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PHARMACEUTICAL SCIENCE
Review Article

Understanding Type 1 Diabetes and Its Treatment – Some Remaining Difficulties

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Received: April 10, 2015; **Published:** December 21, 2015

Abstract

Diabetes is a condition in which there is a complete or relative deficiency of insulin. Type 1 diabetes, sometimes called juvenile or insulin dependent diabetes, necessitates insulin injection. It is a devastating disease and we are still far from understanding the causes. This paper discusses some of the problems in our understanding of the aetiology of the condition and explains the difficulty in definition and the limitations in dividing diabetes into Type 1 and Type 2 diabetes. New treatments are discussed including the role of education, transplantation, stem cell production for the development of new B cells, immunotherapy and the artificial pancreas.

Keywords: *Type 1 Diabetes; Glycated Haemoglobin; Insulin; C-Peptide; Insulin Detimir; Insulin Degludec; Insulin Glargine; Lispro Insulin; Islet Autoantibodies; Immunotherapy; Islet Cell Transplantation*

Abbreviations: DAFNE: Dose Adjustment for Normal Eating; NOD: Non Obese Diabetic; HLA: Human leukocyte antigen;

Introduction

The explosion in the prevalence of diabetes has triggered a remarkable interest in the disease and its prevention. The recent estimates on the prevalence of diabetes are astounding. Three hundred and eight two million people have diabetes in the world and 5.1 million people died of diabetes according to the Diabetes Atlas 6th edition. The fascination in these figures arises not only in the human suffering diabetes causes, but also in the economic burden that diabetes poses on any individual or health system. Although the majority of people with diabetes have what is termed type 2 diabetes, the definition of diabetes remains elusive and necessitates committees such as those convened by the WHO to define and then re-define the condition. These committees therefore have the ability to influence the numbers of people with diabetes rather like a referee at a football match!

Diagnosis of diabetes

Diabetes is defined as an absolute or relative deficiency of insulin. The diagnosis may be made by a formal glucose tolerance test, by a raised fasting blood sugar or by a raised 2 hour postprandial blood sugar. The diagnosis may also be made by a raised HbA1c, a surrogate measure of blood sugar over a 6 week period [1-3]. It is immediately clear that if so many different ways to diagnose the condition are available there must be a problem in the definition and many papers have shown that diagnosis by one method may leave a certain percentage of patients undiagnosed if another method is used. The difficulty stems from using blood sugar as a surrogate marker of insulin deficiency. Had dyslipidaemia been used and the condition called diabetes lipidus rather than mellitus, another category of patients would be defined as having diabetes [4]. The distinction between type 1 and type 2 diabetes is also fraught with difficulties and changing the name from juvenile or insulin dependent diabetes to type 1 has not made the diagnosis any more reliable. Patients who present with hypoglycaemia and ketoacidosis make up the majority of type 1 diabetes but many patients require insulin to control their hypoglycaemia in spite of never having had ketoacidosis.

The cause of Type 1 diabetes

Professor Bjorn Nerup expressed some of the difficulties in understanding the cause of type 1 diabetes at the 2014 annual European association for the study of diabetes (EASD) meeting when he described a lifetime of research into the cause of diabetes, chasing 3 causes which might together explain the cause of type 1 diabetes. The first was infection. He suggested that infection damages the beta cell and it is suggested that this is the initial insult to the beta cell in the pathway to type 1 diabetes. However after 40 or more years since this theory was suggested, we do not know which infection. Evidence that coxacci B4 virus, the mumps virus and many more viruses might be a common cause of the damage to the beta cell is missing but after 40 or more years one might suspect that some evidence would have been produced to validate this theory [5]. The second tenet of the theory of type 1 diabetes causation is autoimmunity. The major suggested antibodies are GAD 65, insulin and islet cell antibodies. Unfortunately rise and fall in these antibody titers have been found to occur in people who do not develop diabetes and when these antibodies are tested *in vitro* they do not appear to be particularly harmful to beta cells [5]. Genes have been thought to be very important in many diseases and diabetes was no exception. The early discovery that the HLA system was involved in both protection and susceptibility to diabetes heralded the way for a genetic cause of diabetes [6,7]. Sadly the discovery of the genome and the huge genome wide surveys, have not confirmed an important role for genes in diabetes. Professor Nerup ended his lecture by suggesting that the paradigm of the causation of diabetes that he spent a lifetime investigating might possible be incorrect and that young investigators starting off on a career in diabetes research might try a different paradigm rather than following old men down a path leading nowhere.

Rodriguez-Calves and von Harrath [8] disagree under the umbrella of Victor Hugo [9], whom they quote as having written "Perseverance, secret of all triumphs" Their article "Enterovirus infection and Type 1 diabetes" discusses the finding of Krogvoid., et al. [10] who have shown in the pancreata of 6 patients who recently developed diabetes the presence of a low grade enteroviral infection. Only 1.7% of the islets contained some of the overall 60 intensely enterocapsid protein 1 positive (VP1+) cells detected. Rodriguez-Calvo and Von Herrath [8] go on to say that one might think that 60 VP1 cells in 2492 islets are not much and explain why the number might be sufficient to cause diabetes. In the conclusion, they suggest that perhaps enteroviral and may be other infections are critical triggers to the development of diabetes. The paradigm may be correct!. Not all the islets in biopsy specimens of patients with Type 1 diabetes are destroyed. Insulin containing cells may make up as much as 20% of the total β-cell mass [11,12] so there may be room to stop the destruction and to stimulate new β cell growth/function. Pathological/immunological examination of the pancreata of Type 1 patients have shown that the insulitis in the islets which seems usually β-cell specific rather than including Alpha and delta cells is associated with CD8 + macrophages (CD68+) CD4+ T cells and B lymphocytes (CD20+). In theory attack against any of these cells might defeat the β cell destruction. A good review of preclinical studies has recently been published [13]. The authors make the point that juvenile very young diabetes may be different from adult Type 1 diabetes and we lack useful biomarkers to follow the pathological process. Since the cause of type 1 diabetes remains uncertain one is still left with the definition that the disease is due to a lack of insulin but the role of insulin resistance in type 1 diabetes is now a topic of research. Donga., et al. [14] have recently carried out a meta analysis on insulin resistance in patients with Type 1 diabetes assessed by glucose clamp studies. They conclude that insulin resistance is a prominent feature of patients with type 1 diabetes and involves hepatic peripheral and adipose tissue, a reason to consider rejection of the Type 1 and Type 2 classification of diabetes, Type 2 diabetes usually being referred to as the insulin resistant diabetic condition

Type 1 diabetes treatment

Treatment of type 1 diabetes was revolutionised by the discovery of insulin. The dispute as to who actually was responsible for the discovery has never been fully resolved but that is not unusual in important discoveries. The wonderfully entertaining history of the discovery of insulin by Bliss [15] and the more erudite book by Alberto DeLivre [16] make for exciting reading which brings tears to the eyes as it is hard to realise the impact of this life saving treatment. Since then insulin has been refined and new methods for delivery of insulin have been devised. Glargine 300 units/ml is the newest addition to the insulin stable. A recent trial showed similar glucose control to insulin Glargine (100 units/ml) in a 6 month trial when added to a bolus regime. Hypoglycaemia at night was less but only in the first 8 weeks of the trial [17]. Insulin degludec is another recently launched long acting insulin. A meta analysis of phase 3A trials showed that patients who achieved a HbA1c of < 7% had fewer overall and nocturnal hypoglycaemic events on the insulin degludec when compared

to insulin glargine in both type 1 and type 2 diabetic patients [18]. Insulin detimir is a slightly older addition to the insulin stable. It is long acting insulin analog with slower tissue distribution due to high plasma protein binding [19]. A comparison of detimir with lispro protamine and glargine suggested that insulin detimir was not as effective as the other 2 insulins in reducing glucose variability and lispro protamine led to fewer hypoglycaemic events [20]. Insulin detimir is often used in pregnancy and has been shown not to cross the human placenta [21]. Insulin detimir has been shown to be non inferior to NPH insulin in pregnancy when comparing glycaemic control and foetal outcome but there were less hypoglycaemic events in the detimir group [22].

The ease of administration of insulin has been a wonderful advancement and the advent of disposable, very fine bore; needles have made life so much easier for diabetic patients. The advent of blood glucose meters and the disbanding of urine testing have all gone towards improving the quality of life for diabetic patients. The advent of insulin pumps, and more recently real time tissue (rather than blood) sugar, and of course the promise of a reliable closed loop system in the near future makes for exciting reading [23,24]. However, it is sad to read a recent paper by Lind., *et al*. [25] on glycaemic control and excess mortality in type 1 diabetes. The study was a registry based observational study to determine the excess risk of death according to the level of glycaemic control in a Swedish population of patients with diabetes. The study included patients with type 1 diabetes registered in the Swedish national register after January 1st 1998 and patients and controls were followed till December 31st 2011. The mean age at baseline was only 35.8 years. It is sad to read that 8% of the patients had died as compared to 2.9% of the control subjects. There was a clear relationship between glycated haemoglobin levels and the risk of death from any cause and from cardiovascular causes. Even more upsetting, the type 1 diabetic patients with a glycated haemoglobin level of 6.9% or lower had a risk of death from any cause or from cardiovascular causes that was twice as high as the matched controls. This is in a country that has a magnificent health service; one wonders what it might be like for people with type 1 diabetes who live in a less advantaged country. A recent Norwegian analysis suggests that in the last decade or so the outlook for children diagnosed with Type 1 diabetes has improved [26]. In Ireland we pride ourselves on having a good service for people with diabetes, yet a disturbing report on the care of type 1 diabetes has recently been published [27]. This retrospective audit of a young adult type 1 diabetes population in the west of Ireland, found that the average HbA1c was poor at 81 mmol/mol (9.5%). 32% of these patients had diabetes related complications. The authors found that engagement with services was poor with an average of 3 missed clinic appointments over a 3 year period. It seems we are a long way off from being able to treat type 1 diabetic patients in a satisfactory manner.

The necessity for finding new ways to improve diabetic control is obvious. Education would seem a platform but efforts to improve the education of diabetic patients have not solved the compliance issue. The Berger Method [28] and many similar methods such as the Dose Adjustment for Normal Eating (DAFNE) program [29] have resulted in only modest improvement of HbA1c and these programs are very time consuming for patients and are not taken up by many of those to whom they are offered. Watts., *et al*. [30] report on the effectiveness of training primary care providers to set up mini clinics to treat difficult to control diabetic patients in remote or rural areas. The education program was delivered by a multidisciplinary team over a year using video conferencing technology. Thirty nine patients who had HbA1c of > 9% were seen in 2 mini clinics over 15 months. HbA1c fell from a mean of 10.2% to 8.4%. The DAFNE Program is a much researched program to train Type 1 patients [29]. It is usually a 5 consecutive day course and has been shown to reduce hypoglycaemic events and improve well being. However, only modest improvement in HbA1c occurs. A recent update describes the comparison of 5 consecutive days of training as compared to 5 days over a 5 week period [31]. Of 213 patients who were randomised only 160 completed the course with no significant difference in outcome between the 2 arms of the study. In the diabetes education program (PRIMAS) for type 1 diabetes, HbA1c was only reduced by 0.4 % points [32]. A seven year follow up of the DAFNE program [33] showed only a 3mmol/mol (0.3%) difference. Mean HbA1c at the end being 67mmol/mol (8.3%) viz 70 mmol/Mol (8.5%). The effectiveness of the Kids in Control of Food structured education course (KICk-OFF) for 11-16 year olds with Type 1 Diabetes recently reported no significant improvement in HbA1c after 24 months of intervention [34]. It seems intensive education programs are helpful in improving quality of life, a difficult concept to measure, but not in improving HbA1c to any great extent and it is the high blood sugars reflected in HbA1c that results in the devastating vascular complications.

To improve type 1 diabetes treatment we will need to put more effort to develop strategies to prevent the disease, to prevent the complete destruction of the beta cells early on in the disease and in those patients who have already lost the majority of their β-cells

to continue our efforts to succeed in β-cell transplantation or an artificial pancreas. A recent review [13] starts with the statement that "standard of care for the treatment of Type 1 diabetes is much the same as it was in the early 1920s simply more insulin options". The new knowledge that some β-cells are still alive and secreting C peptide even in long standing Type 1 diabetic patients [11]. Has renewed interest in discovering therapies to heal the β-cells. The much studied immune model of type 1 diabetes, the non-obese diabetic (NOD) mouse, has paved the way for immune treatment trials in humans [13]. However, differences in β-cell replication, islet structure, severity and composition of the immune infiltrate in the islets and the main t-cell subset involved make the non obese diabetic (NOD) mouse information difficult to translate to humans.

Although it has been difficult to discover genes that cause type 1 diabetes, some progress has been made. The human leucocyte antigen (HLA) system was the first break through many years ago. It appears that HLA class 2 DR4-DQ8 haplotypes are found in most children who develop type 1 diabetes. With this genetic profile if the children develop serum auto antibodies (2 or more), then 75% of children will develop diabetes within 10 years and almost all within 20 years [35]. Although auto-antibodies against insulin are the first auto-antibodies to be detected before the onset of type 1 diabetes, other autoantibodies arise after this, before the onset of diabetes [36]. Interferon γ producing CD 4+ T cells directed against proinsulin [37] and CD8+ macrophages against insulin are thought to be the cause of insulinitis [38]. It has been shown that in an animal model of type 1 diabetes that oral insulin will reduce the development of diabetes through the induction of insulin specific CD3+ regulatory T cells [39]. The Pre-POINT randomised clinical Trial [5] reported a study of 25 children who were islet auto antibody negative aged 2-7years old with a family history of type 1 diabetes and susceptible HLA class 2.genotypes. The children received oral insulin or placebo, to see whether oral insulin might induce insulin immunotollerance. The authors report that indeed this was possible without incurring hypoglycaemia. This leads the way to a trial to see whether the immune tolerance, triggered by oral insulin, might stop islet autoimmunity and prevent the onset of diabetes in these very susceptible children [12]. Attempts to alter the inflammatory process once diabetes has been diagnosed may be too late in the destructive process but if the prediction of future onset of type 1 diabetes is so difficult in the majority of patient's then perhaps early alteration of islet inflammation may preserve some useful β cell function. Keymeulen., et al. [40] have reported on 4 years of treatment with CD3 humanised monoclonal antibody, They showed that a weeks treatment at the early stage of onset of Type 1 diabetes resulted in a delay in the rise in insulin requirements and a reduced deterioration of metabolic variables in younger patients. A study of 58 Type 1 patients who had had diabetes for 4-12 months duration and evidence of residual C peptide, has shown that a 14 day course of Teplizumab, a humanised anti CD3 monoclonal antibody, improved C peptide response after 12 months (17.7% higher). The treated patients required less insulin but improvement in HbA1c as compared to the controls was not demonstrated [41]. Two excellent reviews of immunotherapy in Type 1 diabetes have recently been published [42,43].

Islet cell transplantation

Islet cell transplantation for the treatment of Type 1 diabetes has been curtailed by the scarcity of human pancreata. The results have been only moderately successful and the explosion of interest following the successful results from the Edmonton group were considerably modified when they reported their 5 year follow up in which most of the patients were back on insulin [44]. The side effects of the necessary immunotherapy to prevent rejection has also dampened enthusiasm for transplantation, except in certain circumstances such as hypoglycaemia unawareness and very unstable diabetes [45]. The use of pig islets for xenotransplantation is beset with difficulties but recent advances have made Xenotransplantation a possible source of islets to replace human islets which are so scarce [46].

Whole pancreas transplantation has been very successful but again paucity of donors and the morbidity and mortality associated with the procedure has also limited the procedure to very specially selected patients who also need renal transplant. Again the immunotherapy to prevent rejection is an important barrier to wide introduction of the procedure even if pancreata were easily available [47].

The advent of stem cells as the precursor of a functioning β cell has been a very exciting development and the discovery of methods to develop β cell sfrom stem cells on an industrial scale has opened up new prospects on the cure of Type 1 diabetes [48,49]. ViaCyte have now manufactured a clinically approved graft that contains human pancreatic endoderm cells in a durable semi permeable macro

encapsulation device (Encaptra). Clinical studies are in progress following encouraging animal trials [50,51]. It is possible that these encapsulation devices will allow for much reduced immunosuppression as the capsules are designed to stop entry of large protein molecules responsible for the immune attack on the β cell.

Conclusion

Type 1 diabetes presents problems in definition, diagnosis and treatment. Reversal of the metabolic derangement is possible with pancreas transplantation or islet cell transplantation but many difficulties remain. The development of the artificial pancreas Is hampered by lack of a reliable blood glucose sensor. Identification of subjects who will develop diabetes, and prevention when identified, seems a long way away for the majority of Type 1 diabetic subjects. Rescue of B cells in the very early stage of Type 1 diabetes with immunomodulation holds great promise as even a small amount of remaining endogenous insulin makes diabetes much easier to control.

Bibliography

1. Guan X., *et al*. "The changing relationship between HbA1c and FPG according to different FPG ranges". *Journal of Endocrinological Investigation* (2015).
2. Kapadia CR. "Are the ADA hemoglobin A (1c) criteria relevant for the diagnosis of type 2 diabetes in youth?" *Current Diabetes Reports* 13.1 (2013): 51-55.
3. Alqahtani N., *et al*. "Use of Glycated Hemoglobin in the Diagnosis of Diabetes Mellitus and Pre-diabetes and Role of Fasting Plasma Glucose, Oral Glucose Tolerance Test". *International Journal of Preventive Medicine* 4.9 (2013): 1025-1029.
4. Shafrir E and Raz I. "Diabetes: mellitus or lipidus?" *Diabetologia* 46.3 (2003): 433-440.
5. Noble JA. "Immunogenetics of type 1 diabetes: A comprehensive review". *Journal of Autoimmunity* 64 (2015): 101-112.
6. Rich SS and Concannon P. "Summary of the Type 1 Diabetes Genetics Consortium Autoantibody Workshop". *Diabetes Care* 38 suppl2 (2015): S45-S48.
7. Poirier JG., *et al*. "Resampling to Address the Winner's Curse in Genetic Association Analysis of Time to Event". *Genetic Epidemiology* 39.7 (2015):518-528.
8. Rodriguez-Calvo T., *et al*. "Enterovirus Infection and Type 1 Diabetes: Closing in on a Link?" Diabetes 64.5 (2015): 1503-1505.
9. Hugo V, L'homme qui rit. A. Michel, Paris, France, 1869 [in French]
10. Krogvold L., *et al*. "Detection of a Low-Grade Entero viral Infection in the Islets of Langerhans of Living Patients Newly Diagnosed With Type 1 Diabetes". *Diabetes* 64.5 (2015): S1682-S1687.
11. Wang L., *et al*. "Persistence of Prolonged C-peptide Production in Type 1 Diabetes as Measured With an Ultrasensitive C-peptide Assay". *Diabetes Care* 35.3 (2012): 465-470.
12. Bonifacio E., *et al.* "Pre-POINT Study Group. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial". *JAMA* 313.15 (2015): 1541-1549.
13. Rekers NV., *et al*. "Wesley JD Immunotherapies and immune biomarkers in Type 1 diabetes: A partnership for success". *Clinical Immunology* 161.1 (2015): 215-216.
14. Donga., *et al*. "Insulin resistance in patients with type 1 diabetes assessed by glucose clamp studies: systematic review and meta-analysis". *European Journal of Endocrinology* 173.1 (2015): 101-109.
15. "Michael Bliss The discovery of Insulin. University of Chicago Ill (1984) ISBN-13;978-)-2660-5899-3.
16. DeLivre A., *et al*. "The discovery of Insulin. Continued controversies after ninty years". *Endocrinología y Nutrición* 58.9 (2011): 449-456.
17. Becker RH., *et al*. "New insulin glargine 300 Units • mL-1 provides a more even activity profile and prolonged glycemic control at steady state compared with insulin glargine 100 Units • mL-1". *Diabetes Care* 38.4 (2015): 637-643.
18. Einhorn D., *et al.* "Patients achieving good glycaemic control (HbA1c <7%) experience a lower rate of hypoglycaemia with insulin degludec than with insulin glargine: a met-analysis of phase 3A trials". *Endocrine Practice Journal* 21.8 (2015): 917-926.

19. Raslova K "An update on the treatment of type 1 and type 2 diabetes mellitus: focus on insulin detemir, a long-acting human insulin analog." *Journal of Vascular Health and Risk Management* 6.1 (2010): 399-410.
20. Derosa G., *et al*. "Glucose-lowering effect and glycaemic variability of insulin glargine, insulin detemir and insulin lispro protamine in people with type 1 diabetes". *Diabetes, Obesity and Metabolism* 17.6 (2015): 554-559.
21. Suffecool K., *et al*. "Insulin detemir does not cross the human placenta". *Diabetes Care* 38.2 (2015): e20-e21.
22. Herrera KM., *et al*. "Randomized controlled trial of insulin detemir versus NPH for the treatment of pregnant women with diabetes". *American Journal of Obstetrics & Gynecology* 213.3 (2015): e1-e7.
23. Russell SJ., *et al*. "Outpatient Glycemic Control with a Bionic Pancreas in Type 1 Diabetes". *The New England Journal of Medicine* 371.4 (2014): 313-325.
24. Haidar A., *et al*. "Comparison of dual-hormone artificial pancreas, single-hormone artificial pancreas, and conventional insulin pump therapy for glycaemic control in patients with type 1 diabetes: an open-label randomised controlled crossover trial". *The Lancet Diabetes & Endocrinology Lancet Diabetes Endocrinology* 3.1 (2015): 17-26.
25. Lind M., *et al*. "Glycemic control and excess mortality in type 1 diabetes". *The New England Journal of Medicine* 371.21 (2014): 1972-1982.
26. Gagnum V., *et al*. "All-cause mortality in a nationwide cohort of childhood-onset diabetes in Norway 1973-2013". *Diabetologia* 58.8 (2015): 1779-1786.
27. Casey R., *et al*. "Young adult type 1 diabetes care in the West of Ireland: an audit of hospital practice". *QJM* 34.5 (2014): 903-908.
28. Muhlhauser I., *et al* "Evaluation of an intensified insulin treatment and teaching programme as routine management of type 1 (insulin dependent)diabetes. The Bucharest-Dusseldorf Study". *Diabetologia* 30.9 (1987): 681-690.
29. DAFNE Study Group. "Training in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes: doseadjustment for normal eating (DAFNE) randomised controlled trial". *BMJ* 325.7367 (2002): 746-749.
30. Watts SA., *et al*. "Improved glycemic control in veterans with poorly controlled diabetes mellitus using a Specialty Care Access Network-Extension for Community Healthcare Outcomes model at primary care clinics". *Journal of Telemedicine and Telecare* (2015).
31. Elliott J., *et al*. "A cluster randomized controlled non-inferiority trial of 5-day Dose Adjustment for Normal Eating (DAFNE) training delivered over 1 week versus 5-day DAFNE training delivered over 5 weeks: theDAFNE 5 × 1-day trial". *Diabetic Medicine* 32.3 (2015): 391-398.
32. Hermanns N., *et al.* "The effect of a diabetes education programme (PRIMAS) for people with type 1 diabetes: results of a randomized trial". *Diabetes Research and Clinical Practice* 102.3 (2013): 149-157.
33. Gunn D., *et al*. "Glycaemic control and weight 7 years after Dose Adjustment For Normal Eating (DAFNE) structured education in Type 1 diabetes". *Diabetic Medicine* 29.6 (2012): 807-812.
34. Price KJ., *et al*. "Effectiveness of the Kids in Control of Food (KICk-OFF) structured education course for 11-16 year olds with Type 1 diabetes". *Diabetic Medicine* (2015): 12881.
35. Ziegler AG., *et al.* "Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children". *JAMA* 309.23 (2013): 2473-2479.
36. Ziegler AG and Nepom GT. "Prediction and pathogenesis in type 1 diabetes". *Immunity* 32.4 (2010): 468-478.
37. Arif S., *et al*. "Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health". *Journal of Clinical Investigation* 113.3 (2004): 451-463.
38. Unger WW., *et al*. "Roep BODiscovery of low-affinity preproinsulin epitopes and detection of autoreactive CD8 T-cells using combinatorial MHC multimers". *Journal of Autoimmunity* 37.3 (2011): 151-159.
39. Bresson D. "Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing Tregs". *Journal of Clinical Investigation* 116.5 (2006): 1371-1381.
40. Keymeulen B., *et al*. "Pipeleers DFour-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass". *Diabetologia* 53.4 (2010): 614-623.

41. Herold KC., *et al*. "Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial". *Diabetologia* 56.2 (2013): 391-400.
42. Pozzilli P., *et al.* "Combination immunotherapies for type 1 diabetes mellitus". *Nature Reviews Endocrinology* 11.5 (2015): 289-297.
43. Ben Nasr M., *et al*. "The rise, fall, and resurgence of immunotherapy in type 1 diabetes". *Pharmacology Research* 98 (2015): 31-38.
44. Ryan EA., *et al*. "Five-year follow-up after clinical islet transplantation". *Diabetes* 54.7 (2005): 2060-2069.
45. Bruni A., *et al*. "Islet cell transplantation for the treatment of type 1 diabetes: recent advances and future challenges". *Journal of Diabetes, Metabolic Syndrome and Obesity* 23 (2014): 211-223.
46. Park CG., "Hawthorne WJ3The use of pig islets for xenotransplantation is beset with difficulties but recent adcvances have made Xenotransplantation a possible source of islets to replace human islets which are so scarce.Current status of islet xenotransplantation". *International Journal of Surgery* (2015): S1743-9191(15)01075-4.
47. Meirelles Júnior RF., "Pancreas transplantation: review". *Einstein (Sao Paulo)* 13.2 (2015): 305-309.
48. Schulz TC. "A scalable system for production of functional pancreatic progenitors from human embryonic stem cells". *PLoS One* 7.5 (2012): e37004.
49. Schulz TC. "Concise Review: Manufacturing of Pancreatic Endoderm Cells for Clinical Trials in Type 1 Diabetes". *Stem Cells Translational Medicine* 4.8 (2015): 927-931.
50. Boettler T., *et al*. "Pancreatic tissue transplanted in TheraCyte™ encapsulation devices are protected and prevent hyperglycemia in a mouse model of immune-mediated diabetes". *Stem Cells Translational Medicine* 4.8 (2015): 927-931.
51. Motté E., *et al*. "Beta Cell Therapy Consortium EU-FP7.Composition and function of macroencapsulated human embryonic stem cell-derived implants: comparison with clinical human islet cell grafts". *American Journal of Physiology: Endocrinology Metabolism* 307.9 (2014): E838-E846.

Volume 2 Issue 2 December 2015