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A ‘cure’ for diabetes and its complications

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The new millennium has brought continued hope for a ‘cure’ with major developments in immunology and understanding of pathogenesis of diabetes and its complications. Intervention studies to slow the progression of beta-cell destruction by using insulin to induce immune tolerance have shown promise in some subsection analysis but without overall benefit for primary outcome. The ongoing INIT 11 study delivers insulin nasally. The TRIGR study eliminates cow milk in babies at risk with promising effect for antibody generation. Stem cells also offer promise. Islet cell transplantation from cadavers is minimally invasive and has shown great promise, but has been slowed due to lack of donors and immunosuppression needs. For those with established diabetes, randomised controlled trials have demonstrated minimising and slowing of progression of diabetes vascular complications. These interventions include intensive glycaemic treatment, blood pressure lowering and lipid lowering agents. Good randomised controlled clinical trials provide the best evidence of these benefits, which must then be followed by more research to better understand mechanisms of disease pathogenesis and treatment effects.

The elusive cure for diabetes is a dream of many individuals and their families who live with diabetes. Increasing numbers of children are now diagnosed with diabetes, with the prevalence in Australia recently reported to be one in 724 children aged less than 15 years, and one in 391 adolescents aged ten to 14 years [1]. Much clinical research is making this dream closer to delivery. Family members are enrolling in natural history and clinical intervention trials to help understand, delay and hopefully stop the disease.

Research to cure diabetes is now multipronged. A number of studies have focused on predicting risk, which then has allowed development of studies to intervene in the

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prediabetes period, before the glucose levels are abnormal. Treatment within the first 100 days after the clinical diagnosis to switch off the immune process or boost the immune defenses is currently under investigation. Research aimed at curing established diabetes uses cell-based therapies including transplantation of islets, the insulin-producing beta cell. Such therapies currently require immunosuppression to prevent rejection and recurrence of the initial diabetogenic process.

Type 1 diabetes is an autoimmune disease, meaning that the body fights itself by activation of the immune system and not recognising the pancreas beta cells as 'self' but as 'foreign'. Our understanding of this loss of 'self-tolerance', is pivotal to development of a cure with many intervention trials now aimed at 'inducing self-tolerance'. In the development of diabetes there is activation of both arms of the immune system: humoral immunity (antibody production by B lymphocytes) and cellular immunity (T lymphocytes). We can measure antibodies with relative ease but measurement of T-cell function is more difficult. Most intervention studies use antibodies to identify the population at risk for diabetes, and use antibodies as a surrogate marker of progression. However T-cell activation precedes antibody production.

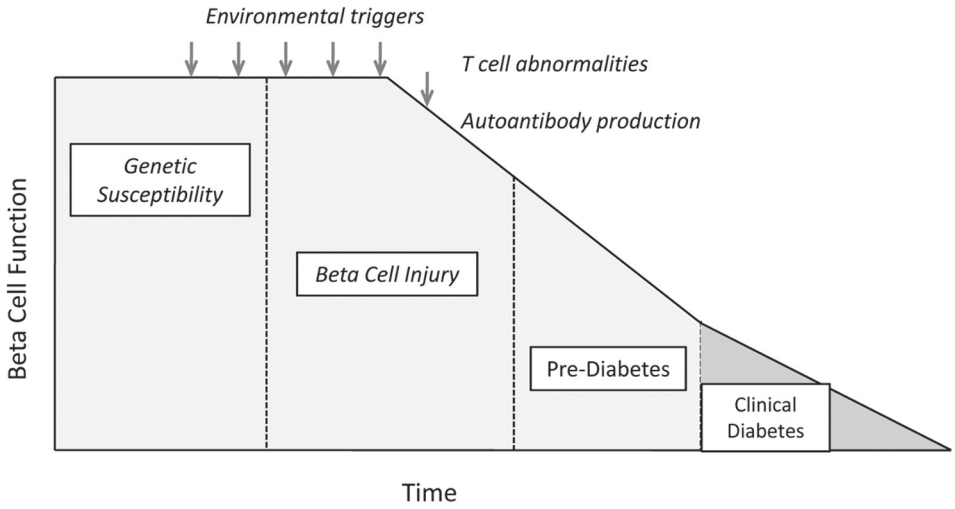

Figure 1. Progression of beta-cell failure in type 1 diabetes.

We now know that the autoimmune process occurs in individuals who are genetically predisposed and that this process appears to be triggered by environmental factors, including viral infection (Figure 1). The autoimmune reaction results in loss of beta-cell mass and hence function which occurs over months to years, during which time prevention may be possible (preclinical preservation). The insulin response to glucose given intravenously is a well-established method to measure beta-cell function in this preclinical phase, prior to abnormal glucose levels. After clinical diagnosis and/or presentation with hyperglycaemia, there is still some functioning pancreas or beta-cell function. This remaining function results in the well-recognised 'remission phase' during which exogenous insulin requirements can

be reduced substantially. Clinical diabetes intervention aims to preserve beta-cell function. This can be measured by C peptide production in response to stimuli, as C peptide is secreted by the beta cell at the same time as insulin.

For those with established diabetes, as detailed in other chapters in diabetes complications (Twigg and McLennan), the risks of long-term end-organ diabetes complications can be reduced by risk factor minimisation targeted at modification of lipids, blood pressure as well as glucose (Figure 2).

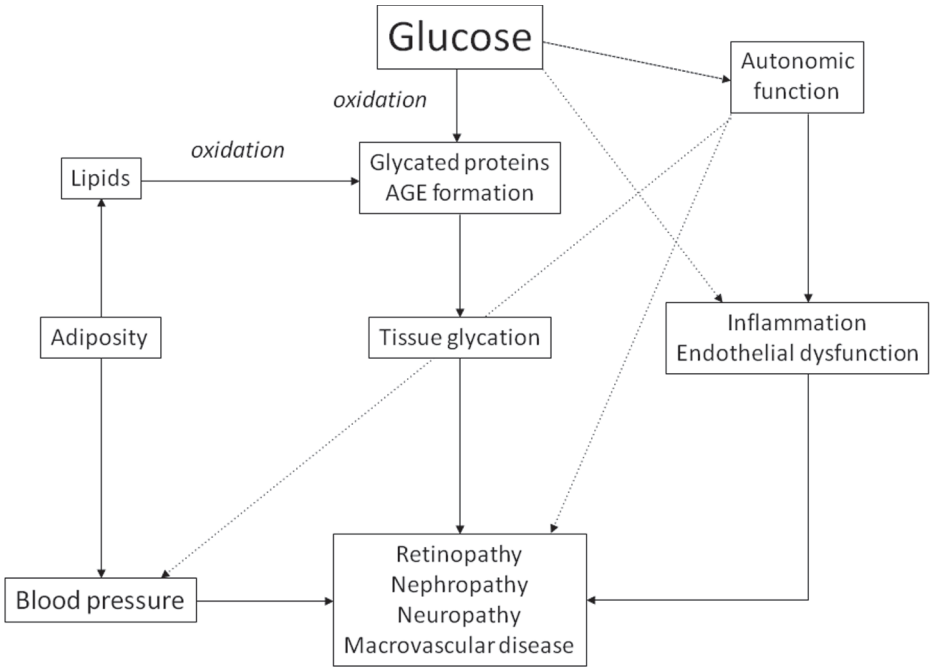

Figure 2. Overview of pathogenesis of long-term vascular complications and modifiers of glucose action.

Studies to understand the genetic and environmental risks

The Type 1 Diabetes Genetics Consortium (T1DGC) is an international project designed to identify genes that modify the risk of diabetes. Families with two or more members with type 1 diabetes have provided blood for this purpose, through four regional centres (Asia Pacific, Europe, UK and North America). More than 2500 families to date have contributed. It is sponsored by US National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) and the Juvenile Diabetes Research Foundation (JDRF) [2]. The major genetic risk is still located on the HLA region, but others genes have been identified, including other immunoregulatory genes [3]. The Australian National Health and Medical Research Council (NHMRC) is currently funding the Australian Childhood Diabetes DNA Repository (ACDDR), for family members with type 1 and type 2 diabetes, who contribute

saliva from which genetic material is derived for subsequent analysis. Further the Type 1 Diabetes TrialNET is an international consortium aimed at understanding natural history, in particular antibodies, in family members and undertakes prevention and intervention trials. Family members can enroll for antibody screening at 200 sites worldwide, in North America, Australia and Europe.

The charmingly named TEDDY study (The Environmental Determinants of Diabetes in the Young) aims to determine infectious agents, dietary factors or other that may impact on diabetes development in children at high risk based on HLA genotyping. Recruitment has been from three centres in the US (Colorado, Washington and Georgia/Florida) and three centres in Europe (Finland, Germany and Sweden). Children from the general population as well as children from families placing them at high risk are recruited [4].

Infections have long been considered to be possible environmental triggers for the autoimmune process. Transmission of enteroviruses occurs by shedding from the respiratory or gastrointestinal tracts. A recent meta-analysis of 26 case-control studies showed a tenfold increase of enterovirus infection in patient at diabetes onset compared to controls [5]. In addition children were nearly four times more likely to have autoimmunity to the pancreas when there was molecular evidence of enterovirus (by finding RNA or viral protein in blood, stool or tissue). This meta-analysis was based on studies from 12 countries, mostly of children but some studies included adults up to the age of 53 years.

While there is a clear distinction between type 1 and type 2 diabetes in terms of pathogenesis, greater weight gain may also be an initiator and/accelerator for autoimmunity and type 1 diabetes. Younger age of diabetes onset is associated with higher body mass index (BMI) at diagnosis, particularly in very young children [6, 7]. Retrospective case control studies have demonstrated increased linear growth and weight gain in early childhood, independent of feeding, with type 1 diabetes [8–11]. The Australian BabyDiab study has followed babies with a first-degree relative with diabetes (parents or siblings) from birth to six years of age. Children with weight above average had more than twofold increase in antibody development than thinner children [12]. Those that gained the most weight during infancy also had an increased risk of islet autoimmunity. Insulin resistance, and associated inflammation, may underlie the role of overweight in the development of type 1 diabetes [13]. Insulin resistance predicted progression from islet autoimmunity to type 1 diabetes [14] and was greater in young children with islet autoimmunity in the German BabyDiab study [15]. Therefore, insulin resistance associated with overweight may be a key factor in both the development and progression of islet autoimmunity. The process may begin in utero; maternal weight before pregnancy and more than 15 kg weight gain during pregnancy predicted risk of islet autoimmunity in their offspring [16].

Vitamin D is an anti-inflammatory steroid with multiple immunomodulatory effects that can promote immune tolerance, however its role in type 1 diabetes remains controversial. A systematic review of observational studies examined whether vitamin D supplementation in infancy reduced the risk of type 1 diabetes in later life [17]. Infants who were supplemented with vitamin D had 29% lower risk of developing type 1 diabetes compared to those who

were not supplemented. There was also some evidence of a dose–response effect, with those using larger amounts of vitamin D being at lower risk of developing type 1 diabetes. However, there are no randomised controlled trials examining the effect of vitamin D supplementation and type 1 diabetes risk.

Immunomodulatory trials

With most autoimmune diseases the main focus of therapy is to treat the abnormal immune response with immunosuppression. Diabetes, however, presents later in the autoimmune process when it is estimated more than 90% of islet function is destroyed, so immunosuppression may not be comparable to other autoimmune diseases some of which are relapsing. Nevertheless, recently a number of larger multicentre studies have reported the outcomes of short-term immunosuppression in new onset diabetes. Many of these treatments are currently marketed for the treatment of rheumatoid arthritis with good safety profiles. Therapy has aimed to modulate T-cell action, or reduce B cells with some success.

One TrialNET study used abatacept, which modulates T-cell co-stimulation, preventing full T-cell activation. In this study 112 newly diagnosed patients were randomised to either treatment or placebo injections at day 1, 14, 28 and then monthly for two years. There was a positive effect of the intervention with preservation of C peptide reserve for 9.6 months compared to the placebo treated group but this declined in parallel, meaning the effect was short term [18].

Another TrialNET used anti-CD20 monoclonal antibody, rituximab, which depletes B cells. In 78 newly diagnosed patients aged between eight and 40 years, the intervention compared to placebo showed positive effects for preservation of C-peptide production, glucose control and lower insulin dose [19].

The Protégé study used teplizumab, anti-CD3 therapy, which binds to T-cells and modulates their action. Enrolled were 516 subjects aged from eight to 35 years, within 12 weeks of diabetes onset in 83 centres across North America, Europe, India and Israel. The primary endpoint was a significant treatment effect at one year of insulin dose <0.5 units/kg/day with HbA1c <6.5%. Whilst this outcome was not achieved, a positive treatment effect was found in children aged from eight to 11 years, and earlier treatment within six weeks of diagnosis [20].

Inducing immune tolerance: antigen-based therapy

Glutamic acid decarboxylase (GAD) is a principle target (or antigen) for autoimmunity antibody production in diabetes. Consequently GAD therapy (two or three GAD injections or placebo) has been trialled after onset of clinical diabetes in patients aged three to 45 years. The initial pilot study in adolescents with two injections of GAD was promising, but the 15-site North American clinical trial was unsuccessful in preserving beta-cell function in the 145 participants [21].

For those in the prediabetes phase, the autoimmune process may be turned off by a process of 'immune tolerance'. Induction of immune tolerance has been sought by using GAD and by insulin itself to which antibodies are made in the prediabetes phase.

Prediction to progression of diabetes has focused on measurement of diabetes-associated antibodies in family members. Models are derived whereby there is a 26%–50% chance of progression to diabetes within five years based on the presence of positive antibodies.

The first trial to use insulin itself as the intervention was the Diabetes Prevention Trial – Type 1 Diabetes (DPT-1) [22]. In one arm low-dose insulin was given by injection to those at highest risk to progression (greater than 50%), and in the other arm insulin was given orally to those with moderate risk of progression. Unfortunately 241 of 670 developed diabetes at a mean age of 13.9 years. The numbers required to screen, in this case using the islet cell antibody, were very high: 103,000 relatives of T1 D patients. Whilst the analysis of the total group of participants did not show a positive effect of the intervention, subsequent analysis has provided evidence that insulin can induce 'self-tolerance' during prediabetes. Those with higher antibodies at baseline showed a significant treatment effect with a lower rate of developing diabetes than placebo treated individuals: 6% vs 10% per year, with a delay of 4.5 years, but after cessation the risk of developing diabetes reverted to that of the placebo treated group [23].

A better method of inducing tolerance potentially is delivering the treatment through the mucosa [24]. The Intranasal Insulin Trial 11 (INIT 11) is currently underway in 11 sites in Australia and New Zealand. The principal investigators are Len Harrison and Peter Colman in Melbourne, and the study is sponsored by Melbourne Health, funded by JDRF and NHMRC through the Diabetes Vaccine Development Centre (DVDC). Risk for diabetes is based on family members found to have two positive antibodies on screening. These individuals are then staged to determine sufficient beta-cell reserve: the insulin response to an intravenous glucose load. Prior to the onset of diabetes the first phase to be lost is the initial response to intravenous glucose. Those in the unsuccessful DIPP study had little insulin reserve, so the time to intervene in the disease process was probably too late, to salvage beta-cell function [25].

Better tests of T-cell function, in particular the T-cell regulatory (TReg) function, are required to help determine the early stages of the autoimmune process during which time intervention may be most successful. Screening of large numbers of family members is required to detect those at risk.

Dietary trials to modify the environment

Changing the environment may prevent the autoimmune process. Babies at high risk of developing diabetes may be amenable to dietary intervention. Cow milk has been investigated in observational studies and early introduction may be a trigger for the autoimmune process.

In Finland babies at risk of diabetes were randomised to delay in cow milk supplementation. This led to a delay in development of diabetes related autoantibodies [26]. This has led to the Trial to Reduce IDDM in Genetically at Risk (TRIGR), a multinational study, including Australia. Recruitment of 2160 infants is complete (enrolment 2002 to 2007) and the intervention completed mid 2007, with follow-up planned over ten years. Infants have been screened by cord blood or heel prick for HLA susceptibility, protection genotype and those without the protective genotype randomised to delay in introduction of cow milk protein [27].

Another possible triggering antigen may be gluten. In Germany delay in introduction of gluten has been investigated in a randomised control study (BABYDIET) of 150 infants: prior to two months of age, after six months and after 12 months. However recent outcome data have not shown a delay in onset of autoimmune markers, with 12% of children developing diabetes related antibodies at age of three years, seven developing diabetes, including four in the late exposure group [28]. This was a pilot study and compliance with the intervention was poor at 30%. Three times more infants are needed to properly examine this hypothesis.

Haemopoietic stem cell transplantation as a treatment for diabetes

Another more controversial form of therapy is haemopoietic stem cell transplantation (HSCT) to treat new onset T1D. HSCT involves mobilisation of haemopoetic stem cells (CD34+ cells) from the bone marrow, ablation of the recipients immune system followed by reinfusion of the autologous stem cells. The aim is to destroy the auto-reactive islet immune T-cells and to reconstruct the immune system without autoimmunity. The strategy has been used successfully in other autoimmune diseases where it is generally reserved for patients that are resistant to other treatments. In 2007 a study was undertaken in Brazil where HSCT was used to treat T1D within six weeks of diagnosis [29]. Twenty-three patients, aged between 13 and 33 years, were enrolled in the study. The investigators reported an improvement in diabetes with 20 of the 23 becoming insulin free for varying periods of time (one to 52 months). Eight of the 20 had relapsed during the time of reported follow-up. The remainder were still being followed at the time of the last report [30]. There was also evidence of improvement in C-peptide, and reduced HbA1c. There was a reduction but not an elimination of islet autoantibodies which suggests that the autoimmune disease was suppressed but not eliminated. This study, although instructive, needs to be interpreted with caution [31]. The authors themselves readily admit this. First, the study was an early-phase, proof-of-concept study, and was not a randomised controlled trial. The well-known honeymoon period that can occur at the onset of T1D complicates the interpretation of the results. The follow-up is relatively limited and the long-term benefits and side effects are unknown. We do know that a third of patients had relapsed and returned to insulin within three years, which is a relatively small gain for such aggressive therapy. Whilst there were no deaths, HSCT is not benign therapy. Two patients developed pneumonia and others developed endocrine abnormalities, including nine of 17 males developing oligospermia. A final point that makes investigations of this nature difficult in T1D is that all the recipients were young, which increases the anxiety and implications around long-term complications

such as malignancy which may yet develop. Despite all these caveats the trial should be seen for what it is, the first of many attempts to cure T1D with cellular therapy rather spending a lifetime treating the consequences of islet destruction.

HSCT has been used as an adjunct to other treatments in T1D [32]. Not only is bone-marrow a source of haemopoetic progenitors, it is also a source of mesenchymal and endothelial stem cells. There is experimental evidence that these stem cells enhance immunomodulation and tolerance, as well as promote engraftment and enhance tissue repair. As a result HSCT is being evaluated in several autoimmune and degenerative conditions and has been proposed as adjunct therapy for other treatments in T1D. In fact there are more than 20 registered trials of HSCT in T1D. Several of these trials are using HSCT as a tolerising strategy to reduce or eliminate chronic immunosuppression in islet transplantation. In other studies HSCT is being trialled as a therapy to prevent progression of complications such as peripheral neuropathy. In all cases the study are exploratory proof-of-concept studies. While ongoing no study has conclusively demonstrated sustained benefit.

Clinical islet transplantation

Clinical human islet transplantation is a new and emerging therapy for a select group of patients with severe metabolic complications as a result of their diabetes. If successful it can provide near perfect blood glucose control without insulin injections and has brought forward the prospect of a cure for this debilitating chronic disease. Currently islet transplantation can be considered a novel therapy for a new indication and the objective benefits and potential risks remain to be fully established by clinical trials. At present the two main indications for islet transplantation are severe hypoglycaemia unawareness, refractory to conventional treatment and islet transplantation in conjunction with a kidney transplant. On the positive side, islet transplantation has been shown to abolish severe hypoglycaemic episodes and patients can expect to have an improved quality of life and reduced risk of end-organ complications. On the negative side, patients must take lifelong immunosuppression with all its short- and long-term complications. Despite these caveats, islet transplantation has created great interest amongst physicians as well as patient groups and several major clinical trials are ongoing. If nothing else, patient enthusiasm for these early trials has highlighted the lifelong burden and deficiencies of current insulin regimens.

Clinical islet transplantation is still a difficult procedure to perform. Islet isolation from a human pancreas requires a skilled laboratory team, a specialised GMP (good manufacturing practice) facility, and has been a limiting factor in the spread of the procedure beyond specialised institutions. Despite the fact that islet transplantation had been performed successfully in rodents, translation into the clinic has been difficult. Researchers in Edmonton were the first to develop islet transplantation into a reliable clinical therapy [33]. Their successes was based on the following principles:

- Selection of the appropriate group of patients for transplantation
- Using a non-toxic effective immunosuppressive regimen

- Isolating appropriate numbers of viable islets
- Transplanting sufficient numbers of islets to control blood glucose levels.

The basic premise of the Edmonton protocol has been that successful islet transplantation is dependent on the isolation and ongoing survival of the maximum number of islets possible. This has meant that most patients require two or more islet transplants to achieve insulin independence. Patients must not be overweight and therefore should be on a low-insulin dose compared to body weight. Once transplanted, islets face rejection and possibly autoimmune disease recurrence and hence islet recipients must take lifelong immunosuppression.

Added to this, pancreatic islets come from organ donors and there is a large mismatch between the number of people with type 1 diabetes and the number of organ donors. For instance in Australia there are 150,000 people suffering from type 1 diabetes and only 250 to 300 organ donors annually. Under the most optimistic of circumstances only 150 of these would be suitable or available for islet transplantation. For this reason definite sources of islet-cell replacement are being developed. These include pancreatic islets from pigs, so-called xenotransplantation, and stem cells. Although early phase I studies have been undertaken in humans, both these sources of islets remain in preclinical development.

Clearly human islet transplantation is not going to be an option for most people with diabetes. This and the current uncertainty has put pressure on transplant units to develop selection criteria that optimises the chances of a successful outcome whilst at the same time ensuring that the benefits of insulin independence are worth the long-term risks of life-long immunosuppression. The patients, who best satisfy these dual objectives, are those with severe hypoglycaemic unawareness. These patients no longer have warning signals of hypoglycaemia, and low blood sugar levels are not detected until they reach dangerously low levels. These patients require constant and intensive medical supervision. If it persists, it can lead to significant cognitive impairment and, in severe cases, can be fatal [34]. Not surprisingly patients who suffer from this problem have poor metabolic control with wide and sudden swings between hypo- and hyperglycaemia. In these circumstances successful islet transplantation will normalise blood glucose levels and prevent hypoglycaemic episodes [35].

Patients who have successfully achieved insulin independence following islet transplantation certainly have a life-changing experience. After many years of difficulty controlling diabetes with insulin, they find themselves insulin independent with perfect control and normal HbA1C. In our experience patients who were once housebound are able to return to active work. There is an obvious improvement in the patient's mood and quality of life. Even patients, who are not insulin independent but have documented evidence of islet graft function, achieve marked improvements in glycaemic control. Physiologic background basal insulin secretion appears to be sufficient to eliminate severe hypoglycaemia and often this is sufficient to dramatically improve the patient's quality of life. Furthermore, there is evidence that C-peptide secretion provides some protection against cardiac complications even in patients that still require exogenous insulin therapy. A study of kidney transplant

recipients with type 1 diabetes who subsequently received an islet transplant, found that those that were C-peptide positive had improved cardiac outcomes compared to those with graft failure [36].

Thus far it would seem that the main negatives for patients are the side effects from immunosuppression. Despite this, there have been surprisingly few opportunistic infections and no deaths. Most immunosuppressive regimens include Tacrolimus and Sirolimus. Most transplanted patients suffer nagging complications such as mouth ulcers, ankle oedema and headaches. These are common side effects of Sirolimus and are often self-limiting although they have been serious enough to lead to withdrawal from the study in some cases. Many patients also need to commence lipid-lowering therapy for hypercholesterolaemia. More worrying are the side effects from Tacrolimus. In particular nephrotoxicity is a concern and it may be exacerbated by concomitant Sirolimus therapy. Many recipients have had a significant reduction in renal function, some even commencing dialysis therapy as a result. Many patients also need to start or increase anti-hypertensive medication [37].

For the islet transplantation to be really successful the majority of patient need to achieve long-term islet graft function. At the moment it is too early to determine whether this can be achieved easily. Many of the initial cohort of patients from Edmonton are back on insulin, although most have retained some graft function with good glycaemic control. This gradual loss of function has proven to be a difficult problem for investigators as it is not clear whether it is due to chronic rejection, disease recurrence or 'islet exhaustion'. This problem is exacerbated by the fact that there is no easy way to monitor graft function. Monitoring graft function by tissue biopsy has been a major reason for the improved survival of renal, liver and cardiac transplantation and the lack of a similar test for islet grafts is proving to be a major hurdle in determining the natural history of islet transplantation.

In 2002 the National Pancreas Transplant Unit at Westmead Hospital began a trial of pancreatic islet transplantation. Initially we are selecting patients with type 1 diabetes who suffer from severe hypoglycaemic unawareness that has failed to respond to intensive management by an endocrinologist. Of the six patients transplanted, three were able to cease insulin injections [38]. The good news for these patients is that they no longer suffer life-threatening 'hypos' and this has resulted in a substantial improvement in their quality of life. However, the immunosuppressive drugs cause significant side effects, and our view remains that this treatment should be reserved for those patients with the most severe metabolic complications from their diabetes. These promising findings led to the Australian Government funding a second multi-centre Australian trial which was led by the Westmead Hospital Centre. In this second trial 16 patients have been transplanted with measurable graft function in all patients bar one. Eight patients were insulin independent and all patients had abolition of their hypoglycaemic episodes. A new immunosuppressive regimen was evaluated which was beneficial at preventing rejection and better tolerated. These patients were compared with a similar cohort of patients who had severe hypoglycaemia and who were managed with intensive medical follow-up and insulin pumps. Those patients undergoing islet transplantation had improved blood glucose control and abolition of hypoglycaemia whereas those patients on insulin pumps had no reduction of hypoglycaemia.

These are early days in the development of this therapy and much needs to be done before this treatment can be offered to a wider group of patients. These trials are an important proof of principle and bring us a step closer to finding a cure for type 1 diabetes. We can help some patients but the treatment comes at a price. Areas requiring improvement include a need for more reliable islet isolation procedures, better and safer anti-rejection drugs and long-term studies to show that this treatment provides real benefits for patients. In addition to solving these technical problems, we need to develop alternative sources of donor islet tissue. Some researchers are developing stem cells. Our unit, in collaboration with others, is using gene therapy to develop pig islet cells for transplantation into humans. Over the past decade there has been substantial progress in cell-based therapy to treat type 1 diabetes. The current trials of human pancreatic islet transplantation have demonstrated that this type of approach can abolish the need for exogenous insulin and provide real benefits to patients. In addition the ongoing development of an indefinite source of islets provides hope that cell-based therapies could become the norm for type 1 diabetes rather than a life-changing therapy for a few.

Prevention of long-term complications

Both type 1 and type 2 diabetes lead to long-term complications of vascular disease affecting the eye, kidney, nerves and larger blood vessels of the heart and limbs. The biological contributors are similar but in type 2 diabetes there is possibly a greater impact of accompanying high blood pressure and abnormal lipids. For those with type 1 diabetes the features of the metabolic syndrome increase their risk of complications substantially, threefold in a recent study [39]. We are understanding more about the relative contributions to vascular disease and also the contribution of the autonomic nervous system which innervates the vasculature [40, 41].

Statins are of proven benefit to reduce vascular outcomes in line with the benefit demonstrated in those without diabetes [42], but its is unclear whether it is appropriate to initiate in all young people, especially when not considered safe during pregnancy. The Adolescent Type 1 Diabetes Cardiorenal Intervention Trial (AdDIT) will help determine whether adolescents at high risk to progression will benefit from Angiotensin converting enzyme inhibitors or statins [43]. This is a multinational study in the UK, Canada and Australia currently recruiting 500 at-risk adolescents as defined by the urinary concentration of the protein, albumin.

As well as statin therapy to reduce cholesterol, recent studies have examined triglyceride-lowering treatment in diabetes [44, 45]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial was a large randomised trial of fenofibrate to prevent vascular complications, in particular cardiovascular disease in T2DM patients. A total of 9795 T2DM subjects aged between 50 and 75 years were recruited from 63 centres in Australia, New Zealand and Finland between February 1998 and November 2000 and study closeout was early 2005. Subjects were randomised to receive either 200 mg co-micronised fenofibrate or matching placebo for an average of five years follow-up. The primary endpoint of non-fatal MI + CHD death was not significantly reduced by fenofibrate (HR =

0.89: 0.75 –1.05; p = 0.16), however, most other macrovascular endpoints were significantly reduced by treatment, including hospitalisation of ACS, non-fatal MI alone, coronary or carotid revascularisation [46]. Fenofibrate also profoundly reduced microvascular events, including eye complications (by 37%), albuminuria (18%) and loss of renal function (3.7 kidney-years saved), together with amputations (36%) [47–49].

Work is now underway exploring the blood biomarkers and genetic markers associated with both the cardiovascular and microvascular complications seen in the FIELD study, as well as with any predictors of benefit of treatment, supported by National Health and Medical Research Council (NHMRC) of Australia grants and the main study sponsor, Abbott, who market fenofibrate globally. Basic science (cellular and animal) research work is being conducted in parallel, in order to understand how fenofibrate might protect so powerfully against microvascular disease in type 2 diabetes. A better knowledge of how fenofibrate might mediate these large benefits may offer hope for even more potent treatments against diabetic complications in future.

Design of clinical trials for diabetes treatment and complications

Evaluations of potential new therapies for improving the treatment of type 1 or type 2 diabetes mellitus use classical methods of clinical trials design. This means that after short-term exposure trials in normal subjects (phase 1) and then patients (Phase 2) to establish the pharmacology of a new drug and then the activity of such an intervention and immediate safety of use, larger randomised trials, usually with placebo control are conducted in a parallel-group schema (Figure 3). Follow-up is over some months (Phase 2) in order to explore acceptance and compliance with planned dosing schedules, and more reliably establish the magnitude of benefits on glycaemic control, blood pressure, or similar. Thereafter, over some years usually (Phase 3), clinical trials designed to yield harder clinical endpoints such as changes in renal function, progression of retinopathy, or cardiovascular event reductions are conducted, to determine whether the sought benefits of the treatment are in fact borne out.

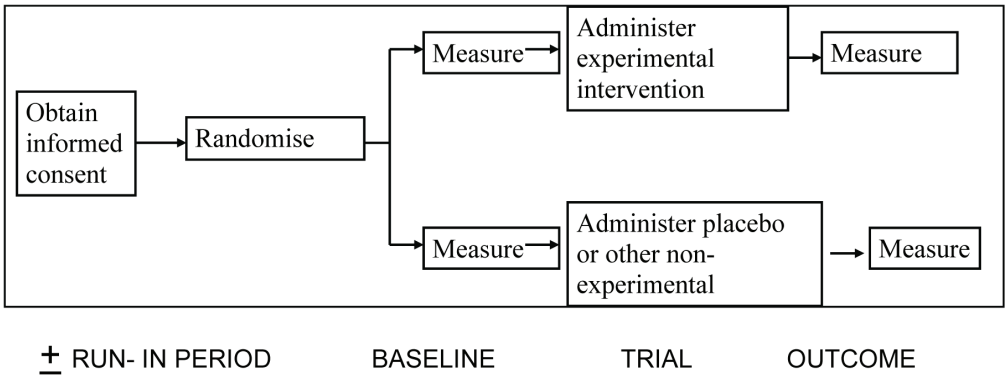

Figure 3. Parallel group randomised trial design

Table 1. Traditional clinical trial phases and their purposes.

Phase 1	Determines the action, distribution, metabolism and toxicity of different dosages of a drug in humans, healthy volunteers or patients with the disease being studied for whom alternative treatment options may have been exhausted. Only a few patients participate. Alternatively, it may be a small pilot study to establish safety and calibration of a device.
Phase 2	Determines the levels of efficacy and safety on a broader group of patients with the condition of interest.
Phase 3	Compares the intervention with the current standard treatment in a large number of patients (often hundreds or thousands) who suffer from the condition of interest. Patients are randomly allocated to either the new intervention or the standard treatment. A phase 3 trial usually determines whether the new intervention is sufficiently superior to the current or standard treatment to replace it or be a viable alternative. A phase 3 trial may also reveal further side effects of the intervention or identify subgroups for whom the intervention is particularly beneficial.
Phase 4	Long-term surveillance after drug approval may detect rare adverse effects or those that are apparent only over a long period. Unexpected benefits may also be identified, requiring further phase 3 trials for confirmation.

At the stage of a phase 3 trial, the results of which are of most direct potential applicability to clinician practice, most of the more common safety issues associated with an investigational treatment will have been identified. However, the possibility of previously unknown rarer side effects being recognised with longer exposure time, larger numbers or both remains, and it has not been uncommon for marketed products to be later withdrawn or more restricted in approved uses as more real-life experience becomes available, making these phase 3 trials, and after-market surveillance programs (phase 4) invaluable in identifying real advances for patient treatment policies [50, 51].

When large phase 3 trials of new drug therapy are designed, ideally they are set on a background of standard care, and the new therapy, or its matching placebo, are added to existing care. This often offers the advantage of more closely mimicking how the drug might be used in real life, and does not deny trial participants ongoing use of current best standard care. Occasionally, the new treatment might be proposed to replace an existing treatment, in which case a comparative trial of new versus established treatment may be conducted (in this instance a double-placebo or so-called double-dummy design would be needed in order to preserve blinding, if possible) [52].

Strong randomisation is pivotal to a good trial result, and ever more sophisticated methods to stratify a randomisation by important prognostic patient characteristics is making interpretation of trials results simpler, based on crude numbers of events. Randomisation ensures that the key variables influencing outcomes of interest are balanced between the group allocated to receive the active intervention and the control treatment. Processes used to randomise must be secure and unpredictable in advance, so as to ensure their integrity. This is often achieved by using a central web-based system [52].

Blinding or masking of treatments has become an important hallmark of good trials, when practicable. Because both trial participants or patients and their doctors may have in-built prejudices one way or another about the potential advantages of a new therapy under test, this can lead to inadvertent biases in assessing side effects, complications, outcomes and perceived links to study treatments. For that reason, great lengths are undertaken in order to try to avoid such biases from arising [52]. Part of this process is to mask study treatment with a placebo wherever possible, with matching colour, appearance, consistency, taste and so on, in order to avoid any party knowing which treatment is being received during the study. Of course, if an emergency clinical situation arises, then the opportunity to unmask immediately must be provided within the trial organisation, as the patients' best interests must remain foremost.

For the same reasons, outcome adjudication is also often set out to be performed at arms length from the trial apparatus, by a group of people with no other direct involvement in a study, so that decisions about the nature of outcome events or untoward complications can be judged impartially. Centres such as the NHMRC Clinical Trials Centre have decades of experience in helping investigators set up clinical trials with these considerations built in to the trial protocol and operational structures.

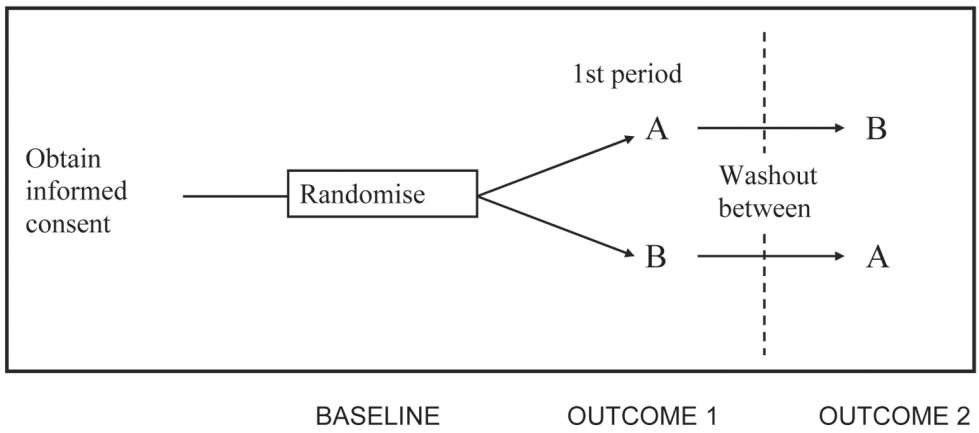

Figure 4. A two-period cross-over trial design.

When new devices are being tested, such as insulin pumps and continuous glucose monitors, blinding of the intervention is usually not possible. Some attempts can be made to reduce differences between groups, for example, whereby all subjects have devices installed, but not all are activated (early activation could be randomised), but generally in these circumstances, investigators rely on impartial assessments of outcomes being possible. This could include instances of hospitalisation or medical intervention being required for documented severe hypoglycaemia, in this example. This sort of trial might lend itself to a cross-over design whereby each subject experiences two phases of treatment with and without device support, each for sufficient time periods that reliable estimates

of any advantages emerge (Figure 4). After pancreatic islet transplantation, insulin dosing requirements for glucose homeostasis may be the primary outcome studied.

There are many opportunities to enhance the design of phase 3 trials in practice. One way is to consider a run-in period prior to randomisation of patients into a trial. Most often, such a run-in period would be 'single-blind' meaning that only the patient would be unaware of what treatment was being offered to them, with study staff knowing that the patient was receiving (most commonly) a placebo at that point [52]. The potential advantages of a run-in period include (i) that the patient has already been entered into the trial routine; (ii) time is allowed to establish that subject characteristics meet all the inclusion criteria, including any special pathology results that are not immediately available; (iii) subjects have an opportunity to discuss trial participation with spouses and their usual doctors and to reflect on whether they wish to make what is often a long-term commitment to a tight schedule of follow-up visits (if they change their mind at this stage they have not upset the randomisation yet); (iv) subjects who are not likely to be good compliers can be identified in advance and excluded from randomisation if desired (though generalisability of final results may be affected if many subjects are excluded in this way); and (v) less commonly, the use of an active rather than placebo run-in period can characterise subjects in terms of their responsiveness to treatment, allowing later analyses identifying whether big-responders gain more benefit from treatment than small responders in some biomarker or other putative measure of treatment mechanism.

Another useful strategy might be to consider a washout period at the end of the study. Where a drug treatment may have exerted a potentially adverse effect in some way, it becomes important to know whether such an effect is reversible when the treatment is withdrawn. So, for example, a number of drugs developed for use in type 2 diabetes, including pan-PPAR agents, have shown renal injury in terms of increased proteinuria and loss of glomerular filtration rate (GFR) sufficient to prevent the drug development proceeding. In some cases, the renal injury has been permanent for some patients [53]. In the FIELD study, use of fenofibrate rapidly increased plasma creatinine levels by around 12%, a signal of some concern clinically, as this could potentially represent a small drop in GFR. Furthermore, the creatinine rise remained present for the duration of the five-year average treatment period, raising legitimate concerns about whether this could adversely affect cardiovascular risk. As it turned out, a washout study in over 600 patients showed that the increase in creatinine was fully reversible even after five years, and also unmasked a more favourable final GFR among subjects who had received fenofibrate rather than placebo during the trial [48].

Results of FIELD analysed by the extent of changes in creatinine during a six weeks active run-in period immediately prior to randomisation, among all 9795 patients further showed that cardiovascular events were reduced most by study treatment among subjects with a larger rise in creatinine (perhaps such an increase is a marker of greater drug bioactivity). Preservation of renal filtration function was no different according to the extent of the creatinine rise before randomisation during active run-in treatment [48].

Pre-specifying a number of secondary and tertiary outcomes in advance is important for how such results are perceived by the general community. Because 'data-dredging' can so easily lead to spurious findings, outcomes that have been planned out in detail before trial results are known carry much more weight both with journals and with peers. A significant time is required to set these out in a protocol ahead of trial commencement [52].

Uniquely in the disease area of type 2 diabetes, there are greater hurdles for new drug development and registration than in other disease domains. For products planning to be accessible in the US, the recently updated Food and Drug Administration (FDA) regulations mean that drugs intended for use in type 2 diabetes must prove their safety with respect to cardiovascular events. While drugs can reach market prior to the results of such trials being known, such trials must be planned or underway to ensure that a hazard from treatment exceeding a 30% increase in CVD risk (HR >1.3) can be ruled out at the end of such a study [53]. This has arisen after several drugs reaching routine clinical use in the market have been found to potentially give rise to CVD harm, most notably rosiglitazone, though the correct interpretation of the data from a number of relevant trials remains contested [54].

Conclusions

We are understanding more about the development of diabetes and its complications. Much exciting work has already been done but many critical studies are underway. We await the outcomes with hope that the increase in type 1 and type 2 diabetes may be reduced and diabetes complications minimised. Randomised clinical trials remain the best way to identify such advances, and must be well planned with clearly defined prespecified primary, secondary and tertiary outcomes to ensure the maximum scientific value is achieved.

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