



REVIEW

Prevention versus intervention of type 1 diabetes

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Abstract Type 1 diabetes (T1D) is a cell-mediated autoimmune disease. New cases of T1D are on the increase and exogenous insulin therapy is the only intervention regularly initiated for T1D patients. Though tremendous strides have been made in prediction of T1D, prevention and intervention strategies have not experienced the same success. In this review, we will discuss some possible reasons why new intervention therapies for T1D have not been implemented into the mainstream treatment regimen for T1D patients. We will also discuss potential caveats for why prevention and intervention trials in T1D may not have experienced the same success as prediction trials.

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1. Introduction

Type 1 diabetes mellitus (T1D), which accounts for approximately 5–10% of diabetes, is the result of cell-mediated beta (β)-cell dysfunction and destruction, leading to severe insulin deficiency [1–4]. Tremendous strides have been made in unraveling the pathogenesis of T1D and in

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predicting clinical disease development. Prediction studies for T1D have shown that the greater the number of islet autoantibodies present, the greater the risk of progression to clinical disease [5–10]. However, a caveat to the detection of the increasing numbers of islet autoantibodies is that the islet autoantibodies are not believed to be the main effectors in the pathogenesis of T1D. Islet reactive T cells are thought to be the primary effectors of β -cell destruction [4,11,12].

Disease models for development of T1D suggest that islet autoimmunity exists in many subjects long before development of clinical manifestations [13,14]. It is during this “pre-clinical” stage that islet autoantibodies appear and β -cell function diminishes. The increasing numbers of islet autoantibodies may signify later stages of autoimmune destruction and consequently underlie their utility in prediction of T1D. In other words, the lack of efficacy of prevention trials may be highly correlated with the timing of implementation with respect to degree or stage of islet autoimmune activity.

2. Prevention trials

Prevention of clinical disease is defined as a procedure implemented before clinical manifestation. Prevention trials are generally classified into 3 categories: primary, secondary, and tertiary prevention [5]. Primary prevention trials in T1D are initiated prior to development or evidence of islet autoimmunity, generally in infants with perceived increased genetic risk. Secondary prevention trials are conducted in children and adults after development of islet autoimmunity has been confirmed. Tertiary prevention occurs after clinical disease has been detected. Tertiary trials are often referred to as intervention trials instead of prevention trials. The different types of prevention trials are summarized in Fig. 1.

The ultimate goals of primary and secondary prevention trials are to arrest or delay clinical disease, whereas the goal of tertiary prevention/intervention is to disrupt the disease process with resultant preservation of β -cell function or stimulation of β -cell recovery. Globally in disease prevention, the greater emphasis should be on primary and secondary trials.

Recruitment of subjects for primary trials is often harder compared to secondary trials due to the lack of autoimmune markers. Once markers of autoimmune destruction are present or development of clinical disease (tertiary) has occurred, identification of subjects for inclusion into studies is simplified. With regard to T1D, the most prevalent biomarkers utilized in assessing risk for disease development are islet autoantibodies. Unfortunately, the presence of islet autoantibodies, particularly multiple islet autoantibodies, signifies a later state of disease development. This may be one critical factor as to why prevention and intervention trials have not experienced the same success as prediction studies. Therefore, a better predictor of early stage islet autoimmunity may be to identify the T cell effectors of β -cell damage. Prevention trials may benefit from being initiated earlier in the autoimmune pathogenesis by implementing assays to detect cellular effectors at screening instead of relying on islet autoantibodies as discussed above.

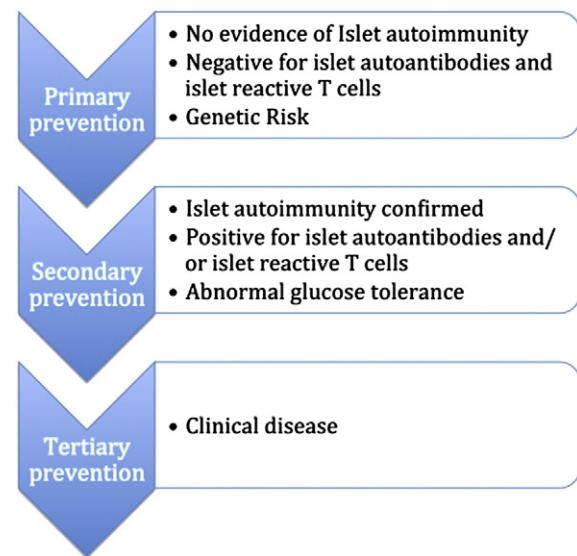


Figure 1 Definitions of primary, secondary and tertiary prevention trials. Prevention trials are categorized as primary, secondary or tertiary (intervention) trials. Primary prevention trials are conducted prior to evidence of disease pathogenesis. In the context of T1D, this generally means the presence of islet autoantibodies. Secondary prevention trials are conducted with subjects that have developed biomarkers of potential disease progression, i.e. the development of islet autoantibodies. Tertiary prevention trials, or intervention trials, are conducted with subjects who have developed clinical disease.

3. Primary prevention trials for T1D

Dietary (nutritional trials) are frequently primary prevention trials. Since subjects enrolled in primary trials do not have biomarkers of autoimmune destruction, suggesting that their risk of clinical T1D is low, the prevention strategy must be benign as most of the subjects would not be expected to progress to clinical disease. Examples of primary prevention trials include:

1. The Trial to Reduce Incidence of Diabetes in a Genetically at Risk study (TRIGR). The TRIGR study is designed to determine if the frequency of T1D can be reduced by preventing exposure to cow milk proteins in infants [15]. This trial has recruited subjects and preliminary results are expected in the fall of 2013. Results of a pilot trial of similar design were reported in 2010 [16].
2. Finnish Dietary Intervention Trial for the Prevention of T1D (FINDIA). The FINDIA study focused on the removal of bovine insulin exposure, from cow's milk formula, in newborns suggesting that bovine insulin may be a diabetes trigger [17]. The results of this pilot study demonstrated that weaning infants to an insulin-free cow's milk formula reduced the cumulative incidence of autoantibodies by age 3 years in children at risk for T1D.
3. Primary Prevention of Type 1 Diabetes in Relatives at Increased Genetic Risk (BABYDIET) study. The BABYDIET study, examined whether delayed exposure to gluten in newborns reduced the risk of diabetes [18]. The results of

this trial showed that delaying gluten exposure until the age of 12 months is safe, but does not substantially reduce the risk of developing islet autoimmunity in at risk children.

4. Primary intervention and oral insulin for prevention of type 1 diabetes in infants at high genetic risk to develop diabetes (Pre-Point). Pre-Point will recruit at risk children and infants for administration of oral insulin in an attempt to stop the development of islet autoimmunity, detected by islet autoantibodies [19]. This is an on-going study, which is currently in the process of recruitment.

Even with negative results, completion of primary prevention trials helps advance the field of diabetes prevention through lessons learned about potential triggers for T1D, recruitment of potential subjects, and identification of at risk subjects. A full listing of on-going and completed trials can be reviewed on the website for Clinical trials sponsored by the National Institutes of Health (NIH) at www.clinicaltrials.gov or www.diabetestrialnet.org.

4. Secondary prevention trials for T1D

Secondary prevention trials occur in subjects who have already developed some marker of autoimmunity or abnormal glucose tolerance, but not clinical T1D. These studies have the advantage of easier recruitment as markers can be used to screen large numbers of potential subjects. Treatment regimens in secondary prevention trials must be deemed of higher value than the perceived risk of developing clinical disease. A few examples of secondary T1D preventions are:

1. The European Nicotinamide Diabetes Intervention Trial (ENDIT). This was a randomized placebo-controlled trial to determine if nicotinamide could prevent or delay the progression of T1D in at risk subjects. Unfortunately, nicotinamide was shown not to prevent diabetes but effects on the immune system were noted [20,21].
2. The Diabetes Prevention Trial Type 1 (DPT-1). In this trial, subjects received either parenteral or oral insulin [22,23]. This trial also failed to demonstrate a benefit of either oral or parenteral insulin therapy in preventing T1D. However, a post hoc subgroup analysis indicated a potential delay in T1D in those subjects with the highest insulin autoantibodies [23]. A trial is currently being conducted by TrialNet to test this important finding.
3. Anti-CD3 monoclonal antibody (teplizumab) for prevention of diabetes in relatives at risk for type 1 diabetes. This study is being conducted by TrialNet and is currently recruiting. This study will test whether teplizumab is able to stop or slow the autoimmune destruction of the β -cells [24].

Unfortunately, to date none of the secondary prevention trials in T1D have been able to prevent the development of clinical T1D in at risk subjects. The question of timing of interventions with regard to the progression of T1D is still of major concern. Was the cause of the failure of the secondary prevention therapy the dose of the intervention? Or, had the β -cell autoimmune destruction progressed too far? Or, was the trial aimed at the wrong targets? Often times the intervention may have worked in animal models and not

carry over into human subjects. Or, studies may have demonstrated success in improving β -cell function in smaller study populations but when implemented in a larger study, the beneficial effects are no longer realized.

As discussed with primary prevention trials, even though results of the studies are negative, advantages to at risk subjects who participate in secondary prevention studies are often realized. Subjects in prevention studies are followed very carefully with regard to developing hyperglycemia and thus are often diagnosed at an early stage of clinical disease. In fact, over 50% of new clinical T1D cases in subjects entered in primary and/or secondary prevention studies are diagnosed based on hyperglycemia without other symptoms. These subjects have much higher levels of preserved C-peptide in contrast to subjects diagnosed upon hospitalization [25]. The benefit of preserved C-peptide may result in fewer complications in future years for these subjects.

5. Intervention of T1D or tertiary prevention

Over the years, numerous tertiary prevention or intervention trials for T1D patients have been implemented. Initial tertiary prevention or intervention trials were directed at abrogating the autoimmune β -cell destruction in new onset T1D patients and focused on systemic immunosuppression using drugs such as cyclosporine [26–29]. These studies demonstrated success in suppressing the autoimmune destruction of the islets but with a high price paid in the form of long-term side effects for patients due to systemic immune suppression and nephrotoxicity [26,27]. Another caveat observed, was the transient effect of the drugs on the autoimmune response. Most of the therapies were effective only while the patients were on the therapy. Because systemic immune suppression carries long-term risks of serious complications, newer intervention therapies have focused on antigen-specific, antigen non-specific (cell or immune system targets), and combinational therapies instead of systemic immunosuppression.

Examples of non-antigen specific tertiary prevention trials for T1D are:

1. CTLA-4 antibody (abatacept) in recent onset diabetes. This trial was conducted to determine if CTLA-4 (a co-stimulatory molecule on immune cells) would preserve insulin production by β -cells. Initial results from this trial suggested that abatacept may be able to delay insulin loss in the treated patients [30]. This drug is currently undergoing further study.
2. Tumor necrosis factor- α (TNF- α) antagonist (etanercept) treatment in children with new-onset type 1 diabetes [31]. This was a randomized, placebo-controlled double-blind pilot study which demonstrated that the etanercept treated group had an increase in their β -cell function compared to the control group. However, an increased risk of serious infections potentially associated with the use of this drug were documented.
3. Anti-CD3 is an antibody targeting a protein on the surface of T cells with the focus on deleting the T cells responsible for the autoimmune destruction and repopulating the immune system with non-destructive or even regulatory T cells [32–34]. Early studies using anti-CD3 in new onset

T1D patients demonstrated promising results in preserving β -cell function but the acceptable dose window balancing efficacy and toxicity appear to be quite narrow. Two recent large Phase 3 trials failed to meet primary endpoints [35,36]. Subsequent trials are currently under-way to clarify these issues.

4. Anti-CD20 (rituximab). Anti-CD20 down-regulates B lymphocytes and has been used in cancer treatments and other autoimmune diseases [37–40]. The use of rituximab in intervention trials in newly diagnosed T1D patients demonstrated some efficacy although the results appear to be short lived [39,40].
5. Autologous Nonmyeloablative Hematopoietic Stem Cell transplantation (HSCT). This is a protocol whereby hematopoietic stem cells are collected from the peripheral blood of patients and cryopreserved for re-injection back into the patient after the patient undergoes immune ablation with cyclophosphamide and anti-thymocyte globulin [41–43]. The results of these trials demonstrated that the majority of T1D patients who participated in this protocol achieved long-term (mean 29.8 months) insulin independence with good glycemic control [42]. Fig. 2 demonstrates the mean $\pm$ SE of the area under the curve (AUC) of C-peptide responses of 20 patients who became insulin independent after HSCT. However, even though long duration increases in C-peptide and insulin independence were achieved, the patients experienced severe acute and long-term side effects due to the treatments [43].

An example of an antigen specific tertiary prevention trial for T1D is:

1. Glutamic acid decarboxylase (GAD)–Alum vaccination in new onset T1D patients. This was a one-year treatment of

GAD alone or in combination with aluminum hydroxide (Alum). This trial did not show any evidence of preserving insulin production or controlling the immune response [44]. A larger Phase 3 study also demonstrated similar negative results [45].

Intervention therapies would ideally target islet autoimmunity, as desired in prevention, but also preserve or even increase residual endogenous insulin secretion by replacing, regenerating and protecting the existing β -cell mass. The interest in conducting intervention trials may lie even beyond the potential for insulin independence, namely the ease of identifying study subjects and the possibility of diminishing the course of the disease resulting in a milder progression with less complications. Even if full recovery is not possible, patients may experience benefit from participation in the trial. Unfortunately, therapies that diminish or abrogate the autoimmune destruction of the β -cells have the major disadvantage that many therapies require subjects to endure intravenous infusions, hospital stays, illnesses (cytokine syndrome, infections), or long-term side effects (infertility, cancer risk). The most effective intervention to date for achieving long-term insulin independence for T1D patients has been HSCT [41,42]. The HSCT trial demonstrated that C-peptide could be increased several fold and patients could become insulin independent long-term, rather than just the slow decline seen in other trials. It is currently undetermined if the success of AHST treatment on increasing C-peptide responses resides in the transfer of stem cells or the immune suppression (cyclophosphamide and anti-thymocyte globulin). Nonetheless, the price of insulin dependence was accompanied by severely toxic side effects [43]. Similar efficacy with safe, easy to administer treatments would be the goal for intervention trials and a major advance. Combination

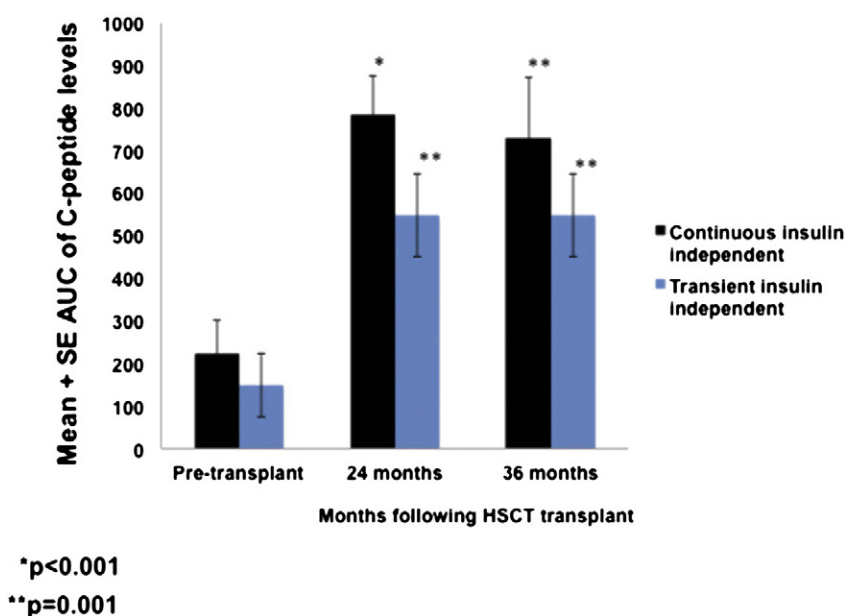


Figure 2 The mean area under the curve (AUC) $\pm$ standard error (SE) of C-peptide responses are shown for results adapted from a clinical trial where 20 patients receiving Hematopoietic Stem Cell transplantation following immune system ablation were followed for a median range of 30 months [37,38]. Twelve of the 20 patients became continuously insulin independent (black bars) whereas, 8/20 became transiently insulin independent (blue bars). Mean AUC was observed to be significantly ($p \geq 0.001$) increased in all patients.

therapies might prove to be the most useful approach to disease modification and intervention. Combinations that modulate both antigen specific and systemic immune suppression might lead to long-ranging benefits and possible insulin independence.

One potential disadvantage of intervention therapies is that after the onset of clinical disease, endogenous β -cell function is markedly compromised. Historically, it was believed that prior to development of clinical T1D, approximately 60–90% of β -cells were destroyed. Utilizing the Network for Pancreatic organ donors with Diabetes (nPOD), several studies have demonstrated persistent β -cell function in even long-standing T1D patients [46–48]. The nPOD results suggested that future therapies may eventually accomplish insulin independency through re-establishment of insulin production from existing β -cells forgoing the need for exogenous insulin replacement, if the autoimmune destruction can be arrested. Another obstacle to intervention trials in T1D, is that conventional treatments for T1D continue to improve making it necessary that intervention strategies be benign and more efficacious. Insulin pens, insulin pumps, new insulin analogs and improvements in glucose monitoring now offer patients greater ability to maintain improved blood glucose levels, and increased patient quality of life. Personalized artificial pancreases, which automatically regulate blood glucose levels, are being tested with encouraging results [49].

As we have discussed above, efficacy of therapies may depend on the stage of the autoimmune response. Implementation of intervention early in the disease process may be needed to suppress the antigen and epitope spreading associated with T1D pathogenesis [50,51]. Antigen specific therapy in T1D has a number of potential limitations. First, islet antigens important to the T1D disease process have been identified through the use of islet autoantibodies. The pathogenic effectors of T1D, islet reactive T cells, and antibodies do not always recognize the same antigens. Thus, the use of autoantibodies to identify the islet proteins used for antigen specific therapies may not identify the most important target antigens. In fact, many of the islet antigens important to T1D disease pathogenesis may remain as yet unidentified. Moreover, even if important islet antigens are identified, therapies altering immune responses to these antigens may not be successful in affecting the T1D disease progression if employed, too late in the pathogenesis of T1D. Therefore, two important issues are associated with the use of antigen-specific therapies, namely which antigens to employ (are the antigens chosen the correct ones) and how should the antigens be administered.

Autoimmune diseases, such as T1D, and cancer are linked by overlapping immune recognition of self proteins [52]. In autoimmune diseases tolerance to self antigens is lost and specific cells are destroyed by the immune system. In T1D these are the pancreatic beta cells. Whereas, in cancer, the recognition of abnormal or non-self antigens is tolerated; consequently cancers are not eliminated by the immune system. Abnormalities of immune regulation may underlie both autoimmune diseases and cancer with deficiencies in immune regulation being commonly observed in autoimmune diseases and exaggerated regulation being characteristic of some cancers. The absence of both autoimmune diseases and cancer requires a delicate and dynamic balance of just the right amount of regulatory mechanisms [53,54]. And herein

lies a caveat to immunomodulatory treatment of autoimmune disease. It may be very difficult for immune therapy to restore just enough regulation to suppress autoimmune mechanisms long term but at the same time not result in too much regulation and an increased risk of cancer and/or infections. It is likely that only immunomodulatory therapy that is disease specific could accomplish this delicate balance, especially long term, which may be decades for an autoimmune disease like T1D, which commonly presents in childhood. Currently, antigen based therapies have the greatest likelihood of being disease specific, but efficacy of antigen based therapies of autoimmune diseases, including T1D, remains elusive. When efficacious immunomodulatory therapies for T1D are identified, the possible long term increased risk of cancer will have to be considered when compared to the risk benefit ratio of conventional (at the time) insulin replacement therapy.

Down-regulation of islet reactive T cells has been difficult without systemic immune system down-regulation. Studies showing the use of myeloablation have demonstrated marked increased C-peptide and insulin independence suggesting that long-term improvement insulin responses may be possible but not without drastic re-modeling of the immune system. These studies bring up major ethical issues that need to be addressed for the future of T1D trials and treatments. Namely, what are the ethical boundaries that should be considered in conducting clinical trials with therapies that will never be available outside of research? Is it ethical to conduct or continue to finance clinical trials where the only likely outcome is to further knowledge on the pathogenesis of the T1D disease process? In other words, should we continue to conduct clinical trials using treatments, which have a high probability of never being embraced by the medical community, the T1D patient population and their families?

Other important ethical considerations are what price will or should patients pay with respect to re-educating or remodeling their immune systems in an effort to delay the onset of or cure clinical disease? What are the acceptable limits for long or short-term side effects necessary to reach insulin independence? What can researchers or physicians ethically ask patients to consider in an effort to forward our knowledge of a potential cure when insulin replacement treatment options are becoming so advanced and efficacious?

Finally, when considering therapies or prevention trials, researchers and physicians should keep in mind that not all ages of individuals will likely benefit from the same treatment or intervention. As a person ages, so does the immune system opening up the possibilities of differing immune system players or autoimmune targets in the development of T1D. Thus, therapies may need to be tailored to specific age groups of patients taking into account the stage of islet autoimmunity present.

6. Conclusion

Prevention, prediction, and intervention of T1D have come a long way in recent years. Fig. 3 summarizes the different types of prevention and intervention trials. We can now accurately predict family members at risk of T1D and we are well on our way to establishing screening protocols for use with the general public. With the establishment of the Type

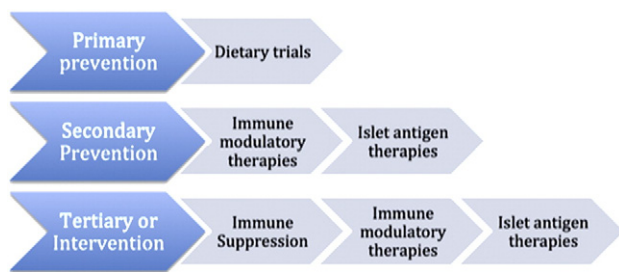


Figure 3 Examples of primary, secondary and tertiary prevention trials. Dietary or nutritional trials are considered primary prevention trials. Secondary prevention trials include therapies that are designed to attenuate the disease pathogenesis. In the contact of T1D, this involves attenuation of the islet autoimmune response. These therapies can be in the form of immune regulatory therapies or islet antigen specific therapies. Tertiary intervention trials are conducted after the development of clinical disease so can also include systemic immune suppression.

1 Diabetes TrialNet Clinical trials group (<http://www.diabetestrialnet.org>) and the Immune Tolerance Network (<http://www.immunetolerance.org>) to aid in developing and implementing clinical trials for prevention, and intervention, future trials may yet identify some answers to many difficult questions. However, the future still holds a number of ethical questions that need to be considered for future clinical trial research with the advances in therapies for diabetes patients. Unfortunately, even with the knowledge that preservation of β -cell function is imperative to successful treatment of T1D, none of the therapies to date have been embraced by physicians, families of T1D patients or T1D patients themselves. Unless a therapy can be demonstrated more convenient, less painful, and devoid of long-term side effects it is not likely that future patients will choose the intervention over the choice of available insulin pump therapies currently available. Prevention and intervention of T1D have made enormous advances in the past decade but the field of diabetes research and clinical trials is quickly approaching the point where new therapies and interventions must be capable of competing with the available insulin therapies in order to have a potential of being implemented.

Conflict of interest statement

The authors report no conflict of interest.

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