12 weeks post-transplant [166]. Sulphamethoxazole 400 mg and trimethoprim 80 mg is given once daily for 6 months for *Pneumocystis jiroveci* prophylaxis. If allergic to sulphonamide, it may be substituted with monthly pentamidine inhalations (300 mg).

5.2 Anti-inflammatory and beta-cell-preserving strategies

Based on Hering *et al.*, most centers have adopted the use of anti-TNFalpha blockade in the peritransplant period [35, 148, 153]. Farney *et al.* initially described this approach in mice [169]. Etanercept is given as 50 mg i.v. pre-transplant, then 25 mg s.c. on days 3, 7, and 10 post-transplant [35]. We recently found strong potentiation of marginal mass human islet engraftment in immunodeficient mice when etanercept is combined with an anti-IL1 beta receptor antibody, anakinra [170]. Matsumoto *et al.* suggested that this combination might be beneficial in a small series of islet recipients [171]. At present, this combination is being explored in an expanded series in Edmonton.

Korsgren's group in Sweden have explored a series of strategies designed to decrease an innate immune injury to islets (IBMIR) triggered through islet expression of tissue factor, leading to platelet aggregation, inflammation, and injury [172, 173]. Circulating thrombin-antithrombin complex and C-peptide release from islet lysis correlating with the IBMIR response, and acute loss of labeled human islets detected by positron emission tomography, confirm a major role for this pathway in limiting islet engraftment and survival [174, 175]. Peri-transplant insulin and heparin may partially modify the response [99], but more potent strategies are being tested, including surface binding of heparin to islets [176], low molecular weight dextran, and direct thrombin inhibitors. Balancing risk of bleeding against improved islet engraftment and survival will be critical as these strategies move forward.

Our basic laboratory has been interested in the control of islet apoptosis as a means to augment initial islet survival. Using a spectrum of different pan-caspase inhibitor compounds, we consistently demonstrated in preclinical models that in the presence of these agents, only 10-30% of the usual marginal islet engraftment mass was required to reverse diabetes with mouse, pig, and human islets, when given for just two weeks during the early islet engraftment period [177-182]. One agent (IDN-6556, Conatus Pharmaceuticals Inc., San Diego, USA) appeared to be most promising in a large animal pig islet autograft model [183], and has now moved to early pilot clinical trials in Edmonton.

Glucagon-like peptide 1 (GLP-1) analogues, especially exenatide, have been explored extensively by the Miami, Vancouver, and Illinois groups, as a means to facilitate both single-donor and supplemental islet engraftment [184-190]. These agents need to be continued indefinitely to maintain improved metabolic control in islet transplantation. Up to one third of patients cannot tolerate exenatide due to severe nausea. We have tested an alternative, long-acting once-daily GLP-1 analogue, liraglutide, as this agent has had much lower rates of nausea and intolerance in trials in type 2 diabetes. Liraglutide improves marginal mass human islet engraftment in mice, protects against immunosuppressant related beta-cell toxicity [191], and when tested in a large animal pig autotransplant model, liraglutide helped to resist metabolic graft failure over time [192]. When added to human islets in culture (1 µM/l), liraglutide improved islet recovery and engraftment in

mice [193]. Novo Nordisk is currently conducting a 16-center international placebo-controlled RCT to further evaluate these findings in the clinic [194].

5.3 Maintenance immunosuppression

The choice of maintenance immunosuppressive agents has been especially challenging in clinical islet transplantation. The therapy must be sufficiently potent to suppress auto- and alloimmunity, safe, well tolerated and '*islet friendly*' to avoid beta-cell toxicity [21, 144]. The added risk of nephrotoxicity is a particular challenge, as patients with longstanding T1D have underlying nephropathy, and are at increased risk of renal failure [195, 196]. The Edmonton Protocol reduced calcineurin inhibitor (CNI) exposure through the use of high-dose sirolimus (levels 12-15 µg/l for 3 months, then 10-12 µg/l) [18]. While high rates of one-year insulin independence were observed in Edmonton and other leading islet centers [197], it became apparent that high-dose sirolimus was difficult to titrate when extended to an international multicenter trial [198-200]. High rates of mouth ulceration [100, 101], ovarian cysts [201-203], fatigue, diarrhea, occasional severe small bowel ulceration [204], pneumonitis [205], edema [108, 206], and proteinuria [207, 208] have made this approach less attractive. We have largely abandoned the use of sirolimus at the University of Alberta for islet transplantation, finding that higher-dose tacrolimus and MMF are far better tolerated [121]. We have observed a substantial improvement in five-year insulin independence with alemtuzumab induction and tacrolimus/MMF maintenance, suggesting that this strategy provides adequate immunoprotection and function in practice, despite theoretical concerns of islet toxicity.

Strategies that eliminate CNI entirely while providing ongoing immunosuppressive protection have become a major priority in the field of islet transplantation. Posselt *et al.* found that thymoglobulin induction and monthly maintenance costimulation blockade infusion with belatacept provided an effective CNI-free regimen requiring only sirolimus or MMF [38]. Posselt *et al.* and Turgeon *et al.* have both explored an anti-leukocyte functional antigen-1 antibody (anti-LFA1 mAb, efalizumab) in place of costimulation blockade [38, 209]. While encouraging, these observations are limited to a small number of patients, and the anti-LFA1 mAb has been withdrawn due to exceedingly low rates of progressive multifocal leukoencephalopathy in psoriasis patients (3 confirmed cases and an additional case suspected in >

Table 2. Centers with five-year insulin independence rates >50%

Center	Approach	5-year rate	Year	Reference
Minnesota	Anti-CD3 + etanercept	70% (at 7 yr)*	2011	Hering <i>et al.</i> [35, 152]
Minnesota CITR	T cell depletion + anti-TNF	50%	2012	Bellin <i>et al.</i> [148]
Edmonton	Alemtuzumab + Tac + MMF + anakinra + etanercept	60%	2012	Shapiro <i>et al.</i> [21, 121]
UCSF	ATG + efalizumab/belatacept + SRL or MMF	80% (at 4 yr)*	2012	Posselt <i>et al.</i> [38]
UIC	Tac/SRL or MMF + exenatide + etanercept	60%*	2012	Gangemi <i>et al.</i> [190]
Lille, France	Tac/SRL	50%*	2012	Vantyghem <i>et al.</i> [142]
Geneva, GRACIL	ATG + Tac/SRL	50%*	2012	Berney <i>et al.</i> [142]

Legend: * 5-year insulin independence data updated from previous publications in oral presentation or by personal communication, American Transplant Congress Symposium "Advances in Islet Transplantation" June 6, 2012, Boston, USA, and at the International Islet and Pancreas Transplant Association (IPITA) meeting, 2011, in Prague, Czech Republic. Abbreviations: ATG – antithymocyte globulin, CITR – Collaborative Islet Transplant Registry, MMF – mycophenolate mofetil, SRL – sirolimus, Tac – tacrolimus, TNF – tumor necrosis factor alpha, UCSF – University of California San Francisco, UIC – University of Illinois at Chicago.

46,000 treated) [210-212]. An ideal CNi-free immunosuppression is not available for more widespread and long-term testing in clinical islet transplantation at present.

5.4 Long-term outcomes

The five year results with the original Edmonton Protocol demonstrated a steep loss of insulin independence by the third year, but persistent C-peptide and ongoing long-term protection from hypoglycemia in most treated patients [213]. The cause for inexorable loss of complete insulin independence over time is likely multifactorial, but recurrent autoimmunity and allograft rejection are major causes, exacerbated by a marginal initial engraftment mass.

Senior *et al.* updated the status of the Edmonton patients with a decade of follow-up, and of 138 patients in that analysis, 79% had full or partial graft function with correction of HbA1c and protection from hypoglycemia, and current protocols provided 60% insulin independence beyond 4 years [211]. Similar results have been reflected in the CITR database [214]. This remarkable level of glycemic stabilization is rarely achieved by insulin pumps or by continuous glycemic monitoring.

The encouraging news that more potent induction and maintenance immunosuppression, combined with pre-emptive anti-inflammatory strategies, are having positive impact on the current five-year insulin independence rates [148, 149]. Indeed, as summarized in **Table 2**, there are now at least 6 independent centers, reporting 50-70%

five-year rates of insulin independence with different induction and maintenance approaches [35, 151-153, 215, 216]. For islet-alone transplantation, this represents a milestone advance, as the results at five years equate at least to the registry data for pancreas-alone transplantation [151, 217]. However, islet transplantation will not replace pancreas transplantation until the single-donor rates for islet transplantation improve further. Payne, Griffin, and others demonstrated, at least in small and large animals, a single-donor islet preparation could treat up to three recipients [218, 219]. This has yet to be achieved routinely in humans, but could have major bearing on the current limited islet supply and more broader application in T1D. The first demonstration of this concept was a living donor islet transplant carried out with islets prepared from a distal pancreatectomy [220].

Progress has occurred in single-donor islet engraftment, and 10 programs have reported small cohorts of patients with single-donor success (**Table 3**). Understanding the factors leading to increased islet potency will be essential to expanding this routinely [221]. Predictive islet potency assays (e.g. oxygen consumption rate, mitochondrial and beta-cell viability) will be an essential component in the selection of optimal islet preparations.

6. Impact of islet transplant upon secondary complications

It is not questioned that the restoration of near-perfect glycemic control will stabilize and potentially reverse secondary diabetic complications.

Table 3. Single-donor islet protocols

Center	Approach	Year	Reference
Minnesota	Anti-CD3 + etanercept	2005	Hering <i>et al.</i> [35]
Pennsylvania	Edmonton-like	2003	Markmann <i>et al.</i> [254]
Emory	Efalizumab + MMF	2010	Turgeon <i>et al.</i> [209]
San Francisco	ATG + efalizumab + SRL or MMF	2010	Posselt <i>et al.</i> [255]
San Francisco	ATG + belatacept + SRL or MMF	2010	Posselt <i>et al.</i> [38]
Edmonton	Peritransplant insulin + heparin	2010	Koh <i>et al.</i> [99]
Kyoto	Living donor islet transplant	2005	Matsumoto <i>et al.</i> [220]
Baylor	ATG + anakinra + etanercept	2011	Matsumoto <i>et al.</i> [256]
Vancouver	Exenatide	2007	Ghofaili <i>et al.</i> [189]
Miami	Exenatide	2009	Faradji <i>et al.</i> [184]
UIC	Exenatide	2008	Gangemi <i>et al.</i> [190]

Legend: ATG – antithymocyte globulin, MMF – mycophenolate mofetil, SRL – sirolimus, UIC – University of Illinois at Chicago.

Such degree of control is rarely if at all achievable with subcutaneously injected insulin. Intensive insulin is clearly superior to standard management, but is far from perfect, and the more intensive the administration, the higher the risk of severe and recurrent hypoglycemia. These are the hard lessons learned from the DCCT registration trials of intensive insulin therapy [222, 223]. In whole pancreas transplantation, the near-perfect glycemic control achieved has had major benefit in stabilizing coronary artery disease, intimal carotid thickness, diabetic glomerulopathy, neuropathy, retinopathy, and other secondary complications of T1D, and decreases cardiovascular mortality [224-236].

Compelling data is emerging in islet transplantation providing a similar protective impact. The Milan group showed that positive C-peptide can stabilize both macro- and microangiopathic changes in T1D, irrespective of the insulin independence status [237-243]. The Vancouver group conducted a prospective cohort crossover study, comparing the intervention of islet transplantation with optimal medical therapy, and found reduced progression of retinopathy and a trend towards improved nerve conduction velocity [244, 245]. Despite the presence of CNi, there was a reduced decline in renal function [244]. Danielsen *et al.* found that a surrogate marker of atherosclerosis, carotid intimal thickness, was significantly reduced after 12 months of insulin independence, but emphasized a need for multimodal management to achieve this, including optimal management of hy-

perlipidemia and hypertension [246]. Large-scale RCTs without crossover are needed to corroborate these important positive findings.

7. Alternatives to islet transplantation

While islet transplantation remains an attractive therapy, it is acknowledged that transplant tolerance, or stem-cell derived and xenogeneic islets are not on the proximate horizon [247]. Alternative means to stabilize glucose control are being explored, including high-tech closed loop insulin pumps and implantable sensor technologies that may serve as bridge or destination therapy, avoiding a need for transplantation entirely. Such approaches may still be cumbersome for patients, and glycemic monitoring and insulin delivery in the subcutaneous site may still suffer from the challenges of depot injection, delayed absorption, and imperfect dynamic matching for perfect moment-to-moment control which is attainable in the native pancreas or from transplanted islets.

Several strategies for prevention and early intervention in T1D have met with recent disappointment, despite enormous promise generated in mouse models, as discussed in other articles in this current Special Issue. Shoda *et al.* pointed out that over 463 interventional treatments have been shown to prevent or reverse autoimmune diabetes in NOD mice [142]. Attempts to move these to the clinic have met with frustration despite large-scale clinical enrollment and major cost [248]. The NOD

mouse model clearly has major limitations as a surrogate for clinical autoimmune T1D. Better models or empiric interventions that clearly work in patients are now needed. Voltarelli *et al.* developed an approach in Brazil for 'immunological reset' designed to eliminate autoreactive lymphocyte clones using non-myeloablative autologous hematopoietic stem cell transplantation [249-251]. Of 20 new-onset T1D subjects, 12 were rendered insulin independent for 31 months, but there were side effects from opportunistic infection and oligospermia resultant from heavy immune conditioning with thymoglobulin and cyclophosphamide. If these approaches can be further modified to improve the safety profile, while restoring more permanent self-tolerance, they would obviate a need for cellular replacement therapy.

8. Summary and conclusions

Islet transplantation has moved forward from a state of experimental curiosity in mice to a robust therapy for highly selected patients with T1D, refractory to stabilization by alternate means. This remarkable progress has occurred over a span of 40 years, and could not have occurred without extensive dynamic international collaboration. Currently, more than 1,000 patients have received islet transplants since the Edmonton Protocol series was reported in 2000. The long-term results of islet transplantation in selected centers now match the registry data for pancreas-alone transplantation, with 6 sites reporting five-year insulin independence rates $\geq 50\%$.

Islet transplantation has been criticized for the use of multiple donor pancreas organs, but progress has occurred in single-donor success, with 10 sites reporting increased single-donor engraftment. The next wave of innovative clinical trial interventions will address IBMIR, apoptosis, and inflammation, and will translate to further marked improvement in single-donor success. Effective

control of auto- and alloimmunity is the key to long-term islet function, and high-resolution cellular and antibody-based assays will add considerable precision to this process. Advances in immunosuppression, with new antibody-based targeting of costimulatory blockade and other T-B cellular signaling, will have further profound impact on the safety record of immunotherapy.

Islet transplantation is one of the safest of all organ transplantation procedures, and an ability to deliver cells by a non-surgical low-risk approach is an important attraction of this therapy. Clinical trials will move forward shortly to test out new human stem cell-derived islets, and in parallel, trials will move forward testing pig islets for compatibility in patients. Induction of immunological tolerance to self-islet antigens and to allografts is a difficult challenge, but potentially within our grasp. Ultimately, strategies to prevent T1D, and induce self-regeneration of islets within the native pancreas would replace the need for cellular transplantation entirely. More intensive research is needed to address these avenues.

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