

25 YEARS OF THE RICORDI AUTOMATED METHOD FOR ISLET ISOLATION

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ABSTRACT

The year 2013 marks the 25th anniversary of the Automated Method for islet isolation. The dissociation chamber at the core of the Automated Method was developed by Dr. Camillo Ricordi in 1988 to enhance the disassembling of the pancreatic tissue via a combined enzymatic and mechanical digestion while preserving endocrine cell cluster integrity. This method has ever since become the gold standard for human and large animal pancreas processing, contributing to the success and increasing number of clinical trials of islet transplantation worldwide. Herein we offer an attempt to a comprehensive, yet unavoidably incomplete, historical review of the progress in the field of islet cell transplantation to restore beta-cell function in patients with diabetes.

“...This cell has a small and polygonal structure. Its cytoplasm is perfectly brilliant and free from granules, with a distinct nucleus that is round and of discrete dimension. This type of cell clusters, generally in large number, is diffusely scattered in the glandular parenchyma. These clusters have generally a diameter of 0.1-0.24 mm and may be easily distinguished in fragmented pancreatic preparations...”¹. With these words, the German anatomist and anthropologist **Paul Langerhans** described for the first time the pancreatic islets in his dissertation published in 1869 summarizing the results of his research as medical student in Berlin in the laboratory of **Rudolf von Virchow**'s¹. In 1893, the French pathologist and histologist **Gustave-Édouard Laguesse** associated the Langerhans' name to that of islets (“*îlots de Langerhans*”) having observed similar structures in the human

pancreas². In those years, insulin had not yet been discovered (until 1922, by **Fredrik Bating** and **Charles Best** from London, Ontario) and part of the scientific discussion was polarized on whether the pancreas produced or not “a substance able to destroy glucose”. Thus, the research was primarily focused on attempts to demonstrate that pancreatic fragments transplanted into diabetic animals could cure the disease. The first success was reported in the medical literature in 1882 by doctors **Oscar Minkowski** and **Joseph von Mering** at the University of Strasburg demonstrating transient improvement of glycosuria following subcutaneous transplantation of autologous pancreatic fragments in a pancreatectomy-induced diabetic dog³. The following year (1883), doctor **P. Watson Williams** and surgeon **William H. Harsant** at Bristol Royal Infirmary in the UK attempted the first transplant of three fresh sheep pancreatic fragments in the subcutaneous space of a 15-year old boy with ketoacidosis, who eventually died and histopathological assessment demonstrated “fibrous stroma” in the grafts⁴.

In the subsequent years, most of the research aimed at demonstrating the hypothesis of the presence in the pancreas of beneficial secretion through transplantation of fragments in sited alternative to the subcutaneous space. In 1896, Italian surgeon **Roberto Alessandri** at the Royal University in Rome reported for the first time the transplant of autologous pancreatic fragments in the dog spleen after pancreatectomy, though without achieving measurable graft function⁵; similar results were reported by others⁶⁻⁸. **Alessandri** also tried for the first time the intrahepatic site with poor success^{5, 8}. Despite these failures, the attempts increased and it is noteworthy the series of small pancreatic fragment allografts performed into liver, spleen, peritoneal cavity and subcutaneous space of experimental animals by doctor **Donato Ottolenghi** in 1901 in

Turin, Italy⁷; despite the extremely small size of the fragments utilized, all grafts underwent necrosis and resorption within a couple of days, even if in a few cases preserved insular tissue was observed at histopathological evaluation. In 1903, doctor **James Allan** at Glasgow Royal Infirmary attempted another xenotransplant using feline pancreatic fragments in a patient with diabetes who died two weeks later with ketoacidosis⁹. The British surgeon **Fredrick Charles Pybus**, in light of initial success obtained with adrenal grafts in the treatment of Addison disease, attempted in 1916 the transplant of allogeneic pancreatic tissue into patients with diabetes¹⁰; considering that previous attempts with xenogeneic tissue had failed, he recovered a human pancreas immediately after the death of a trauma victim and transplanted slices into the abdominal subcutaneous space of two males with diabetes (32 and 37 years old, respectively). Despite transient reduction of glycosuria in one patient, none of the grafts yielded reversal of diabetes¹⁰. The disappointing outcome and the subsequent discovery and implementation of insulin therapy for diabetes tempered the interest and limited the development of further research on the transplantation of pancreatic tissue in the following years.

An important intuition that impacted significantly the progress of experimental research was the hypothesis that function and viability of the endocrine pancreatic graft was impaired by the presence of exocrine acinar tissue. In order to overcome the potential detrimental effect of acinar tissue in pancreatic grafts, two hypothetical solutions were developed. One of the approaches considered transplanting a tissue “enriched” of islets such as fetal or neonatal pancreas, since the development of the exocrine and endocrine pancreas is not synchronous with endocrine cells appearing early during organogenesis¹¹ and being able to synthesize and secrete insulin and glucagon in the period preceding exocrine differentiation. Studies in experimental animals demonstrated that the survival of the graft was influenced by donor age¹¹⁻¹³ and that survival over 56 days could be achieved with fetal and neonatal pancreatic grafts implanted in the cheek of hamsters¹³. The other methodological approach pursued was to separate the endocrine tissue from the exocrine component before the transplant.

The idea to physically separate the endocrine component from the exocrine pancreas was originally proposed by Russian doctor **Leonid W. Ssobolew** from Saint Petersburg in 1902¹⁴, but was not pursued at least for almost 60 years before the isolation of the islets of Langerhans was reported in the medical literature¹⁵. In fact, the development of pancreatic islet isolation techniques was character-

ized by a first era relying on microdissection under the microscope as described in 1964 by doctor **Claes Hellerstöm** at Uppsala University in Sweden¹⁵, though with poor results both in terms of yields and quality (namely, substantial functional impairment). Considering the paucity of endocrine pancreatic tissue, the research focused on transplantation in experimental animal models did not progress much in this period. A renewed impulse to the field followed the discovery of the action of collagenases on pancreatic fragments and the introduction of the enzymatic processing. Polish doctor **Stanisław Moskalewski** described in 1965 a novel method isolate the islets from the minced Guinea pig pancreas using collagenase action that resulted in loss of acinar tissue and freeing of pancreatic islet clusters¹⁶. His method was further improved by U.S. doctors **Paul E. Lacy** and **Mery Kastianovsky** at Washington University in Saint Louis, MO, with the introduction of intra-ductal injection of cold saline buffer to obtain the distension of the pancreas prior to mincing and enzymatic digestion followed by had-picking under the dissecting microscope¹⁷. Clearly, this approach did not allow obtaining adequate islet yields for transplant experiments into animals, with the purification step being the limiting factor of the process. A step forward toward overcoming this hurdle was the introduction of density gradient purification that was initially based on sugar or albumin. Subsequently, the use of discontinuous gradient purification with Ficoll by **Arnold Lindall and Coll.** at University of Minnesota contributed to achieving higher yields after islet isolation even though initially the cell product obtained with this technique was not functional¹⁸. Only when Ficoll was dialyzed and lyophilized in doctor **Lacy's** laboratory vital islets could be obtained for experimental transplant studies. Indeed, Lacy established the two phases of islet cell processing: (i) islet cluster dissociation and dispersion followed by (ii) islet purification from the pancreas. The technique became the standard for rodent islet isolation for the following decade that led to remarkable volume of studies addressing pancreatic islet metabolism, physiology and immunobiology. In 1972, U.S. doctors **Walter F. Ballinger II** and **Lacy** reported the first successful reversal of experimental diabetes in rats following intraperitoneal implantation of 400-600 islets and also that retrieval of transplanted tissue resulted in the reoccurrence of diabetes, as well as the presence of both alpha and beta-cells in explanted tissue at histopathological analysis¹⁹. A further step forward in experimental islet transplant was the 1973 study by doctor **Charles B. Kemp** and

Coll. at Washington University describing that the technique of islet embolization into the liver of recipient rats through the portal vein improved the efficiency of transplanted islets compared to the intraperitoneal site with recovery of glucose homeostasis within 2-3 days from implant²⁰. This study set the basis for the choice of the intraportal islet infusion technique in the clinical setting that still today remains the transplant site of choice.

The islet isolation technique developed in the rat by doctor **Lacy** resulted in a significant increase of experimental studies in rodents. However, for several years the attempts to extend the Lacy isolation protocol to large animal pancreas (*i.e.*, dog, nonhuman primate and human) yielded poor results with no reports of purified islet cell preparations until 1977²¹. In the mid 1970's the approach of avoiding the purification process for large animal pancreas because of the big islet loss gained favor. Several reports in the literature describe attempts to use pancreatic fragments containing unpurified islets to cure experimental diabetes in large animals^{22,23}.

An important innovative approach to enhance the isolation protocol for large animal pancreas was described by doctors **Atsushi Horaguchi** and **Ronald C. Merrell** at Stanford University using a dog model²⁴. Their protocol consisted of three phases: (i) the cannulation of pancreatic duct with intra-ductal injection of collagenase solution to better digest the fibrotic structures; (ii) mechanical dissociation with digestion at 37°C; and (iii) filtration of the pancreatic digest through a 400 µm filter mesh²⁴. With this approach, islet recovery was estimated of 57% with a purification of approximately 10%; thus, it became possible obtaining adequate islets for transplantation from a single donor. In the same period, doctors **Garth L. Warnock**, **Ray V. Rajotte** and **A.W. Procyshyn** at the University of Alberta demonstrated that improvements in the technique of pancreatic micro-fragment transplantation into the splenic sinusoids could result to the achievement of sustained normoglycemia in diabetic dogs^{25,26}. This allowed for the development in experimental models of immunosuppression as well as of cryopreservation protocols by doctor **Rajotte and Coll.** for the storage pancreatic fragments at -196°C²⁷.

At the end of 1970's, the technique by Horaguchi and Merrell applied to the human pancreas led to initial attempts of unpurified pancreatic micro-fragment transplantation in the clinical arena by doctors **John S. Najarian**, **David E.R. Sutherland**, **Arthur J. Matas**, **Fred C. Goetz** and *Coll.* at the University of Minnesota^{28,29}, though resulting in poor metabolic control³⁰ and did not solve the numerous clinical is-

sues associated with inadequate immunosuppression, suboptimal endocrine mass transplanted *vis-à-vis* the complications associated with the lack of purification of the grafted tissue (namely, portal hypertension and disseminated intravascular coagulation).

The first report of successful transplantation of allogeneic pancreatic fragments into patients with Type 1 diabetes was reported in 1979 by doctors **Felix Largiadèr**, **E. Kolb** and **Ulrich Binswanger** at Zurich University^{30,31}; one of the patients, a 22 years old with T1D and severe retinopathy and nephropathy underwent simultaneous allogeneic kidney along intrasplenic pancreatic micro-fragments (obtained from two donors) transplantation under anti-lymphocyte serum, azathioprine, cyclophosphamide and prednisone treatment and showed sustained normoglycemia 1 year post-transplant. Over the months post-transplant, besides improvement of renal graft function, a progressive reduction of exogenous insulin requirements was observed, with achievement of insulin independence by 8 months that lasted for ten months, when rejection of the kidney was associated with hyperglycemia recurrence. The patients died a month later and intrasplenic pancreatic islets could be detected in the necrotic specimens^{30,31}. More substantial, both in terms of numbers and measurable success, was the clinical experience with autologous intrahepatic islet transplantation as a palliative treatment of pain in patients with chronic pancreatitis undergoing total pancreatectomy performed by doctors **Najarian**, **Sutherland**, **Matas** and **Goetz** at the University of Minnesota^{28,29,32,33}.

In the 1980's, new progress were reported with the islet isolation techniques from dog and human pancreata by doctor **Daniel H. Mintz's** group at the University of Miami and doctor **Derek W. Gray and Coll.** at Oxford University³⁴⁻³⁷. Briefly, the protocol consisted of intraductal injection collagenase injection, dispersion of the pancreatic tissue by mechanical agitation and passing through a series of graded needles followed by purification using filtration and centrifugation on density gradient solution – a method that would allow yields of approximately purity of 20-40% from human glands^{35,36}. The technique showed some promise in canine islet transplantation models, particularly for the autografts while it required more than one donor in the allografts³⁷⁻³⁹. Using modifications of this method, doctors **Rodolfo Alejandro**, **Daniel H. Mintz** and *Coll.* at the University of Miami initiated in 1985 a pilot clinical trial of 5 allogeneic islet transplantation in four C-peptide negative patients with Type 1 diabetes with evidence of retinopathy, nephropathy and neuropathy as islet after kidney (IAK) and simultaneous islet-kidney (SIK) transplantation (negative

serum crossmatch and ABO compatible donor:recipient combination)^{36,40}. Novelty introduced in the field through this clinical trial include: preservation of the pancreas by hypothermic pulsatile perfusion with cryoprecipitated silica-treated human plasma, the use of transhepatic portal vein cannulation for three procedures with monitoring of portal vein pressure before and after islet infusion, as well as modulation of islet immunogenicity by treatment with anti-Ia monoclonal antibody *in vitro*. The longest islet graft function was measured for 26 and 18 weeks in two transplants, and graft failure invariable occurred in all cases possibly consequent to inadequate levels of immunosuppression³⁶.

During the same years modifications in the canine islet isolation procedures allowed to obtain adequate numbers in volumes of pancreatic tissue to be safely infused in the portal system without inducing portal hypertension while treating diabetes were introduced by doctors **Mark S. Cattral**, **Warnock**, **Norman M. Kneteman**, and **Rajotte** at the University of Alberta by combining intraductal perfusion with collagenase of the pancreas, gentle dissociation and purification of density gradients^{41,42}.

A turning point for clinical islet transplantation was the introduction of the "Automated Method" of

pancreas dissociation by doctor **Camillo Ricordi**, who, after obtaining his medical degree from the University of Milan, received an NIH Research Trainee Award in 1986 to join doctor Lacy's team at Washington University. The method consisted of a mechanically enhanced enzymatic digestion based on a dissociation/filtration chamber allowing pancreatic fragments and islets freed from gland to be removed promptly from the system to avoid overdigestion while preserving cluster integrity (Figures 1 and 2). The method was first published in 1988⁴³ and has represented ever since the gold standard for virtually all research centers working on human⁴⁴ and large animal islets⁴⁵, besides its application also for the isolation of other tissues⁴⁶. Shortly after the introduction of the automated method, the initial success with islet transplantation in humans were reported by doctors **David W. Scharp**, **Lacy**, **Ricordi** and *Coll.* at Washington University with a first series of patients with T1D and established or incipient nephropathy to ascertain if insulin independence could be attained and if immunosuppression could be discontinued one year after transplantation without rejection. Three subjects received approximately

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Automated Method for Isolation of Human Pancreatic Islets

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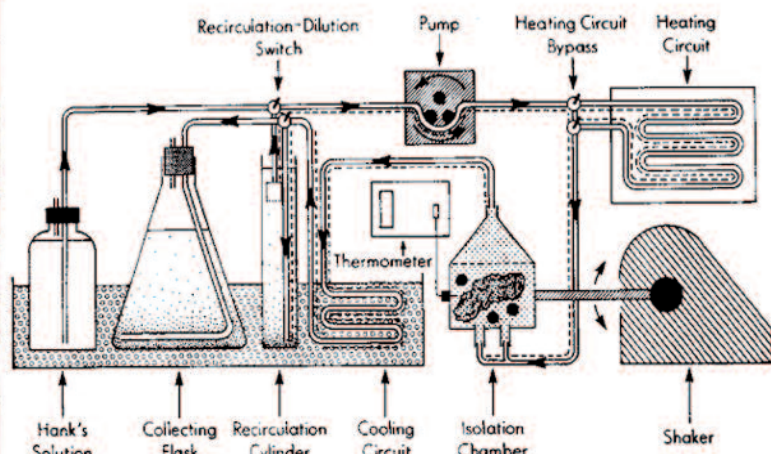


Figure 1. The headings of the manuscript describing the Automated Method and a picture of Dr. Ricordi with one of the first human pancreas shipments from NDRI that was processed in Saint Luis.

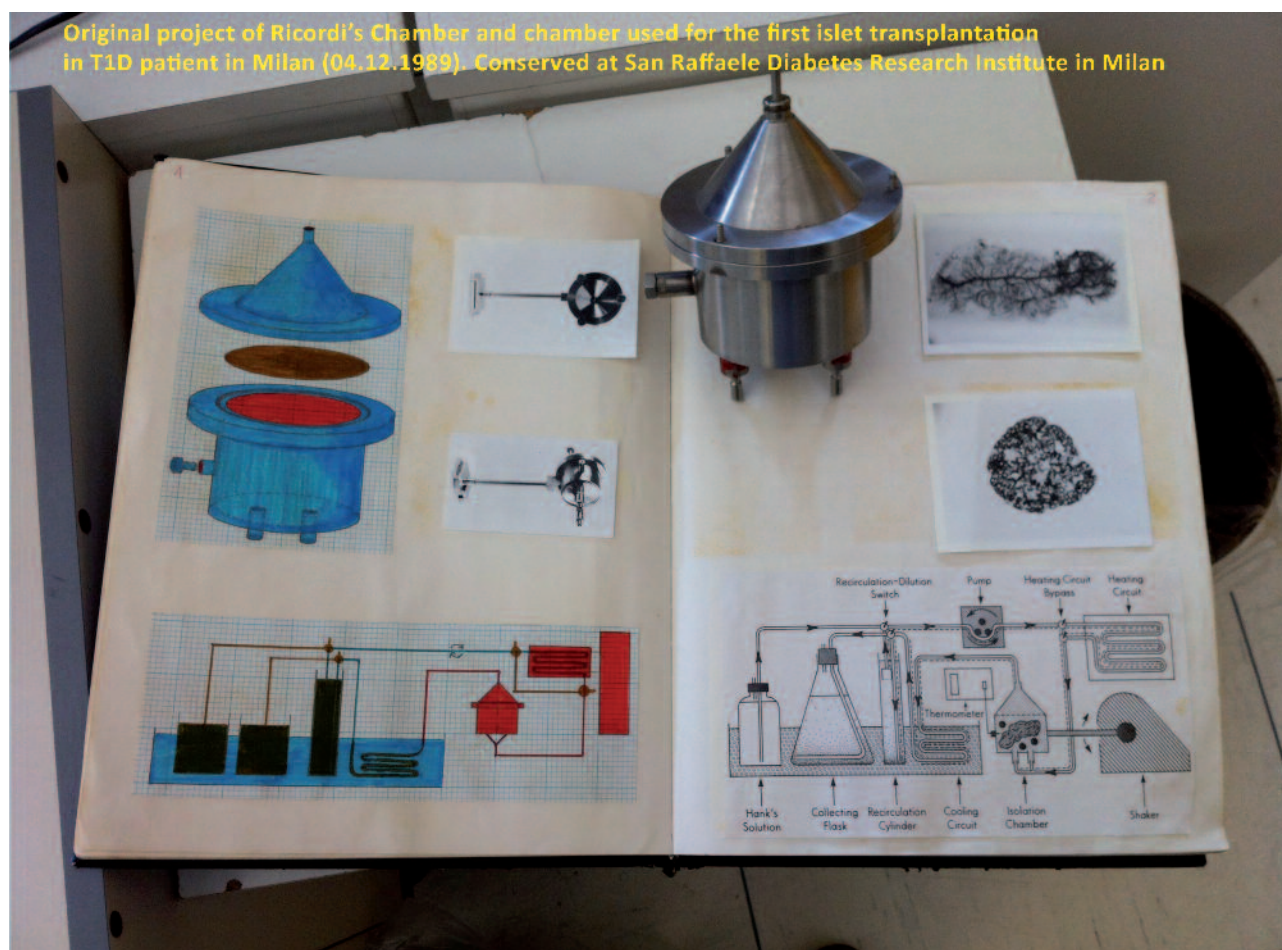


Figure 2. One of the initial prototypes of the stainless steel Dissociation Chamber and Dr. Ricordi's Laboratory Notebook describing the concept and drafts of the Automated Method for islet isolation. Photo by L. Piemonti (Milan, 3rd March 2013).

6,000 islet equivalents per kg of body weight but all lost graft function despite ongoing immunosuppressive regimen (azathioprine, prednisone and cyclosporine)⁴⁷; additional islet transplants were performed with some degree of success thereafter⁴⁷⁻⁴⁹. Using a more elaborated islet isolation technique⁵⁰, doctors **Warnock, Rajotte and Coll.** at the University of Alberta reported a good production of C-peptide in two recipients with T1D following SIK (from the same donor). They received conventional triple immunosuppression that had to be reduced in both cases due to CMV infection 6 week post-transplant with subsequent loss of islet graft function.

Further improvements were introduced in the late 1980's. Amongst these were techniques aimed at optimizing the efficiency of the purification of large animal and human islets using a semiautomated method of density gradient separation using computerized cell separator by doctors **Stephen P. Lake and Coll.** from the Leicester Royal Infirmary

in UK⁵¹ and doctors **Alejandro** and **Mintz** from the University of Miami⁵²; improved gradients that incorporated cold preservation solutions by student **Barbara J. Olack** and Coll. at Washington University⁵³; techniques to stain islet cell clusters with zinc dye by doctor **R. Alejandro and Coll.**⁵⁴ and by doctor **W. A. Hansen** and Coll. from Hagedorn Research Laboratory in Gentofte, Denmark; as well as the publication of consensus papers aimed at standardizing human islet assessment⁵⁵ contributed to progress in the field.

In the 1990, the introduction of novel techniques to improve the efficiency of isolation techniques resulting in high yields of pancreatic islets contributed to the development of numerous clinical protocols around the World. In the early 1990's, doctor **Ricordi** moved to the University of Pittsburgh to direct the Cellular Transplantation Division of the Transplantation Institute headed by doctor **Thomas Starzl**. Also doctors **Alejandro** and **Mintz** from the

University of Miami joined forces with doctors Ricordi and Starzl's Team to help optimizing protocols and accelerate the progress in the field of islet transplantation. The first series of sustained insulin independence was obtained in nine patients undergoing excision of liver and pancreas (that would result in surgery-induced diabetes) and receiving allogeneic liver and islet transplantation from the same cadaveric donor; the first clinical case of sustained insulin independence following allogeneic islet transplantation was a 15 years old girl whose visceral organs were excised for cancer who received multi-visceral organ (liver, small bowel and islet) transplantation^{56,57}. These trials allowed improving the transplant technique with the introduction of the use of slow infusion (by gravity) of the islet graft kept in suspension into an infusion bag to reduce the risk of portal hypertension^{56,58}.

In 1990, doctors **Scharp, Lacy and Coll.** at Washington University reported the first case of transient exogenous insulin independence obtained following transplantation of 800,000 cultured allogeneic islets with 95% purity (pool of two allogeneic islet preparations) isolated using the automated method into a patient with T1D receiving Minnesota anti-lymphocyte serum, azathioprine and cyclosporine⁴⁸. Ten days after transplantation, the patient achieved normoglycemia (albeit with glucose intolerance) and could discontinue exogenous insulin therapy for 2 weeks⁴⁸. Insulin independence following islet transplantation from a single donor obtained using the automated method was reported by doctor **Carlo Socci and Coll.** at the San Raffaele Institute in Milan, Italy in a patient with T1D transplanted on April 25 1990⁵⁹. Subsequently, insulin independence and/or consistent graft function after islet transplantation was reported by Centers across the World using also cryopreserved⁶⁰ along to fresh allogeneic islets, paving the way for a possible clinical application of cellular therapies to restore beta-cell function in patients with T1D.

In 1994, German doctors **Bernard J. Hering, Reinhard G. Bretzel and Coll.** at Justus-Liebig University in Giessen reported 30% insulin independence after allogeneic islet transplantation in patients treated with cyclosporine and steroids and receiving an anti-oxidant therapy and intense intravenous insulin management in the peritransplant period⁶¹. In 1997, doctors **Antonio Secchi, Socci, Guido Pozza and Coll.** at the San Raffaele Institute in Milan reported a 45% insulin independence rate by administering elevated islet numbers (~11,000 islet equivalents per kg of body weight) under cyclosporine and steroid immunosuppression⁶². Also, **Alejandro, Ricordi, Joshua Miller, Mintz, and Coll.** at the University of Miami reported long-term

(6 years) function in patients with T1D recipients of allogeneic islets and kidneys⁴⁰, two of which maintained detectable C-peptide for 13 years⁶³. The development of better purified and characterized enzyme blends characterized by reduced endotoxin contamination⁶⁴⁻⁶⁷ resulted in higher reproducibility, when compared to other collagenase blends available at the time (i.e., Collagenase P), contributing significantly to achieving improved islet yields from human pancreata⁶⁸⁻⁷⁰. At the same time, initial attempts of fetal porcine islet cell cluster transplantation were performed in uremic patients with diabetes receiving a renal allograft by doctors **Carl G. Groth, Olle Korsgren, Anita Tibell and Coll.** at the Karolinska Institute in Stockholm, Sweden⁷¹. The grafts were implanted intra-hepatically or under the renal capsule under conventional immunosuppression with anti-thymocyte globulin or 15-deoxyspergualin, and showed detectable c-peptide in the urines for up to 200-400 days⁷¹ without evidence of porcine endogenous retrovirus infections⁷². In 1992 doctor **Ricordi** funded the Cell Transplantation Society (CTS) during the first meeting that was held in Pittsburgh, PA. The CTS that is now a section of The International Transplantation Society (TTS), has steadily grown gaining an important role for advancements of the field of islet transplantation and for cellular transplantation by and large.

The Islet Transplantation Registry (<http://www.med.uni-giessen.de/itr/>) collected the experience on a total of 267 transplants performed in several Centers voluntarily reporting the outcome of their trials from 1990 until 2001⁷³. Collectively, the outcome was overall rather modest with only 12.4% of the 267 transplants achieving insulin independence for periods greater than a week and only 8.2% for over one year⁷³. A new era in the field of islet transplantation begun with the introduction of the 'Edmonton Protocol' in 1999 by doctors **A.M. James Shapiro, Jonathan R.T. Lakey, Edmond A. Ryan, Gregory S. Korbutt, Ellen Toth, Garth L. Warnock, Norman M. Kneteman, and Ray V. Rajotte** at the University of Alberta in Canada⁷⁴. The trial showed 100% insulin independence at 12 months in 7 consecutive individuals with brittle T1D and was characterized by: (i) implantation of large numbers of freshly isolated (no culture) cadaveric allogeneic islets (mean of 11,574 islet equivalents/kg per recipient obtained from multiple donors), (ii) the use of human albumin instead of fetal bovine serum in the media utilized for cell processing, and (iii) the use of a steroid-free immunosuppressive regimen based on sirolimus, tacrolimus and anti-IL-2 antibody⁷⁴. This protocol was subse-

quently reproduced in a multicenter international trial⁷⁵ that yielded 58% insulin independence at one year and revealed the important impact of the center experience in islet cell processing and patient management on clinical outcomes⁷⁶.

In the meanwhile, the 'Edmonton Protocol' was adopted with or without various modifications at several institutions contributing to increasing the numbers of islet transplants performed worldwide. Overall, the results from these single center trials showed consistent achievement of metabolic improvements with normalization of HbA1c and glycemic excursions using low exogenous insulin doses and complete insulin independence after transplantation of adequate islet numbers, paralleled by a significant reduction/elimination of severe hypoglycemic episodes and improved quality of life in patients with unstable diabetes receiving islet transplant alone (ITA), islet-after kidney (IAK) or simultaneous islet-kidney (SIK) transplantation^{50,75,77-93}.

At the evaluation of the extended follow up of patients receiving the 'Edmonton Protocol' it was revealed that progressive loss of insulin independence occurred over time, with need to reintroduce exogenous insulin resulting in excellent metabolic control long term and HbA1c. While approximately 80% of the patients showed sustained graft function (measured as persistence of detectable C-peptide), only 10% of study subjects maintained independence from exogenous insulin five years after transplantation⁹⁴. The possible causes of the progressive loss of graft function are multifold⁹⁵. Since 2005 to date, the clinical research has focused on the major objectives of obtaining and maintaining the longest possible time high rates of insulin independence with lowest islet numbers. At the present time, in at least 5 centers (namely, Edmonton, Minneapolis, Geneva, Lille and Milan) have been reported proportions of insulin independence between 40-50% at five years after transplantation⁹⁶⁻⁹⁹, which are not too far from those of pancreas transplant alone. Furthermore, the data available through the CITR confirm an overall trend toward an improvement of insulin independence at 3 years (approximately 44% of patients) following islet transplantation.

The islet transplant community is rapidly growing. The Collaborative Islet Transplant Registry (CITR; www.citregistry.org) has been established in 2001 and has been collecting data from over thirty Centers in North America, Europe and Oceania through self-reporting their activity. In the most recent CITR Report¹⁰⁰, a total of 571 recipients received 1,072 islet infusions from 1,187 donor pancreata, the majority of transplant being ITA. The

availability of pooled data from different centers is invaluable as it allows analyses of clinical outcomes and provide insights on potential variables associated with higher success rates of islet transplantation^{93, 101-105}. Notably, the community is likely much larger than what currently captured by the CITR, since several Centers in Europe, Asia and South America that have and/or are performing islet transplantation trials in recent years do not necessarily report to the Registry; these include, amongst others: the trial by doctors **Frantisek Saudek and Coll.** at Prague University in Czech Republic¹⁰⁶; by doctor **Shinichi Matsumoto and Coll.** at Kyoto University^{107,108}; the trial by doctor **Pablo F. Argibay and Coll.** in Argentina¹⁰⁹; the trial by doctor **Mari Cleide Sogayar and Coll.** in Brazil¹¹⁰; and the trial introducing Campath-1H in SIK transplant recipients by doctor **Janming Tan and Coll.** at Xiamen University in China¹¹¹.

Amongst the several progresses of the recent years, it is worth mentioning at least a few that contributed moving the field forward. The introduction of CD25 targeting in islet transplant recipients by doctor **Philip Morel and Coll.** at the University of Geneva¹¹². The development of islet transplant consortia to maximize efficiency of the transplant programs, such as the Portland/Minneapolis, the Huddinge/Giessen, the Swiss-French GRAGIL^{89,113-115}, the Miami/Huston and Miami/Dallas¹¹⁶⁻¹²⁰ the Miami/Washington DC^{121, 122}; the Geneva/Athens¹²³ networks. The introduction of iodixanol-University of Wisconsin solution density gradients to enhance large animal¹²⁴⁻¹²⁶ and later human¹²⁷ islet separation by doctor **Michel P.M. van der Burg and Coll.** at Leiden University in the Netherland. The utilization of cultured islets by the University of Minnesota and University of Miami groups¹²⁸⁻¹³⁰. The implementation of anti-CD3 antibody at induction by doctor **Bernard J. Hering and Coll.** at the University of Minnesota¹²⁸. The attempts at inducing hematopoietic chimerism in islet transplant recipients by combined bone marrow-derived stem cell and islet transplantation at the University of Miami^{63,131,132}. The invaluable report describing the histopathological features of bioptic specimens obtained from a patient who passed away >13 years of insulin independence following islet transplantation by doctor **Thierry Berney and Coll.** at the University of Geneva in Switzerland¹³³. The understanding of the critical role of stress-activated signal transduction pathways occurring in the pancreas and islets due to ischemia following donor cerebral death, organ preservation and islet isolation resulting in amplification of acute, nonspecific inflammation on islet yields, quality and immunogenicity reported by several research groups, in-

cluding doctors **Stephen Paraskevas, Laurence Rosenberg and Coll.** in Montreal, Canada¹³⁴, **Saida Abdelli, Christophe Bonny and Coll.** at the University of Lausanne in Switzerland^{135,136}, **Rita Bottino, Massimo Trucco and Jon D. Piganelli and Coll.** at the University of Pittsburgh¹³⁷, amongst others^{138,139}. The discovery of islet production of tissue factor, described by doctors **Lisa Moberg, Bo Nilsson and Coll.** at the Karolinska Institute (140), MCP-1/CCL2 described by doctors **Lorenzo Piemonti, Federico Bertuzzi and Coll.** at the San Raffaele Institute in Milan, Italy¹⁴¹⁻¹⁴⁴, CD40 described by doctor **Ricardo L. Pastori** and Coll. at the University of Miami¹⁴⁵, amongst others. The description that targeting the TNF pathway after transplantation may be beneficial to improve islet engraftment in rodents by doctors **Alan C. Farney and Coll.** in 1993 at the University of Minnesota¹⁴⁶, which was substantiated by the demonstration of the significant release of pro-inflammatory cytokines such as TNF-alpha and the induction of endothelial cell activation upon intrahepatic embolization of allogeneic rat islets or inert beads which could be partially reduced via macrophage depletion as shown by doctors **Rita Bottino, Luis Fernandez, Camillo Ricordi, Luca Inverardi and Coll.** at the University of Miami in 1998¹⁴⁷, thus justifying the subsequent introduction of TNF-alpha signaling blockers in clinical islet transplant recipients by the University of Miami^{130,131,148} and University of Minnesota^{97,129}, which retrospectively was confirmed as one of the key factors associated with the success of islet transplantation¹⁰¹. The demonstration of synergy when combining blockade of TNF-alpha and IL-1-beta signaling by doctors **Morihito Takita, Shinichi Matsumoto, Marlon F. Levy and Coll.** at Baylor Research Institute in Dallas, TX¹⁴⁹. The description of the triggering of an *instant blood mediated inflammatory reaction* involving coagulation factors, platelets and leukocytes immediately after intra-portal islet implantation by doctors **William Bennet, Carl G. Groth, Olle Korsgren and Coll.** at the Karolinska Institute¹⁵⁰, which led to implementation of targeted anti-inflammatory strategies to improve islet engraftment and survival including the introduction of novel anti-inflammatory treatment targeting CXCR1/2 by doctor **Piemonti and Coll.** at the San Raffaele Institute (151). The optimization of pancreas preservation solution containing perfluorocarbons to improve oxygenation during cold storage ('Two Layer Method') by doctors **Yoshikazu Kuroda, T Kawamura, Yoichi Saitoh and Coll.** at Kobe University in Japan (152-157). The introduction of trypsin and protease inhibitors during pancreas preservation by doctor **Kuroda and Coll.** in 1988 and other groups¹⁵⁸⁻¹⁶². The introduc-

tion of a controlled pancreatic distension to increase the reproducibility of islet isolation techniques by doctors **Jonathan R. Lakey, Ray V. Rajotte and Coll.** in Edmonton¹⁶³. The recent evaluation of extra-hepatic transplantation sites for islet cells, including intramuscularly¹⁶⁴ and subcutaneous space¹⁶⁵ by doctors **Keith Reemtsma, Collin J. Wever, Mark A. Hardy and Coll.** at Columbia University in the late 1970's, unfortunately without function, followed more recently by better results reported with the intramuscular site by doctor **Ehab Rafael and Coll.** at the Karolinska Institute¹⁶⁶ and by doctor **Sabrina Dardenne and Coll.** at the University of North France in Lille¹⁶⁷; as well as new pilot experiments with an intra-bone marrow site by doctor **Piemonti and Coll.** in Milan^{98,168}, and the use of intra-peritoneal site mainly for initial clinical attempts to transplant encapsulated porcine or human islets to confer immunoprotection by doctors **Robert B. Elliot, Christina Buchanan and Coll.** at the University of Auckland in New Zealand¹⁶⁹, and by doctors **Giuseppe Basta, Giovanni Luca, Riccardo Calafiore and Coll.** at the University of Perugia in Italy^{170,171}, amongst others. The use of noninvasive imaging techniques to detect islet grafts using paramagnetic beads for magnetic resonance imaging in experimental animals by doctor **Frantisek Saudek and Coll.** in Prague^{172,173} and in the clinical settings by doctors **Christian Toso, Thierry Berney and Coll.** in Geneva¹⁷⁴, and the use of positron emission tomography by doctors **Olof Eriksson, Torbjörn Lundgren and Coll.** in Sweden¹⁷⁵. The use of supplemental islet infusion and or the use of exenatide in patients developing graft dysfunction by the Miami group^{148,176,177}. The introduction of novel approaches to seal the tract of trans-hepatic catheterization of the portal vein to reduce the risk of hemorrhage and improve safety of the islet transplant procedure by doctors **Tatiana Froud, Ricordi, Alejandro and Coll.** at the University of Miami¹⁷⁸. The introduction of exenatide treatment for islet transplant recipients to favor engraftment by the University of Illinois at Chicago and the Miami groups^{179,180}. The use of living-related segmental pancreas donor by doctor **Shinichi Matsumoto and Coll.** at Kyoto University^{107,108}. The introduction of Campath-1H as lympho-depleting agent by doctor **Tan and Coll.** at Xiamen University¹¹¹, the University of Miami¹³² and the University of Alberta^{99,181}.

The field of islet transplantation has significantly evolved since the initial attempts by doctors **Minkowski and von Mering** in 1882, with remarkable acceleration over the last three decades thanks to the incredible effort of the research community

across the Worlds yielding to the achievement of steady improvements in the cell processing and transplantation techniques, patient management and immunotherapy protocols utilized (Figure 3). Preservation of beta-cell function is reproducibly currently attained in recipients of islet autografts, a therapeutic option that should be considered for individuals undergoing total pancreatectomy for non-malignant conditions and as recently reported also for malignant condition¹⁸². Restoration of beta-cell function can be obtained by transplantation of allogeneic islets in both nonuremic (ITA) and uremic (SIK and IAK) subjects with diabetes, allowing for long-term sustained function and associating with improved metabolic control even when required exogenous insulin (*i.e.*, suboptimal islet mass transplanted or development of graft dysfunction). The introduction of **Ricordi's** Automated Method twenty-five years ago has definitely given a remarkable impulse to the field, contributing to the expansion of the number of transplants performed worldwide since the early 1990's. Islet transplantation has been approved as a reim-

bursable procedure in several Countries, including Australia, Canada (selected provinces), France, Italy, Switzerland, the National Institute for Health and Clinical Excellence in the United Kingdom, Sweden and the Nordic Network. In the U.S.A., autologous islet transplantation is currently reimbursed. The completion of registration trials by the Clinical Islet Transplant consortium (CIT-06 and CIT-07) will likely lead to biological licensure by the U.S. Food and Drug Administration shortly. This is an important step, as islet transplant activity in the United States has been severely restricted by limited access to research funds, with the exception of a joint Medicaid/Medicare initiative that is currently supporting the islet-after-kidney trial (CIT-06).

We are currently experiencing an exciting stage of innovation and renewed promise for cellular-based therapies to restore beta-cell function. Novel extra-hepatic transplant sites and tissue engineering approaches are being explored which may allow for improved engraftment and sustained function with cadaveric human, xenogeneic or stem-cell derived islet cells in the near future. It has been and still is an exceptional journey!

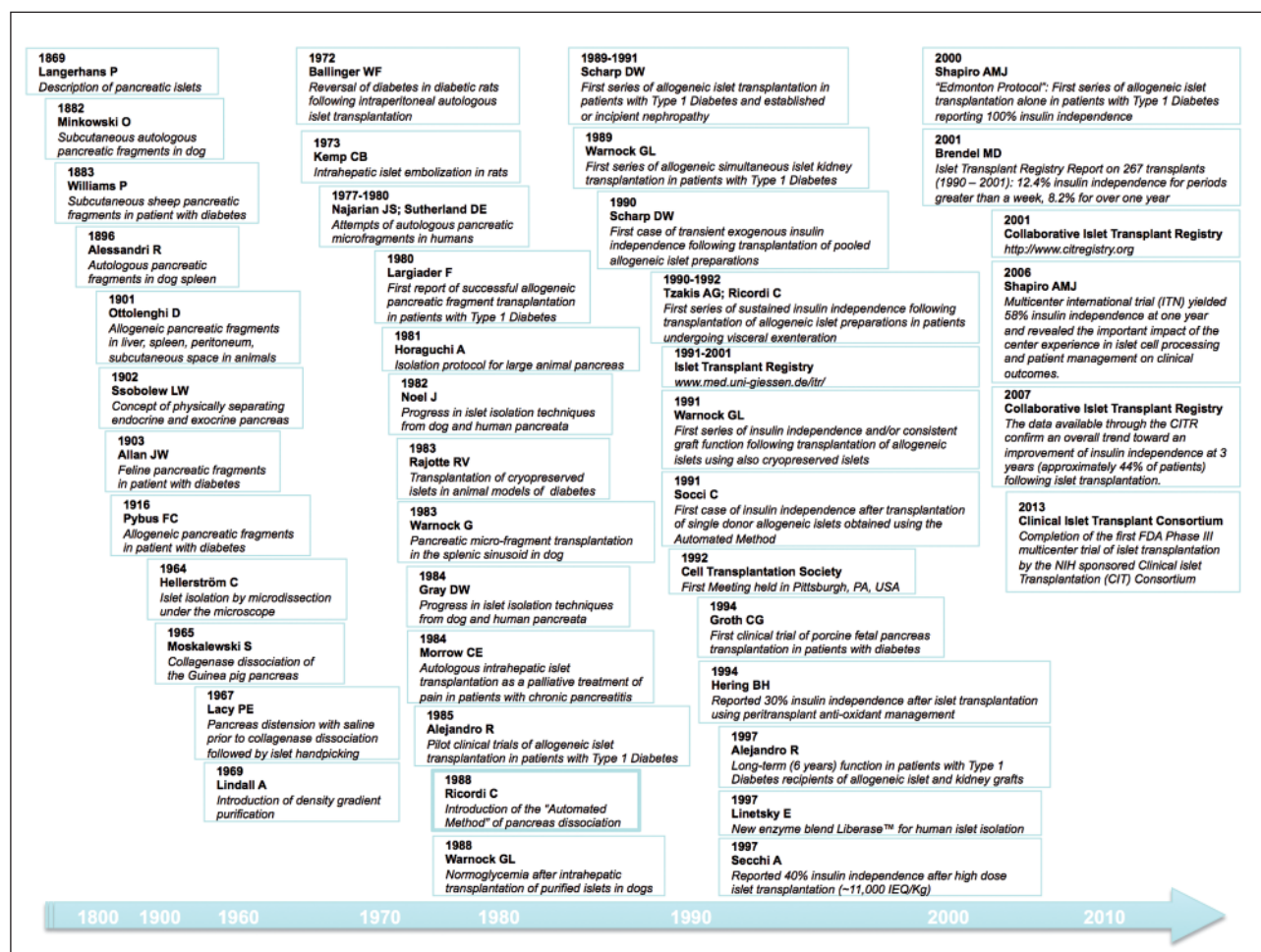


Figure 3. Timeline of pancreatic islet transplantation.

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