Review

MOLECULAR METABOLISM

Primary prevention of beta-cell autoimmunity and type 1 diabetes — The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) perspectives

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ABSTRACT

Objective: Type 1 diabetes can be identified by the presence of beta-cell autoantibodies that often arise in the first few years of life. The purpose of this perspective is to present the case for primary prevention of beta-cell autoimmunity and to provide a study design for its implementation in Europe.

Methods: We examined and summarized recruitment strategies, enrollment rates, and outcomes in published TRIGR, FINDIA and BABYDIET primary prevention trials, and the TEDDY intensive observational study. A proposal for a recruitment and implementation strategy to perform a phase II/III primary prevention randomized controlled trial in infants with genetic risk for developing beta-cell autoimmunity is outlined.

Results: Infants with a family history of type 1 diabetes (TRIGR, BABYDIET, TEDDY) and infants younger than age 3 months from the general population (FINDIA, TEDDY) were enrolled into these studies. All studies used HLA genotyping as part of their eligibility criteria. Predicted beta-cell autoimmunity risk in the eligible infants ranged from 3% (FINDIA, TEDDY general population) up to 12% (TRIGR, BABYDIET). Amongst eligible infants, participation was between 38% (TEDDY general population) and 97% (FINDIA). Outcomes, defined as multiple beta-cell autoantibodies, were consistent with predicted risks. We subsequently modeled recruitment into a randomized controlled trial (RCT) that could assess the efficacy of oral insulin treatment as adapted from the Pre-POINT pilot trial. The RCT would recruit infants with and without a first-degree family history of type 1 diabetes and be based on general population genetic risk testing. HLA genotyping and, for the general population, genotyping at additional type 1 diabetes susceptibility SNPs would be used to identify children with around 10% risk of beta-cell autoimmunity. The proposed RCT would have 80% power to detect a 50% reduction in multiple beta-cell autoantibodies by age 4 years at a two-tailed alpha of 0.05, and would randomize around 1160 infants to oral insulin or placebo arms in order to fulfill this. It is estimated that recruitment would require testing of between 400,000 and 500,000 newborns or infants.

Conclusion: It is timely and feasible to establish a platform for primary prevention trials for type 1 diabetes in Europe. This multi-site European infrastructure would perform RCTs, supply data coordination and biorepository, provide cohorts for mechanistic and observational studies, and increase awareness for autoimmune diabetes.

Keywords Type 1 diabetes; Beta-cell autoimmunity; Prevention; Antigen-based immunotherapy

1. INTRODUCTION

Type 1 diabetes results from an autoimmune destruction of the insulin-producing beta cells within the pancreatic islets of Langerhans. This process is identified by circulating islet autoantibodies to beta-cell antigens and is mediated by a lack of immunological self-tolerance [1,2]. Self-tolerance is achieved by T cell exposure to self-antigens in the thymus or in the periphery (i.e. outside the thymus or bone marrow, in secondary lymphoid tissues such as lymph node, gut and spleen) in a manner that deletes autoreactive effector T cells or induces

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regulatory T cells and regulatory cytokines such as interleukin-10 (IL-10). Immunological tolerance can be achieved by administration of antigen under appropriate conditions [3,4]. Evidence is now emerging in humans that these approaches may be effective in chronic inflammatory diseases such as multiple sclerosis, allergy, and type 1 diabetes [5–8].

GPPAD is the **G**lobal **P**latform for the **P**revention of **A**utoimmune **D**iabetes that was established in 2015 in Germany and UK with the intention to establish an infrastructure for primary prevention trials. More specifically, we aim to develop and launch the first randomized controlled phase II/III trial (RCT) using autoantigen-based therapy and to consider other approaches that might inhibit or prevent the earliest events in newborns that lead to multiple anti-islet autoantibodies. GPPAD will also investigate the feasibility, practicalities, and acceptability of recruitment of newborn children into mechanistic studies and third generation natural history studies. Antigen-based therapy in type 1 diabetes will serve as a model system. However, the planned platform will be adaptable and deployable in the investigation and prevention of other approaches to type 1 diabetes primary prevention, and to other childhood conditions and illnesses, with a major underlying goal of the promotion of better health outcomes early in life and during pregnancy based on improved understanding of the human immune system. GPPAD will focus on primary prevention, defined as the prevention of seroconversion to beta-cell autoantibodies. Primary prevention has a strong rationale. First, neonates who are at increased risk to develop multiple beta-cell autoantibodies and type 1 diabetes can be identified using family history and/or genetic markers at several loci, in particular HLA class II and class I haplotypes. Second, there is a marked peak incidence period of beta-cell autoantibody seroconversion between age 9 months and 2.5 years, providing a finite study follow-up until age 3–4 years and the primary endpoint in a RCT. Third, there is an early autoantibody target, insulin and its precursor preproinsulin, encoded by a gene with a common polymorphism that confers genetic risk for type 1 diabetes by altering neonatal immune tolerance to insulin and its precursors. Primary prevention also offers the opportunity and a platform to have a second chance at prevention (secondary) if children develop beta-cell autoantibodies.

It is widely held that if neonatal tolerance to beta-cell antigens could be enhanced, this could prevent or delay the onset of pre- or asymptomatic type 1 diabetes (defined as loss of tolerance and multiple autoantibodies) and prevent or delay disease diagnosis. The key here is "neonatal", the time when the natural mechanisms of immune tolerance are fully active as the child becomes tolerant to commensal microorganisms and dietary components. Currently, antigen-specific tolerance approaches are attempted in individuals in whom the immune system has matured and in whom an autoimmune memory response is well established. We, however, have laid the foundation for antigen-specific primary prevention by demonstrating in genetically at-risk children aged 2–7 years who are beta-cell autoantibody negative that orally-delivered insulin is safe (does not affect plasma glucose levels) at a dose that appears to engage the immune system in a manner that is consistent with immune-mediated, tolerogenic protection [9].

Hence, we believe that we have three important pillars for primary prevention to move forward – a strategy to identify neonates at type 1 diabetes risk by genetic markers; knowledge when beta-cell autoimmunity starts; and demonstration that antigen-specific therapy is feasible. With these in hand, the task is to develop an infrastructure that can make a significant impact on reducing the numbers of children who develop type 1 diabetes via broad and safe primary prevention therapy. Avenues to achieve the implementation of such a program will be discussed.

2. REVIEW OF PREVIOUS PRIMARY PREVENTION EFFORTS

2.1. The TRIGR study (Trial to Reduce IDDM in Genetically at Risk)

The TRIGR study is a dietary randomized controlled trial aiming to reduce the incidence of beta-cell autoimmunity and type 1 diabetes by weaning to an extensively hydrolyzed formula [10]. The trial was unsuccessful in reducing beta-cell autoimmunity. The study is still ongoing to access risk of type 1 diabetes. It has estimated a cumulative incidence of 9.9% by age 6 years for multiple beta-cell autoantibodies in the control group. The study was powered to detect a 35% change in the end point, and 20% risk to miss a true difference between the groups. Concurring with initial estimates, the risk of positivity for two or more beta-cell autoantibodies was 11.4% (95%CI, 9.4%–13.2%) among those randomized to the control group (conventional formula, $n = 117$), and similarly 13.4% (95%CI, 11.3%–15.5%) among those randomized to the casein hydrolyzate formula ($n = 139$). There were no clinically significant differences in the rate of reported adverse events between the two groups.

Although the outcome of TRIGR was disappointing from an efficacy viewpoint, the TRIGR study has without doubt an unprecedented value in uniquely demonstrating that conducting primary prevention trials for type 1 diabetes is feasible. TRIGR has recruited 2159 infants with HLA-conferred disease susceptibility[10] and a first-degree relative with type 1 diabetes of a total of 5156 (42%) tested for HLA eligibility. Infants were prospectively followed for at least 6 years with high retention and documented protocol adherence.

2.2. The BABYDIET pilot study

The open randomized controlled BABYDIET study aimed to reduce beta-cell autoimmunity by delayed gluten exposure in the first year of life [11]. Evidence came from two natural history studies, BABYDIAB and the Diabetes and Autoimmunity Study in the Young (DAISY), which demonstrated that early gluten exposure is associated with increased risk of beta-cell autoimmunity in childhood [12,13]. Of 1168 newborn children with a first-degree relative with type 1 diabetes screened for eligibility, 169 were found eligible because they had the high risk HLA genotypes[11], and 150 (89%) consented to participate. Participants were followed for at least 3 years with 27 children developing beta-cell autoantibodies (cumulative risk by age 4 years for any beta-cell autoantibody: 15.4% (95%CI, 9.5%–21.3%); for multiple beta-cell autoantibodies: 9.5% (95%CI, 4.6%–14.1%). The study demonstrated no beneficial effect of delaying gluten exposure to 12 months of age when compared to introducing gluten at 6 months of age in the intention to treat as well as per protocol analysis. Only 70% of families adhered to the dietary-intervention protocol while 30% introduced gluten earlier or later than recommended. This study indicates that an open dietary prevention trial is likely to have limitations with respect to protocol adherence, which in consequence will affect the ability to measure efficacy of the intervention.

[10] DQB1*02/DQB1*03:02; DQB1*03: 02/x (x not DQB1*02, DQB1*03:01, or DQB1*06:02); DQA1*05-DQB1*02/y (y not DQA1*02:01-DQB1*02, DQB1*03:01, DQB1*06:02, or DQB1*06:03); DQA1*03-DQB1*02/y (y not DQA1*02:01-DQB1*02, DQB1*03:01, DQB1*06:02, or DQB1*06:03).

[11] DRB1*03-DQA1*0501-DQB1*0201/DRB1*04-DQA1*0301-DQB1*0302;DRB1*04-DQA1*0301-DQB1*0302/DRB1*04-DQA1*0301-DQB1*0302;DRB1*03-DQA1*0501-DQB1*0201/DRB1*03-DQA1*0501-DQB1*0201;DRB1*04-DQA1*0301-DQB1*0302/DRB1*08-DQA1*0401-DQB1*0402;DRB1*04-DQA1*0301-DQB1*0302/DRB1*01-DQA1*0101 -DQB1*0501.

2.3. The FINDIA pilot study

The Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) recruited 1104 infants with type 1 diabetes susceptible HLA genotypes[12] to a randomized double-blind three-arm clinical trial aimed at determining whether the removal of bovine insulin from infant formula milk reduced the incidence of beta-cell autoimmunity at age 3 years [14]. The trial was successful in recruiting all children over a 3.5-year period from three Finnish pediatric hospitals, with an acceptance rate of 97% of those being found eligible (n = 1133). Of the randomized children, 908 (82.3%) provided one follow-up sample, and 196 (17.7%) withdrew from the study; after 3 years of follow-up, around 50% remained in the study. The pilot trial found a significant decrease in the prevalence of one or more beta-cell autoantibodies in the children who had been assigned to the bovine insulin free formula group.

2.4. TEDDY

The Environmental Determinants of Diabetes in the Young (TEDDY) study is a longitudinal cohort study with the primary aim to identify dietary factors, infectious agents, or other environmental exposures associated with an increased risk of islet autoimmunity and type 1 diabetes [15]. It follows children from age 3 months to age 15 years with intensive 3–6 months follow-up visits and blood draws without any intervention. The TEDDY study included children with an increased genetic risk[13]. TEDDY screened 424,788 newborns for eligibility over a period of 6 years including 418,348 from the general population and 6440 from families with first-degree relatives diagnosed with type 1 diabetes. Of general population newborns, 20,133 (4.8%) met the HLA eligibility criteria; of newborns with a first-degree relative, 1456 (22.6%) were found to be eligible. Of eligible children, 8676 (40.2%) families consented to participate in TEDDY and started the long-term follow-up, including 7724 (38.4%) from the general population, and 952 (65.4%) with a first-degree relative.

3. LESSONS FROM PREVIOUS PRIMARY PREVENTION STUDIES IN TYPE 1 DIABETES

- Primary prevention trials in first-degree relatives are feasible and multi-center recruitment is possible.
- Recruitment to primary prevention trials beyond first-degree relatives based on testing for genetic risk is possible.
- Prevention trials that are non-blinded with open access to treatment arm may lead to insufficient protocol adherence.
- Infants with approximately 10% genetic risk for multiple beta-cell autoimmunity can be identified.
- Acceptance by eligible families to participate in prevention or intensive observational studies ranged from 38% to 97%.

4. MECHANISTIC APPROACH TO STUDY IMMUNE EFFICACY IN PRIMARY PREVENTION

4.1. Pre-POINT

Antigen-based therapy using oral insulin exposure at a dose of 1 mg twice a week is efficient to prevent autoimmune diabetes in NOD mice [16]. Insulin was given from week 5 when NOD mice have initial signs of beta-cell inflammation and lymphocytic infiltration. In humans, oral insulin therapy at a modest daily dose of 7.5 mg does not delay progression to type 1 diabetes in first-degree relatives with insulin autoantibodies and ICA with the possible exception of a subgroup of relatives with high levels of insulin antibodies where daily 7.5 mg oral insulin might delay the clinical onset of type 1 diabetes by an average of 10 years [17]. The difference between the mouse and human studies were the timing of treatment and the dose, which was estimated to be around 10-fold higher in successfully treated mice.

The Pre-POINT study is the first to administer an autoantigen to genetically at-risk children prior to any signs of autoimmunity [9]. The rationale is to expose oral mucosa to antigen in order to stimulate the immune system in a safe environment that normally favors a tolerogenic immune response. Thus, the objective of Pre-POINT has been to identify a dose of oral insulin that is safe and engages the immune system towards immune tolerance.

Pre-POINT was performed as a double-blind placebo-controlled dose increasing phase I/II clinical trial. Children aged two to seven years with a family history of type 1 diabetes and type 1 diabetes susceptible HLA class II genotypes and without islet autoantibodies (n = 25) were randomized to receive placebo (n = 10) or insulin (n = 15) orally once a day for 3–18 months. The design included dose escalation so that six children were included in each of the 2.5 mg, 7.5 mg, 22.5 mg, and 67.5 mg insulin dose groups. The highest dose was estimated to be equivalent to the dose required to prevent diabetes development in NOD mice. None of the doses caused hypoglycemia. All doses were well tolerated, and adverse events were similar between placebo and insulin treated children. Daily administration of 67.5 mg of insulin was able to induce a measurable T and/or B cell immune response to insulin. The response differs to the typical responses seen in children who develop diabetes in that the antibody responses are of weak affinity. Moreover, one child developed salivary IgA response to insulin, suggesting a specific response to insulin exposure in the mouth. The T cell responses were most informative, with a preponderance of cells showing regulatory T cell gene expression. These regulatory gene expression profiles are unique for the respective T cells of children treated with the 67.5 mg dose compared to lower doses or placebo (Figure 1). These results are encouraging from a safety viewpoint and indicate that oral exposure to insulin at doses that are approximately equivalent to efficacious doses in rodents may promote tolerance in children.

4.2. Learning from peanut allergy

Around the same time as the Pre-POINT study, a large study aimed at preventing peanut allergy through active exposure to antigen were performed and reported. Unlike previous attempts based on avoidance of peanuts, the consumption trial was successful, providing supporting evidence for antigen-based therapies in disorders such as type 1 diabetes. Relevant to primary prevention, the LEAP trial enrolled 542

[12] DQB1*02/DQB1*0302; DQB1*0302/x (x not DQB1*0301 or DQB1*0602); DQA1*05-DQB1*02 (DR3)/y (y not DQB1*0301, DQB1*0602, or DQB1*0603).

[13] General population: DR4-DQA1*030X-DQB1*0302[1]/DR3-DQA1*0501-DQB1*02 01; DR4-DQA1*030X-DQB1*0302[1]/DR4-DQA1*030X-DQB1*0302[1]; DR4-DQA1*030X-DQB1*0302[1]/DR8-DQA1*0401-DQB1*0402; DR3-DQA1*0501-DQB1*0201/DR3-DQA1*0501-DQB1*0201 (DR4 subtyping must exclude DRB1*0403)First-degree relatives: DR4-DQA1*030X-DQB1*0302[1]/DR3-DQA1*0501-DQB1*0201; DR4-DQA1*03 0X-DQB1*0302[1]/DR4-DQA1*030X-DQB1*0302[1]; DR4-DQA1*030X-DQB1*0302[1]/DR8-DQA1*0401-DQB1*0402; DR3-DQA1*0501-DQB1*0201/DR3-DQA1*0501-DQB1*0201; DR4-DQA1*030X-DQB1*0302[1]/DR4-DQA1*030X-DQB1*020X; DR4-DQA1*030X-DQB1*0302[1]/DR1[2]-DQA1*0101-DQB1*0501; DR4-DQA1*030X-DQB1*0302[1]/DR13-DQA1*0102-DQB1*0604; DR4-DQA1*030X-DQB1*0302/DR4-DQA1*030X-DQB1*0304; DR4-DQA1*030X-DQB1*0302[1]/DR9-DQA1*030X-DQB1*0303; DR3-DQA1*0501-DQB1*0201/DR9-DQA1*030X-DQB1*0303([1] = Acceptable alleles in this haplotype include both DQB1*0302 and *0304; [2] = In this DQB1*0501 haplotype,DR10 must be excluded. Only DR1 is eligible).

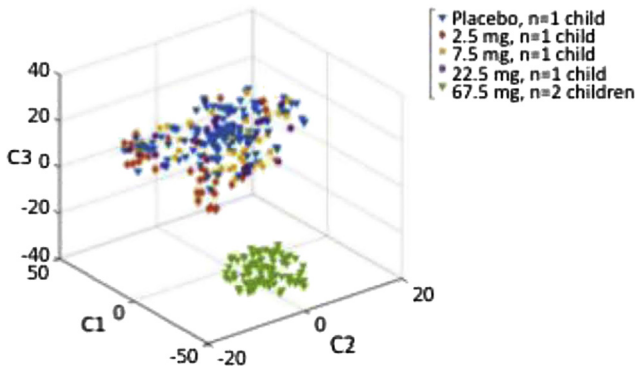

Figure 1: CD4$^+$ T cell immune response profiles against insulin from the Pre-POINT trial [9]. Multivariate gene expression analysis of insulin-responsive CD4$^+$ T cells isolated from Pre-POINT participants with CD4$^+$ T cell responses to insulin. The data show t-distributed Stochastic Neighbor Embedding (tSNE) analysis of pre-processed Ct values for all analyzed genes. A linear model was used to correct for confounding effects, which can mask relevant biological variability. Batch effects (dummy coding each plate/batch) were modeled jointly with dose effects to obtain a corrected gene expression data set. This resulted in two distinct clusters, with one cluster consisting of cells derived from children receiving 67.5 mg insulin (green symbols) and the second cluster consisting of cells derived from children receiving placebo (blue symbols), 2.5 mg insulin (red symbols), 7.5 mg insulin (yellow symbols), and 22.5 mg insulin (purple symbols). C1, C2, and C3 are component 1, component 2, and component 3 of the tSNE. Reproduced from reference #9 with permission from JAMA American Medical Association (License Number 3786541433624).

infants who initially had no preexisting sensitivity to peanuts, but who had an estimated 9% risk for developing peanut allergy by age 5 years [7]. Children randomized to peanut consumption were instructed to eat at least three peanut-containing meals per week in order to consume at least 6 g of peanut protein per week until age 5 years. In comparison, children taking the highest oral insulin dose in Pre-POINT consumed a total of 0.47 g of insulin per week, which, if one assumes that less than 10% of total peanut protein is allergenic, corresponds well to the exposure to allergen in the LEAP trial. The prevalence of peanut allergy at 60 months of age was 13.7% in children who avoided peanut consumption and, remarkably, only 1.9% in children who consumed peanuts ($P < 0.001$). The results are even more striking in a per protocol analysis where 0.4% of non-sensitized children developed peanut allergy in the consumption group. Interestingly, peanut consumption is followed by the development of peanut-specific IgG4 and avoidance by peanut-specific IgE antibody, respectively. T cell studies were not reported. Another impressive aspect of the LEAP trial was that only 12 (2.2%) of the 542 enrolled children did not complete the study at age 5 years. Overall, the primary prevention part of the LEAP trial is encouraging for GPPAD attempts to introduce autoantigen-based primary prevention for type 1 diabetes.

4.3. Pre-POINT-early

Many children with type 1 diabetes develop beta-cell autoimmunity between 9 months and 2.5 years of age. Hence, in order to offer possible efficacy in the broadest sense, treatment for primary prevention will need to start prior to that age. Pre-POINT-Early is a double-blind RCT performed in children aged 6 months to 2 years in order to validate the findings of Pre-POINT and determine safety of the 67.5 mg dose of insulin in very young children. The study was recently initiated in Germany and will recruit and randomize 44 children at a 1:1 ratio to daily oral insulin powder ingested with food (dose escalation: 7.5 mg per day for 3 months; increased to 22.5 mg per day for 3 months; increased to 67.5 mg per day for 6 months) or placebo. Families will self-administer the oral insulin powder packed in capsules. The outcome is again a measurable adaptive immune response to insulin that has features of a regulatory response and does not have the features associated with beta-cell autoimmunity. The trial has 80% power to detect an immune response in 67% of treated children at a two-tailed significance of 0.05. We reason that if Pre-POINT-Early is able to show such an immune response in the absence of safety concerns, there is sufficient rationale to plan a phase II/III trial assessing the efficacy of oral insulin administration to prevent beta-cell autoimmunity. We expect to have results by January, 2018.

5. GPPAD PHASE II/III RCT AND TRIAL PLATFORM

The first GPPAD RCT is currently being planned. The lead therapy is oral insulin with an objective to test the efficacy of therapy to reduce the incidence of beta-cell autoimmunity in genetically at-risk children. This is under the assumption that results from Pre-POINT-Early will demonstrate safety and confirm immune efficacy. The goal is to introduce immune tolerance against autoantigen before the start of beta-cell autoimmunity as primary prevention for type 1 diabetes. There are several reasons why we favor insulin as one of our first choice candidates for primary prevention. There is clear evidence from man [18,19] and animal models [20] that insulin is the key early and primary autoantigen of childhood diabetes. There is also a strong genetic rationale for loss of tolerance against insulin as a primary predisposing factor for type 1 diabetes. Allelic variation in the insulin gene is associated with type 1 diabetes [21] and islet autoimmunity [22] via a mechanism of thymic T cell deletion [23]. Moreover, insulin autoimmunity is closely associated with the HLA DR4-DQ8 haplotype present in over 60% of children who develop type 1 diabetes. There are also practical considerations, given the excellent safety profile thus far whereby both, antibody and T cell immune responses can be directly measured and serve as biomarkers of immune efficacy.

GPPAD will, however, be mindful of alternative approaches. For example, we are aware of increasing evidence for a role of vitamin D in immune tolerance [24], and we will consider mechanistic studies and development of therapies such as proinsulin peptides [25], insulin mimetopes [26], nasal insulin [27], or probiotics [28] and anti- or pro-inflammatory seasonal therapy [29]. The promise is to develop a platform for multiple studies. This platform should include the neonate and infant testing sites and centers, a data coordinating center that provides data base, data analysis, biorepository, and procedures for regulatory approval, a pharmacy, and a communication and dissemination center.

5.1. Target age and population

Infants will be recruited. Start of treatment will not be before the introduction of solid foods, which is usually between age 4 and 6 months. Since there is a marked rise in beta-cell autoantibody seroconversion, and in particular insulin autoantibody seroconversion at around age 9–12 months of age, and a lower incidence of insulin autoimmunity beyond this age [30–32], we suggest that the trial target children below age 9 months. It is noted that a beta-cell autoimmunity can start already at age 6 months in a small minority (<5%) of children who develop type 1 diabetes [30–32].

Participants will be required to have an HLA-DR4/HLA-DQ8 haplotype and have an estimated genetic risk of at least 10%, which can be achieved in several ways: 1. A multiple first-degree family history of type 1 diabetes [33]; 2. A first-degree relative of a patient with type 1 diabetes, a HLA-DR4/HLA-DQ8 haplotype and no protective HLA-DR

or HLA-DQ haplotypes; 3. No first-degree family history of type 1 diabetes, but a combined genetic risk score that we expect corresponds to the upper 0.5% of the general population. This risk score was estimated using T1DGC data [34] and will be refined in much larger cohort of non-diabetic individuals and using data from patients not selected because they have a first-degree relative with type 1 diabetes, that is population-based, prior to finalizing the trial protocol.

The RCT will have two recruitment phases (Figure 2). The first phase will only include first-degree relatives of patients with type 1 diabetes who fulfill the 10% risk criteria. The purpose of this is to extend safety data in infants and to provide a run in period for logistics of the study. It will run for a period of 12 months and is expected to recruit 100 infants to the RCT. The second recruitment phase will extend eligibility to all three categories and include infants without a family history of type 1 diabetes.

5.2. Testing for diabetes genetic risk

Newly born babies in Europe are routinely screened within the first days after birth, using a few drops of blood from the heel onto filter paper cards, for certain genetic, endocrine, and metabolic disorders, and are also tested for hearing loss prior to discharge from a hospital or birthing center. Depending on the country, additional information such as the baby's name, sex, weight, date/time of birth, date/time of heel stick collection, and date/time of first feeding, and contact information of the parents and the baby's primary care physician is also collected.

Testing for type 1 diabetes genetic risk for eligibility into the RCT will be done via filter paper cards, which will be separate from the official newborn screening cards, and offered to families either together with the established newborn screening as supplemental testing with separate consent, or will be offered at regular child check-ups performed at the primary care pediatrician at the age of 3–10 days (U2), 4–5 weeks (U3), or 3–4 months (U4). Information about type 1 diabetes in the family will be obtained. Supplemental diabetes risk assessment and the subsequent possibility to participate in a RCT to prevent diabetes in case of higher genetic risk and any concerns will be discussed with the families. Diabetes risk assessment will be financed through research and will be of no cost for the family.

Genetic testing will be first surveyed in Saxony, Germany, with trial centers in Dresden and Leipzig; additional testing and trial centers in Germany (Hannover in Lower Saxony, Munich in Bavaria); Saxony covers around 36,000 births per year, Lower Saxony 66,000, and Bavaria 110,000. Testing in the UK, and potentially other European countries will follow.

5.3. Genetic eligibility and testing

Genetic testing will be performed by a two-step process. The first is based on HLA and family history of type 1 diabetes. HLA will be determined by three SNPs to identify 1. Infants who have the HLA DR4-DQ8 haplotype amongst first-degree relatives of patients; and 2. All infants, regardless of their type 1 diabetes family history, who have the HLA DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype [35]. Relatives

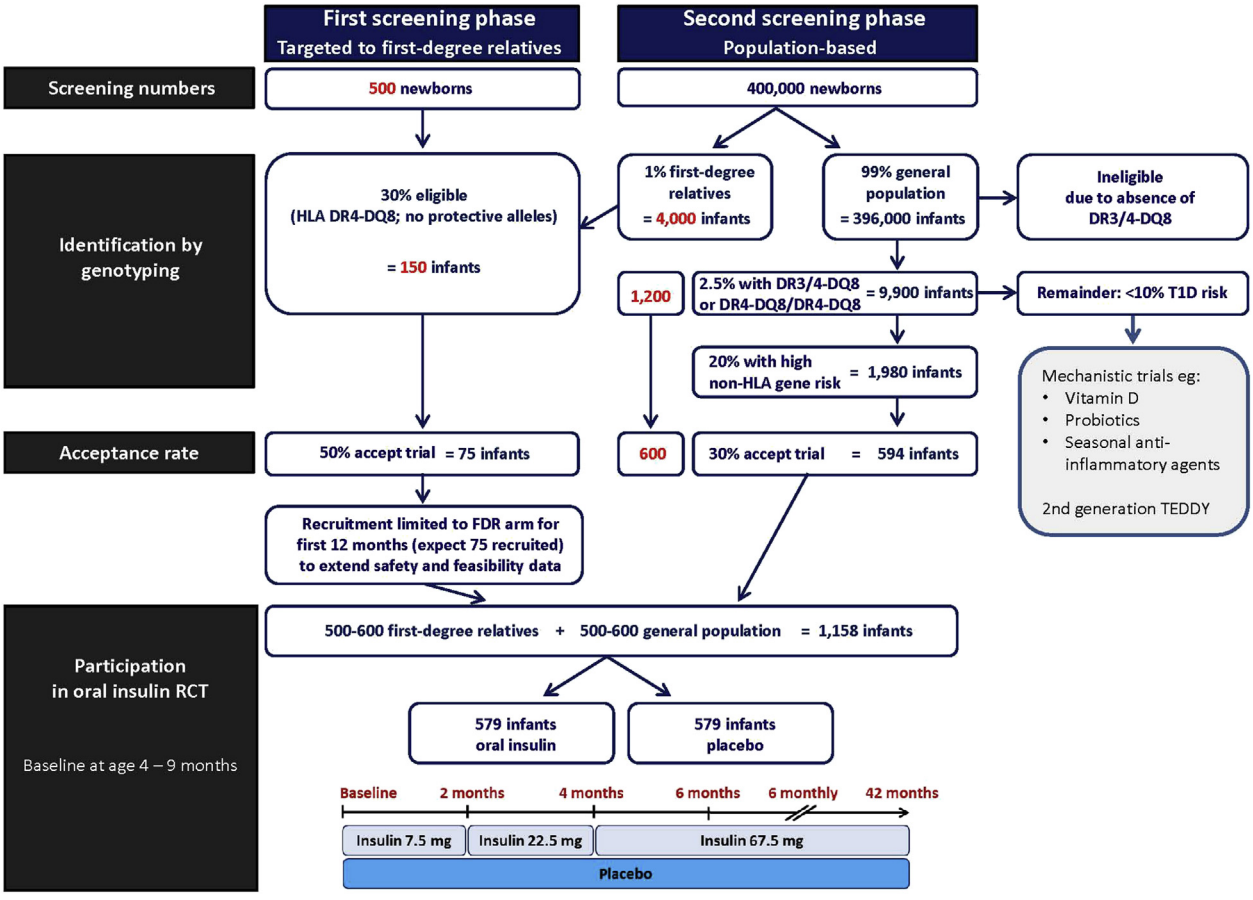

Figure 2: Design of GPPAD randomized controlled trial.

with HLA DR4-DQ8 will then be tested for the protective alleles DRB1*0403, DR11, DR12, DQB1*0602, DR7-DQB1*0303, DR14-DQB1*0503, DRB1*13-DQB1*0603 and will be eligible if none of these are present; outcome risk is estimated to be 12%. Non-relatives with the HLA DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotypes will be tested with a panel of SNPs from HLA class I and non-HLA class II regions to identify infants with a predicted outcome risk of around 10%. The SNP panel is in development and will be based on algorithms similar to those described [34,36]. Genetic typing will be outsourced.

5.4. Outcome

The primary outcome of the RCT is multiple beta-cell autoantibodies, or type 1 diabetes, at the age of 4 years. Multiple beta-cell autoantibody positive is defined as antibodies against two or more beta-cell antigens — insulin, GAD65, IA-2, ZnT8 — in two consecutive samples.

5.5. RCT size and statistical power

It is estimated that 11% of the placebo-treated children will develop multiple beta-cell autoantibodies. In order to have 80% power to detect a 50% reduction in risk of beta-cell autoimmunity with two-tailed alpha of 0.05, 521 children are required in each arm. With an expected drop-out rate of 10%, 1158 infants will need to be enrolled into the RCT.

5.6. Design

The oral-insulin-RCT will be conducted in two recruitment phases (Figure 2).

The first recruitment phase will be restricted to infants who are first-degree relatives of patients with type 1 diabetes and will last 12 months. The target of genetic testing in this phase is 500 infants. Around 30% of all first-degree relatives are estimated to meet eligibility criteria and around 50% are expected to participate in the RCT. Thus, recruitment of around 75 infants is expected in this phase. Based upon the prevalence of type 1 diabetes in Germany, 0.8—1% of all newborns will have a parent or sibling with type 1 diabetes. Thus, around 60,000 births would be required to reach the target in the first recruitment phase.

The second recruitment phase will target all newborns within the genetic testing sites. We estimate that this will require an additional 400,000 newborns to be tested in order to reach the final target of 1158 participants in the RCT. This estimate is based on the assumptions that 1% (4000) of newborns are first-degree relatives of patients with type 1 diabetes, that 30% (1200) of these will be eligible and that 50% (600) will accept to be randomized, and that 0.5% (1980) of the newborns without a relevant family history will have an eligible genetic risk score and that 30% (594) of these will participate. Acceptance rates are estimated from the TRIGR and TEDDY relatives and on the basis that acceptance to participate in general population eligible infants was around 60% of the acceptance amongst the eligible first-degree relatives. These numbers are likely to require adjustment as the RCT proceeds but provide a feasible target and a guideline to the effort required to conduct a phase II/III primary prevention RCT.

5.7. Treatment

Infants will be treated with daily placebo or daily oral insulin at a dose of 7.5 mg for 2 months, followed by a dose of 22.5 mg for 2 months, and followed by a dose of 67.5 mg, which will be given until they reach age 4 years. Children who develop multiple islet autoantibodies have reached the primary trial outcome and will stop treatment. The amount of insulin required per child is around 80 g for 3.5 years of treatment. Around 50 kg of insulin crystals will be needed to complete the trial.

5.8. Opportunities arising from the RCT

A number of opportunities arise from establishing the platform to conduct such a RCT. First, there will be the opportunity to enroll families that do not wish to participate in the oral insulin RCT into observational studies or mechanistic studies. These could include mechanistic studies that contain intervention protocols such as dietary supplements. Important for these studies will be the opportunity to collect samples that can be used to examine cellular immune responses in the first year of life, a resource that is currently rarely available. The platform could also serve to validate findings from the TEDDY study with respect to environmental exposures that may associate with beta-cell autoimmunity. Finally, the design of the oral insulin RCT excludes infants who are HLA-DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8, but do not reach a 10% outcome risk. These are nearly 2% of the tested population and will have an outcome risk of around 3%, which is sufficiently high to consider other interventions such as dietary supplements. Moreover, children who reach the outcome and become multiple beta-cell autoantibody positive could be eligible for trials to revert autoimmunity and prevent progression to clinical hyperglycemia. Thus, the platform would provide a pipeline for multiple parallel primary prevention trials and treatment trials.

5.9. Significance

It is understood that GPPAD has a long-term and multifaceted vision of how to achieve type 1 diabetes prevention and intervention, and that the first RCT and trial platform will be one important component of this vision. If successful, the program will provide the strategy and infrastructure to introduce prevention at the level and scale needed to quickly assess efficacy and quickly integrate successful prevention at the population level. Beyond this, the GPPAD program will increase awareness for autoimmune diabetes and its early diagnosis, and as such has the potential to improve care of children at diabetes risk. The platform may also serve as a model for other childhood diseases.

5.10. Roadblocks of primary prevention

There are challenges and roadblocks which may impede the successful implementation of primary prevention strategies including costs, public awareness, feasibility, and limited evidence of successful therapy.

The costs for primary prevention RCTs are high, and likely to be considerably higher than performing trials in children with established beta-cell autoimmunity. Our unofficial estimate is in the tens of millions of euro for the trial outlined in Figure 2. Part of the reason for the high costs is that even though type 1 diabetes risk in the participating children is at least 20-fold higher than background, we are forced to treat a large number of children who will not develop diabetes. This underlines the need for biomarkers that go beyond genetic susceptibility, and can discriminate infants with higher risk than what can be realized with genotyping. Feasibility of recruitment and follow-up during infancy and early childhood has been established in previous studies (see Sections 2 and 3.). However, experience from these studies indicates that large resources need to be invested into education, awareness, compliance and retention in order achieve this. Thus, the efforts and expenditure required for conducting a primary RCT call for a larger return for the investment. GPPAD will achieve this by integrating the RCT into a platform of mechanistic studies and natural history studies which not only aims to test multiple prevention strategies but also collect precious biomaterial to provide novel insights into disease pathogenesis and biomarker development to meet the changing needs of the scientific and clinical community with respect to reducing the incidence of type 1 diabetes.

Related to this is the awareness that, while we present evidence as to why antigen-based therapies with insulin could be tested in a RCT, there are no efficacy data for primary prevention with oral insulin or any other antigen-specific therapy. Again, the benefit of the platform is the effort is not solely focused on successful prevention with a single RCT, but to include multiple short-term increments in knowledge and a long-term infrastructure for implementing prevention therapeutics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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