STUDY PROTOCOL

Pre-POINT (Primary Oral Insulin Trial)

A dose-finding safety and immune efficacy study for primary mucosal insulin therapy in islet autoantibody negative children at high genetic risk for type 1 diabetes

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**Synopsis**

**Title**  
Primary intervention with mucosal insulin for prevention of type 1 diabetes in infants at high genetic risk to develop diabetes

**Short Title**  
Pre-POINT (Primary Oral INsulin Trial)

**Clinical Phase**  
I/II (dose drug bioavailability, safety and feasibility)

**Sponsor**  
Investigator-initiated trial; supported by Juvenile Diabetes Research Foundation (JDRF) and the German Federal Ministry of Education and Research (BMBF)

**Conducted by**  
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**Study Objective**  
The objective of this study is to determine the feasibility, safety and bioavailability of oral insulin in children with high genetic risk for type 1 diabetes (T1DM) in a dose escalation primary intervention pilot study.

**Significance**  
A dose with proven drug bioavailability to the immune system for use in a phase II/III primary T1DM vaccination trial (POINT study) in genetically at risk subjects.

**Study Design**  
Randomized, placebo-controlled, double-blind/double-masked, multi-center, dose escalation primary intervention pilot study.

**Accrual Objective**  
25 (3:2 randomization to active and control arms)

**Study Duration**  
18 months

**Endpoint**  
The development of immunity to insulin (IgG or IgA antibodies, or T cell response to insulin and/or insulin peptides).

**Inclusion Criteria**  
1. Children aged 2 years to 7 years who:
   A. have a multiplex first degree family history of T1DM (both parents, parent and sib, or two sibs); and a type 1 diabetes susceptible HLA DR4-DQB1*0302 or DR4-DQB1*0304 haplotype and none of the following HLA DR or DQB1 alleles:
      - DR 11
      - DR 12
      - DQB1*0602
      - DR7-DQB1*0303
      - DR14-DQB1*0503
   or
   B. have a sibling with T1DM; and are identical by descent for the HLA DR3/DR4-DQ8 genotype with their diabetic sibling;

2. Islet autoantibody negative at time of recruitment.

**Exclusion Criteria**  
1. Children with any kind of congenital or acquired chronic disease that potentially interfere with the study objectives.
2. Prior or current participation in another intervention trial.
3. Chronic oral steroid use and/or other chronic oral immunosuppressant
Treatment Description
Active arm: Insulin given orally at 2.5 mg, 7.5 mg, 22.5 mg, or 67.5 mg daily. Control arm: placebo administered orally.
Children will be treated for the duration of the Pre-POINT study or until becoming GAD or IA-2 autoantibody positive or until diabetes onset. Average expected duration of treatment: 11 months (minimum 3 months; maximum 18 months corresponding to expected Pre-POINT study duration).

Safety Assessment
1. Hypoglycemia. Capillary blood glucose measured before and 30, 60 and 120 minutes after drug has been administered on the first day of treatment at each dose level; capillary blood glucose measured 60 minutes after drug has been administered on day 2 to 7 at each dose level and monthly thereafter.
2. Laboratory tests. Blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start and end of treatment for each dose. Oral glucose tolerance test (OGTT) if positive for GAD or IA-2 autoantibodies every 6 months.
3. Allergy to the study drug. Total IgE and IgE antibodies to insulin (at start, day 15, 3 months and 6 months for each dose and every 6 months) and family self-reporting.
4. Psychosocial effects of study participation. Questionnaires completed by families prior to and after 3 and 9 months participation in the study.

End of Pre-POINT study phase
A dose-finding committee will determine the end of Pre-POINT if or when:
1. The study drug is shown to be unsafe.
2. The study is no longer feasible or becomes very unlikely to provide information that will allow decisions to be made pertaining to dose to be used in POINT.
3. A safe dose of oral insulin is identified that is shown to cause a change in the immune response to the study drug.
4. All proposed doses of oral insulin have been tested.
5. Other information becomes available relating to the safety, immune bioavailability or efficacy of the study drug that no longer requires the completion of Pre-POINT in order to decide on the POINT protocol.

Proceeding to POINT
Pre-POINT will proceed to the POINT study if and when the dose-finding committee identifies a dose that can be tested for efficacy.
Follow-up visits of Pre-POINT participants will continue after treatment in Pre-POINT has stopped until a decision has been made whether to proceed to POINT. Children who have participated in Pre-POINT and who have not become GAD or IA-2 autoantibody positive and have not developed diabetes will be eligible for POINT in their allocated treatment group (active or placebo).

Participating Centers
4 centers: Germany (Munich, Dresden), Austria (Vienna), UK (Bristol), USA (Denver)
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1. Scientific Background and Rationale

1.1 The medical problem

Type 1 diabetes (T1DM) is an autoimmune disease with worldwide increasing incidence rate. T1DM is one of the most common chronic diseases of childhood, is the cause of serious late-stage health problems including kidney disease, and is associated with large social and economic burden. Preventing/delaying T1DM and/or its long-term complications is therefore of major benefit to society.

Current therapy does not prevent complications. The DCCT showed that complications in patients with T1DM can be reduced if glycemia is kept under control by intensive insulin therapy (1; 2). Unfortunately, such metabolic control is 1. difficult to achieve throughout life, and 2. associated with a substantially increased number of life-threatening episodes of hypoglycemia. It is generally agreed, therefore, that experimental interventions to improve metabolic control in patients with T1DM or prevent T1DM in individuals at risk for T1DM are a high priority.

1.2 Rationale for primary intervention

Experimental interventions applied late in the disease process have had limited success. Interventions at or after T1DM onset are immunomodulatory or immunosuppressive in nature. Some have shown a beneficial effect in reducing insulin requirement to achieve reasonable metabolic control, but the side effects were either too great or as yet uncertain in the case of monoclonal antibody therapy. New drugs may have better benefit/risk ratios, but it is likely that treatments applied after clinical disease onset will require therapies with significant side effects in order to substantially improve metabolic control. Preventative therapies can be applied prior to diabetes onset in individuals who are genetically at risk and who have evidence of autoimmunity indicating a high risk of later development of diabetes. Three large multi-center trials have been performed in this group; two showed no beta cell protection by treatment with nicotinamide or parenteral insulin (3; 4), and the third showed no benefit from treatment with oral insulin in the study group as a whole, but some encouraging effect after post-hoc stratification of subjects (5). It is clear that although not impossible, preventing diabetes onset in subjects with an immune system primed to destroy insulin producing beta-cells is also likely to require potentially toxic therapies. An alternative is to intervene prior to the appearance of autoimmunity.
The infrastructure for performing primary intervention trials in T1DM are established. Identification of neonates with high T1DM risk using genetic typing in affected families can be achieved (6; 7), and families of these children are willing to participate in primary intervention trials that are relatively demanding with respect to lifestyle change, time, and blood collection (8). Early specific markers that predict who will develop T1DM in childhood are available (9; 10), thereby allowing primary intervention trials to be carried out within a relatively short time frame. Thus, provided suitable treatments are identified, the infrastructure to perform primary intervention trials for childhood T1DM is available.

1.3 Identification of appropriate subjects for primary intervention

Neonates with very high T1DM risk can be identified and monitored for the appearance of diabetes relevant autoimmunity. It has been demonstrated that neonates who have a first degree family history of T1DM and the HLA risk genotypes DR3/4-DQ8 or DR4-DQ8/DR4-DQ8 have a 20% or higher risk for developing islet autoantibodies during childhood (7; 11). Risk can be further stratified by selection of neonates with susceptible genotypes at other diabetes genes (11-13), by selection of neonates with multiple family history of diabetes (6), and by selection of relatives that are HLA identical to the proband (14). In some cases, the risk for developing islet autoantibodies can exceed 50%. In the BABYDIAB cohort, 55% (95% CI, 30-80%) of neonates who had a multiplex first degree family history of T1DM and who had either the DR3/4-DQ8 or DR4-DQ8/DR4-DQ8 genotypes developed diabetes relevant autoantibodies (multiple islet autoantibodies – IAA, followed by GAD and IA-2 antibodies) in the first years of life, and the majority of those who developed these antibodies progressed to disease in childhood (6). In the DAISY cohort, siblings of children with T1DM who have the HLA DR3/DR4-DQ8 genotype and are identical by descent for both HLA haplotypes with their diabetic proband sibling had a 65% risk for developing islet autoantibodies by age 7 years and a 50% risk of developing diabetes by age 10 years (14). The overall risk for developing T1DM in these two high risk groups was 50% by 10 years of age. Thus, the development of multiple islet autoantibodies is an early and specific marker that represents a valid end point for primary prevention trials in high risk children, and it is possible to identify children with a 50% or higher risk of developing multiple antibodies in childhood.
1.4 Rationale for antigen-specific primary intervention with oral insulin

(Pro)insulin is a primary autoantigen in children developing T1DM. (Pro)insulin is a beta-cell specific antigen, and has been suggested to be important in driving autoimmune beta-cell destruction in the NOD mouse (15-18). Most recently, it was demonstrated that expression of a non-mutated proinsulin gene in the NOD mouse was essential for disease implying that (pro)insulin is a primary autoantigen in this model (19). Autoimmunity against insulin is also a characteristic feature in the pathogenesis of human childhood T1DM. Insulin autoantibodies (IAA) are present in sera of almost all children at diabetes onset (20). Prospective studies investigating the natural history of T1DM in children from birth such as the German BABYDIAB study (21), the Finnish DIPP study (22), the US DAISY study (7), and the Australian BabyDiab study (23) have shown that IAA are the first or among the first islet autoantibodies detected early in infancy. The early appearance of IAA is often followed by autoantibodies to other beta-cell antigens such as GAD or IA-2, and children who progress to multiple islet autoantibodies are more likely to develop T1DM (7; 9; 21-24). Only IAA of high affinity are predictive of progression to multiple autoantibodies and T1DM, and these are a specific marker of future disease in genetically at risk children (10). In the BABYDIAB cohort, the majority (25 of 27 cases) of children developing T1DM before 10 years of age have detectable high affinity IAA early in life (21). Moreover, children who have these IAA have a 50% risk to develop T1DM before puberty (9). Finally, studies on lymph nodes from patients with T1DM showed that the predominant T cell reactivity was against a peptide of the insulin A chain, suggesting that insulin remains a major autoimmune target years after diabetes onset (25). Altogether, the findings point to insulin as a primary autoantigen in the disease process, and suggest that a deleterious immune response to insulin is an early event that if altered could change the course to disease.

Early antigen (insulin) specific intervention is an attractive and available candidate therapy. Modification of environment, although likely to be effective for some cases, is unlikely to be a universal method of preventing T1DM. The use of non-specific agents is likely to also affect responses to vaccines and infection. In contrast, specifically targeting the immune response to autoantigens may provide protective immunity that does not affect immune responses to other vaccines or pathogens. Insulin is almost always amongst the first autoantigens recognized by antibodies in cases of childhood T1DM (9; 26), thereby representing an obvious target for antigen-specific intervention. Moreover, there is a strong relationship between diabetes-relevant insulin autoimmunity and the presence of HLA DR4-DQ8 allele (10), thereby establishing a molecular basis for antigen-specific intervention. Human insulin has been used
for decades to treat patients by injection, and more recently it has been given via oral, intranasal and inhaled routes in both patients and healthy subjects (5; 27-30). Mucosal administration of insulin is effective in inducing regulatory immune responses that can prevent autoimmune diabetes in animal models (31-35). On this basis, a placebo-controlled, double-blind/double-masked, primary intervention pilot study of mucosal (oral) insulin treatment in high risk children (Pre-POINT study) is proposed.

1.5 Preclinical studies using mucosal insulin

*Oral/intranasal insulin prevents diabetes in animal models.* In experimental animal models, administration of self-antigens to mucosa-associated lymphoid tissues can induce immune tolerance and prevent autoimmune disease (36; 37). Mucosal immunity generates a protective immune response manifested by Th2-type cytokines such as IL-4, IL-10, and TGF-β (38). In the NOD mouse, oral or intranasal administration of insulin or insulin peptides induces regulatory T cells that prevent autoimmune diabetes (31-35). Oral insulin therapy in NOD mice is most successful when given to neonates (39).

1.6 Clinical studies using mucosal insulin

*Mucosal insulin trials in man.* Studies using oral or intranasal antigen therapy in man, although reassuring from a safety perspective (29; 30), have so far not proven to have clinical benefit in human autoimmune diseases (27; 28; 40; 41). Oral insulin has been given to both diabetic and non-diabetic subjects without side effects (5; 27; 28). Children as young as 3 years of age have received oral insulin without side effects. Two studies have been performed administering insulin orally at doses ranging between 2.5 and 7.5 mg per day (together with standard insulin replacement therapy) in patients with new onset T1DM (27; 28). Neither demonstrated obvious benefit with respect to preservation of residual beta-cell function. One of these studies demonstrated increases in the regulatory cytokine TGF-β (42). Human recombinant insulin (7.5 mg per day) has been administered orally to *prediabetic* ICA and IAA positive first degree relatives of T1DM patients without significant beneficial effects (5). A sub-analysis of the data, however, showed significant benefit in those relatives with higher titer IAA (5), raising hopes that antigen therapy may be effective if we optimise the conditions and timing of administration. In this regard, a post-hoc analysis of the oral insulin DPT-1 data showed that after stratification of subjects by age, a potential benefit was best observed in the children aged less than 5 years of age (Dr. J. Barker, Denver, unpublished personal communication). Immunoregulatory effects of antigen vaccination may not be able
to counteract the pathogenic immunity in end-stage autoimmune disease, and asymptomatic individuals prior to preclinical autoimmune disease may be preferred candidates for such immunoregulatory therapy. No study in man has attempted to use autoantigen therapy for primary prevention of autoimmune disease. The advantage of primary prevention is that protection would be present before and at the time of initiation of the autoimmune process. This implies that autoimmunity per se could be prevented, rather than having to reverse a determined disease process.

1.7 Rationale for dose of insulin

In man, oral insulin has been administered at 2.5, 5, and 7.5 mg/day (corresponding to around 0.03-0.8 mg/kg/day for 9 kg to 85 kg participants) with no adverse effects. The potential beneficial effect on diabetes described in the DPT-1 cohort (5) was achieved at a dose of 7.5 mg/day. Based upon FDA conversion charts (http://www.fda.gov/cder/cancer/animalframe.htm), the proposed dose of 7.5 mg in a child aged 6 months to 6 years corresponds to approximately 9 to 18 mg/m²/dose. Previous studies in mice used doses ranging from 1 to 100 mg/kg per day in various time schedules (33; 43-45). In the study of Zhang, the optimal dose for efficacy in the mouse model was around 100 mg/kg given twice a week (33) corresponding to 300 mg/m²/dose or 600 mg/m²/week in man. A partial effect was observed at 10-20 mg/kg per day, but not at 1-2 mg/kg per day. The hypothesized mechanism of action of the study drug in preventing type 1 diabetes is by immunization to induce a regulatory immune response.

The Pre-POINT Study is a dose-finding pilot study that will address feasibility, safety and bioavailability of oral insulin to the immune system before proceeding to a larger phase II/III trial (POINT study). The Pre-POINT pilot study will provide the basis for dose selection in the POINT trial. Pre-POINT will test four doses of oral insulin: 2.5 mg, 7.5 mg, 22.5 mg, and 67.5 mg per day (three-fold increases between doses). The lowest dose (2.5 mg) is equivalent to the average dose used in the DPT-1 study (corrected for weight of subject). The highest dose (67.5 mg) is in the range of an efficacious dose in the mouse (see Table 1).
Table 1. Oral insulin doses used in mice in relation to those used in Pre-POINT.

A. Mouse – fed twice weekly from age 5 weeks, once weekly from age 10 weeks\(^a\)

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Diabetes incidence (%)</th>
<th>Effect</th>
<th>Mouse mg/kg dose</th>
<th>Human equivalent mg/m(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo</td>
<td>12 mo</td>
<td>Per dose</td>
<td>Per Week</td>
</tr>
<tr>
<td>PBS</td>
<td>20.5</td>
<td>49.2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.01</td>
<td>16.7</td>
<td>37.3</td>
<td>None</td>
<td>10</td>
</tr>
<tr>
<td>0.1</td>
<td>11.1</td>
<td>43.8</td>
<td>Slight, NS</td>
<td>100</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>8.0</td>
<td>(P&lt;0.02)</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\) data from Zhang et al, PNAS 1991

\(^b\) Efficacious dose equivalent in man

B. Proposed dose of oral insulin for children in Pre-POINT – given daily.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Age of child</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo</td>
</tr>
<tr>
<td>2.5 mg</td>
<td></td>
</tr>
<tr>
<td>- mg/m(^2)/day</td>
<td>5.9</td>
</tr>
<tr>
<td>- mg/m(^2)/week</td>
<td>41</td>
</tr>
<tr>
<td>7.5 mg</td>
<td></td>
</tr>
<tr>
<td>- mg/m(^2)/day</td>
<td>17.7</td>
</tr>
<tr>
<td>- mg/m(^2)/week</td>
<td>124</td>
</tr>
<tr>
<td>22.5 mg</td>
<td></td>
</tr>
<tr>
<td>- mg/m(^2)/day</td>
<td>53.1</td>
</tr>
<tr>
<td>- mg/m(^2)/week</td>
<td>372</td>
</tr>
<tr>
<td>67.5 mg</td>
<td></td>
</tr>
<tr>
<td>- mg/m(^2)/day</td>
<td>159.3</td>
</tr>
<tr>
<td>- mg/m(^2)/week</td>
<td>1116</td>
</tr>
</tbody>
</table>
1.8 The significance of Pre-POINT

The significance of the Pre-POINT study to T1DM is that if successful, a larger phase II/III primary intervention trial (POINT study) can be commenced in order to determine whether administration of mucosal insulin before the appearance of islet autoimmunity can delay or prevent the development of islet autoantibodies or diabetes in children who have both familial and HLA genetic risk for T1DM. This could lead to the development of a primary T1DM vaccination for genetically at risk subjects. The phase II/III POINT trial will use a dose of oral insulin (minimum 2.5 mg per day up to a maximum of 67.5 mg per day) that will be determined in the Pre-POINT pilot study based on safety and immunogenic effectiveness.

If primary intervention with oral insulin is found to be safe and efficacious in the Pre-POINT/POINT trial, it can be subsequently tested in subjects with lower a priori risk, and potentially be applied in subjects without a family history of T1DM. Primary intervention therapy would offer a number of advantages over intervention at a later stage of the disease process. A major benefit is that public health measures for screening and prevention could be applied to a disease that is currently increasing in prevalence and considered a worldwide burden.
2. Study Objective

The aim of the Pre-POINT study is to determine the feasibility, safety, and bioavailability of mucosal insulin administration (orally) in children with high genetic risk for T1DM in a dose escalation primary intervention pilot study.

3. Participating Centers

The Pre-POINT study is an international multi-center trial. Participation in Pre-POINT will be organized via national groups/Coordinating Centers. Each national group will be asked to agree to the terms of the final consensus protocol, and be responsible for its own internal organizational structure.

The Data Coordinating Center (DCC) will be in Munich shared between the Institut für Diabetesforschung am Helmholtz Zentrum München/ Forschergruppe Diabetes, Technische Universität München (Professor Ziegler) and the Clinical Trials Service and Research Unit, Ludwig-Maximilians University (Professor Hasford).

The following Centers will participate as clinical centers in the Pre-POINT study:

- **Austria**: national coordinator, Edith Schober, Vienna
- **Germany**: national coordinators, Anette Ziegler, Peter Achenbach, Munich, Dresden
- **UK**: national coordinator, Polly Bingley, Bristol
- **USA**: national coordinator, Georgeanna Klingensmith, Denver

4. Clinical Trial Design

4.1 Summary

The Pre-POINT study is designed as a randomized, placebo-controlled, double blind_double masked, multi-center, dose-escalation, primary intervention pilot study in which oral insulin will be administered daily to islet autoantibody negative children at high genetic risk for developing T1DM. The study will be monitored by an external Data Monitoring and Safety Committee. Recruitment will be carried out in both Europe and the USA to identify children who have an estimated genetic risk of >50% to develop islet autoantibodies and T1DM by adolescence. Twenty-five children will be randomized to treatment or placebo groups (3:2 randomization). Pre-POINT will assess safety of treatment with oral insulin at increasing
doses and determine the bioavailability of mucosal insulin to the immune system. A dose-finding committee will evaluate data pertaining to safety and immune bioavailability, and determine the dose of mucosal insulin to be used in a phase II/III primary intervention study (POINT study). Participants in the Pre-POINT pilot study will be on treatment for a maximum duration of 18 months, will then be followed in 6-monthly visits, and may continue to the phase II/III POINT study within their randomization group after consenting for participation in POINT.

4.2 Selection and withdrawal of study participants

4.2.1 Criteria for subject inclusion/exclusion

Pre-POINT will include both male and female subjects regardless of ethnic/racial background. Privacy rights of all children screened or recruited will be guaranteed according to Good Clinical Practice (GCP) guidelines.

4.2.1.1 Inclusion criteria for HLA screening

1. Written informed consent signed by both parents or legal guardians, or according to local ethical review board.
2. Children up to age 7 years who have a full sibling with T1DM
   OR
   Children up to age 7 years who have a multiplex first degree family history (both parents*, parent* and full sib, or two full sibs) of T1DM.

*Parents must have been diagnosed with diabetes before age 40 years and begun insulin therapy within 1 year of diagnosis.

4.2.1.2 Exclusion criteria for HLA screening

1. Children with any kind of congenital or acquired chronic disease that interfere with study objectives.
2. Assessed inability of family to participate in trial.
3. Prior or current participation in another intervention trial. Pre-POINT would also endeavour to avoid recruitment of children participating in other observational studies (e.g. TEDDY).
4. Chronic oral steroid use and/or other chronic oral immunosuppressant.

4.2.1.3 Inclusion criteria for intervention and follow-up
1. Written informed consent signed by both parents or legal guardians, or according to local ethical review board.

2. Children aged 2.0 years to 7 years (randomisation must be performed prior to 8\textsuperscript{th} birthday) who:
   A. have a multiplex first degree family history (both parents*, parent* and sib, or two sibs) of T1DM; and a type 1 diabetes susceptible HLA DR4-DQB1*0302 or DR4-DQB1*0304 haplotype and none of the following HLA DR or DQB1 alleles:
      
      \begin{itemize}
      \item DR 11
      \item DR 12
      \item DQB1*0602
      \item DR7-DQB1*0303
      \item DR14-DQB1*0503
      \end{itemize}

   or
   B. have a sibling with T1DM; and are identical by descent for the HLA DR3/DR4-DQ8 genotype with their diabetic sibling;

3. Islet autoantibody negative at time of recruitment.

*Parents must have been diagnosed with diabetes before age 40 years and begun insulin therapy within 1 year of diagnosis.*

Pre-POINT will include children aged between 2 years and 7 years. The phase II/III POINT study is proposed to include children from age 6 months. The lower age limit has been raised for Pre-POINT because more frequent collection of larger blood volumes will be required in the Pre-POINT protocol. It is anticipated that the lower age limit for eligibility will return to 6 months in POINT. This may require safety validation in a small number of children aged <2 years. Family history and HLA eligibility criteria will be the same for Pre-POINT and POINT.

4.2.1.4 Exclusion criteria for intervention and follow-up

1. Children with any kind of congenital or acquired chronic disease that interferes with their participation.

2. Assessed inability of family to participate in trial.

3. Chronic oral steroid use and/or other chronic oral immunosuppressant
4.2.2 Premature termination from the study

Participants may be prematurely terminated from the study if they withdraw consent from all future study activities, including follow-up, or if they are “lost to follow-up” (i.e. no further follow-up is possible because attempts to re-establish contact with the participants have failed). The Pre-POINT Study Group (including the DSMB and DCC; see chapter 7) must be promptly informed of such events.

4.3 Number of study participants

Twenty-five children will be enrolled (see figure – page 19). Each child will have a 60% likelihood of receiving the study drug.

First dose: 6 in active arm; 4 in placebo arm.

Higher doses: 3 new children in active arm; 2 new children in placebo arm.

Dose-escalation in individual children: In order to minimise the number of study participants required, and to have sufficient children at each study dose, children will be allowed to increase the dose of the study drug once during the dose-finding Pre-POINT study. Children recruited into one of the first two dose evaluations will be moved to a higher dose during Pre-POINT. These children will be moved to the higher dose 6 months after entering into the study. In this way, a total of 6 children will be included at each dose (three of these children will not have received insulin prior to entering the study and three will have received a lower dose of insulin for 6 months). Further dose increases or more frequent dose increases within individual children will not be performed during Pre-POINT in order to reduce the heterogeneity of potential carryover effects, and to limit the volume of blood collected from individual children.

4.4 Recruitment of study participants

Pre-POINT aims to recruit 5 children per 3 months into the study.

Recruitment will be from participating clinical centers in Germany, Austria, UK, and USA. Recruitment will be through a network of pediatric and neonatal clinics using specific advertising with flyers, magazine and journal articles. Patient support groups will be contacted for support and partnership in the recruitment. A web site (www.diabetes-point.org) and a toll-free telephone number will be available for the general public.

Recruitment to the study will have two steps. The first is genetic screening and autoantibody testing to determine eligibility, and the second step is recruitment of eligible subjects into the
trial. Informed consent from both parents or legal guardians is required, or according to local ethical review board for both steps (see Appendix 6). Consent forms will be modified to meet local IRB requirements.

4.4.1 Genetic screening and autoantibody screening

After consent a sample suitable for DNA extraction will be obtained from the consented child with a sample suitable for autoantibody testing. If the child does not have a multiple first degree family history of type 1 diabetes, DNA samples will also be collected from the child’s sibling who has type 1 diabetes and from the parents. Samples that can be used for autoantibody testing include serum from venous or capillary blood. Samples for genetic screening include mouth swabs and blood.

HLA typing will be performed according to the following algorithms:

A. Child has a multiple first degree family history of type 1 diabetes.

   HLA typing of child to determine HLA DR genotype;
   If DR4 then type for DR7, DR11, DR12, DR14, DQB1*0602, DQB1*0303, DQB1*0503

B. Child has a sibling with type 1 diabetes and no other affected first degree relatives

   HLA typing for DR3, DR4, DQB1*0201, DQB1*0302
   If child is DR3/4, DQB1*0201/0302 then type affected sibling for DR3, DR4, DQB1*0201, DQB1*0302;
   If affected sibling is DR3/4, DQB1*0201/0302 then type both parents for HLA DR to determine whether then child and affected sibling are identical by descent.

Children who are eligible after genetic screening will be tested for autoantibodies to insulin, GAD, and IA-2 in one of the core autoantibody laboratories.

4.5 Randomization of study participants

Children can be randomized if they are eligible on the basis of genetic criteria, if appropriate written consent has been obtained and if they have tested negative for insulin, GAD and IA-2 autoantibodies not more than 3 months prior to the randomization date.

Randomization (blinded): Children will be randomized to the study drug or placebo on a 3:2 ratio (see figure below).

No stratification of the study participants will be performed during Pre-POINT.
The Clinical Trial Service and Research Unit at the University of Munich will provide the randomization list for packaging the drugs in a double blind manner. The drug packs will be sequentially numbered. Opaque sealed envelopes will be provided by the Clinical Coordinating Center to the Sponsor and an independent study physician in case an emergency requires information regarding treatment. The integrity of these envelopes will be checked and they will be collected at the end of the study by the study monitors. All eligible children have to be registered and randomized via a web-based program.

Prior to escalation to doses 3 and 4, the DSMB will review unblinded subject data in particular examining frequency of total and significant hypoglycaemic events (see section 8.4 for details).

### 4.6 Treatment in Pre-POINT

Insulin (2.5 mg, 7.5 mg, 22.5 mg, or 67.5 mg per day) or placebo is given orally daily together with food for the duration of the study. The conversion of the mg unit into IU for the 2.5 mg dose of the oral insulin results in 71.8 IU in a 0.5 mL capsule, the 7.5 mg dose has 215.3 IU insulin in a 0.5 mL capsule, the 22.5 mg dose contains 645.8 IU insulin in a 0.5 mL
capsule, and the 67.5 mg dose has 1937.3 IU insulin in the 0.5 mL capsule. See section 6 for study drug information and administration instructions. For each dose, the insulin powder will be prepared as a 0.5 mL capsule containing the study drug plus filling substance (cellulose). Placebo will be identical capsules containing 175 mg filling substance. Capsules will be opened, the contents sprinkled on food such as yoghurt, fruit compotes, or bread, and the food containing the capsule contents consumed immediately. Administration will be performed daily with a recommendation that the medication is given at breakfast. The study drug should not be administered later than one hour before children go to bed. On days when blood glucose measurements are scheduled, the study drug may be given at any time that is convenient for the parents to allow blood glucose to be measured 1 hour after administration until one hour before children go to bed. The study drug should not be administered if the child has an infection or injury in the mouth or throat.

4.6.1 Dose-escalation in individual participants
Children will be allowed to increase the dose of the study drug once during the dose-finding Pre-POINT study. Children recruited into one of the first two dose evaluations will be moved to a higher dose 6 months after entering into the study (see figure above). A total of 6 children will be included at each dose (3 children will not have received insulin prior to entering the study and 3 children will have received a lower dose of insulin for 6 months). Further dose increases or more frequent dose increases in individual children will not be performed during Pre-POINT. Children who are randomized to placebo will remain in the placebo group throughout Pre-POINT.

*First dose* (2.5 mg oral insulin/day):
6 children in active arm
4 children in placebo arm

*Second dose* (7.5 mg oral insulin/day):
3 new children in active arm
2 new children in placebo arm
3 children increase the dose from 2.5 mg to 7.5 mg oral insulin per day after 6 months of treatment (the first 3 children who were enrolled at the first dose)

*Third dose* (22.5 mg oral insulin/day):
3 new children in active arm
2 new children in placebo arm
3 children increase the dose from 2.5 mg to 22.5 mg oral insulin per day after 6 months of treatment (the last 3 children who were enrolled at the first dose)

*Fourth* dose (67.5 mg oral insulin/day):
3 new children in active arm
2 new children in placebo arm
3 children increase the dose from 7.5 mg to 67.5 mg oral insulin per day after 6 months of treatment (the 3 children who were newly enrolled at the second dose and have not increased their dose before)

*Escalation will occur in both treatment and placebo group so that participants and study investigators will remain blinded to treatment throughout the study.*

Prior to escalation to doses 3 and 4 the DSMB will review unblinded subject data in particular examining frequency of total and significant hypoglycaemic events (see section 8.4 for details).

4.6.2 Expected timeline and duration of treatment in Pre-POINT

The dose-finding Pre-POINT study will aim to recruit 5 children per 3 months into the study. All children will be treated daily for a minimum of 3 months at the recruitment dose. Fifteen (9 receiving study drug and 6 receiving placebo) of the 25 participants will be moved to a higher dose after 6 months of treatment, and remain on that dose for the duration of the Pre-POINT study. Children who reach the endpoint of the proposed phase II/III POINT study (GADA or IA-2A positive or diabetes) will stop receiving treatment. All other children will be treated for the duration of Pre-POINT. If all proposed doses are tested and the recruitment into Pre-POINT study is completed within 15 months as expected, children will be treated for an average of 11 months and a maximum of 18 months during Pre-POINT. At the end of the dose-escalation phase, treatment will stop and children will be followed with 6-monthly visits. Children may restart treatment in POINT if they are still eligible (GAD and IA-2A antibody negative and have not developed diabetes). The study endpoint for a child is reached 30 days after termination of treatment.

4.7 Follow-up

Clinical visits with blood draw and collection of a saliva specimen will occur at 15 days, 3 months, 6 months and 6 monthly thereafter for each treatment dose. The trial is planned to run for 18 months. The Study Group will assess whether the treatment and follow-up will
continue after this period within the phase II/III POINT study. During the transition period between Pre-POINT and POINT, children who participated in Pre-POINT will continue to be followed with 6-monthly visits.

Blood draws will not exceed 25 ml at any one visit.

See Appendix 1 for planned study schedule.

4.8 Outcome measures in Pre-POINT

1. Safety of the study drug.
2. Immune response to insulin.

See Appendix 1 for planned study schedule of Pre-POINT.

4.8.1 Safety monitoring

1. Hypoglycaemia. A. Blood glucose measured before and 30, 60 and 120 minutes after drug has been administered on the first day of treatment at each dose level. Venous (preferably) or capillary blood (if venous blood draw fails) will be used for measurements. Blood glucose measurements on the first day of administration will be performed at a clinical center using a hexokinase method on an analyzer. B. Capillary blood glucose measured 60 minutes after drug has been administered on day 2 to 7 and monthly thereafter. It is recommended that, whenever possible, the child should be fasting between administration of the study drug and glucose measurement. Blood glucose will be measured on capillary blood obtained from a finger prick. These measurements will be performed at home by the families using a glucometer, which is provided by the Study Group. Electronically saved blood glucose measurements will be printed out at each monitoring visits and sent to the study center. Families will also be instructed to report suspected hypoglycaemic events.

All hypoglycaemic events will be graded as follows by the study center:

A. Presumed hypoglycaemia: Event with symptoms commonly associated with hypoglycaemia that ARE reversed by treatment with oral carbohydrate, but NOT documented with a blood glucose measurement at the time of the event.

B. Definite hypoglycaemia: Event with either

   BI. blood glucose measurement <50 mg/dl (<2.8 mmol/l) performed at the time of the event with or without symptoms commonly associated with hypoglycaemia,
BII. symptoms commonly associated with severe hypoglycaemia (e.g. loss of consciousness, convulsion, stupor) that are reversed by treatment with intravenous glucose or subcutaneous glucagons.

2. **Study visits.** Monitoring visits to a study pediatrician or a study center will be conducted at the start of treatment at each dose, after two weeks (Day 15) of treatment at each dose, after 3 months and 6 months of treatment at each dose, and then every 6 months until the trial is completed.

3. **Clinical examination and Laboratory tests.** Height and weight of the child will be recorded at start (Day 1) and every 6 months at the study visits and sent to the DCC. Blood samples will be taken for blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start (Day 1) and end of treatment with each dose.

4. **Allergy to the study drug.** Total IgE and IgE antibodies to insulin will be measured in plasma/serum obtained at the start of treatment at each dose, after two weeks (Day 15) of treatment at each dose, after 3 and 6 months of treatment at each dose, and then every 6 months until the trial is completed. Allergy/intolerance to the study drug will also be monitored by self-reporting by families. Parents/guardians will be instructed to look out for possible allergic reactions to insulin (allergic conjunctivitis, rhinitis, urticaria, angioedema, anaphylaxis) and immediately report this to the clinical coordinator at the study center.

5. **Psychosocial effects of study participation.** Questionnaires completed by families prior to and after 3 and 9 months participation in the study (see Appendix 3).

6. **Monitoring prior to dose escalation.** Prior to escalation to doses 3 and 4 the DSMB will review unblinded subject data in particular examining frequency of presumed and definite hypoglycaemic events.

**4.8.2 Recording of Safety Monitoring data**

All data forms will be sent to the regional Clinical Center. The data forms will be checked by the Clinical Center and families called if necessary (incomplete or incorrectly completed forms). The Clinical Center will report the data from the forms into the Pre-POINT web system. All paper data forms will be stored by the Clinical Center.

**4.8.3 Bioavailability of insulin to immune system**
The proposed mechanism of action is that through mucosal exposure to the study drug a protective immune response to the insulin autoantigen will be achieved, and that this response will subsequently be favoured if events that normally lead to a beta-cell destructive insulin autoimmunity arise. Although other mechanisms of action could be operative, the major focus of the mechanistic studies will be to determine whether administration of oral insulin leads to an immune response to insulin that has characteristics consistent with protection. The mechanistic studies will examine both B and T cell responses to insulin.

4.8.3.1 B cell responses

Although the nature of the events that give rise to IAA in genetically susceptible infants is unclear, we have demonstrated that the IAA preceding T1DM are of IgG isotype, typically initiating with IgG1 followed by expansion to include IgG4 clones, are reactive against proinsulin and rapidly become high affinity. We postulate that mucosal exposure to insulin will lead to differences in the humoral response and potentially include IgA-insulin antibodies, lower affinity insulin antibodies, and insulin antibodies that do not react with proinsulin. Since IgA responses may be restricted to mucosal surfaces, these will also be measured in saliva.

*B cell responses to insulin.* IgG and IgA antibodies to insulin in serum, and IgA antibodies to insulin in saliva samples measured before commencing treatment, and at every clinic visit (15 days, 3 months, 6 months and 6 monthly thereafter for each treatment dose).

4.8.3.2 T cell responses

In order to understand the nature of the immune intervention, we will study T cell responses both quantitatively as well as qualitatively in order to determine the effect of the therapy on the endogenous T cell response as well as trying to monitor the effectiveness of the therapy in changing the nature of the response by cytokines. T cell response measurements are part of a specific sub-study of the Pre-POINT clinical protocol, funded by the National Institute of Allergy and Infectious Diseases (NIAID) through the Autoimmunity Centers of Excellence (ACE).

Cell mediated responses to insulin and proinsulin peptides will be performed in consultation with Dr Gottlieb, Denver and Dr Peakman, London.

The ELISpot assay to proinsulin peptides as described by Arif (46) will be used. Measurements will be performed at the University of London in the UK for specimens.
collected in UK, in Munich for specimens collected in Germany and Austria, and in Denver for specimens collected in the USA. IFNγ and IL-10 responses to study drug and proinsulin peptides will be measured. These will be measured at the start of treatment, after 15 days, 3 and 6 months of treatment at each dose and at the end of treatment.

Antigen-specific therapy mediates its effect via the generation of various types of T regulatory cells. In human type 1 diabetes, it appears that the frequency of the CD4+CD25+FoxP3+T reg cell in circulation is normal while functional studies have differed in whether they may have a qualitative defect. Flow cytometry for evaluating T reg populations will be performed at core laboratory facilities in the UK (London) and Germany (Dresden, Munich) for specimens collected at European sites and in the USA (UC Denver) for North American and Canadian sites. Flow cytometry analyses to examine the frequency of CD4+CD25+FoxP3+, CD4+CD127loFoxP3+, activated CD4+, and activated CD8+ will be performed using previously described cell surface and intracellular staining methodologies. Blood samples will be collected at each site and shipped to each core facility for processing.

Cells that remain after the ELISpot and the Flow cytometry measurements will be stored frozen. These will be used to measure naïve and memory T cell responsiveness to study drug and to GAD65 by a CSFE dilution assay after all study samples have been collected.

**4.8.4 Recording of immune marker data**

Autoantibody and cell-mediated immune results will be reported directly to the Data Coordinating Center. The Data Coordinating Center will check results and enter them into the Pre-POINT data base. The Data Coordinating Center will generate reports for the positive islet autoantibody results in serum and notifies the appropriate Clinical Centers.

**4.8.5 Genetics**

Since polymorphism for the insulin gene (IDDM2) modifies T1DM risk in children and the study drug is insulin it is possible that the study drug has different effects with respect to IDDM2 genotypes. Genotyping of the IDDM2 insulin gene VNTR will therefore be performed in all trial participants.

**5. Psychological impact of study participation on families**
Genetic screening for disease risk raises a number of psychosocial and ethical issues (47-51). Parents may react with concern, anxiety or even depression when informed that their child is at increased risk of developing diabetes (50; 51). The psychosocial impact of participation in the Pre-POINT study on families will therefore be assessed at start and then after 3 and 9 months of treatment, and will continue to be assessed in the phase II/III POINT study after 21 and 33 months of treatment. Psychological variables will be recorded using a questionnaire (see Appendix 3) and will provide information on anxiety, coping and lifestyle changes of participating families. Similar questionnaires are used in other prospective studies from birth such as The Environmental Determinants of Diabetes in the Young (TEDDY) study. The demographic and psychosocial information may also be used to identify the pre-study family characteristics that discriminate study completers from study drop-outs.

Pre-POINT/POINT will assess:

1. *Parent’s worries and thoughts about the infant’s risk.*
   
   Parents will be asked to estimate their child’s risk over the course of the child’s life time (e.g. the child will never get diabetes, will get diabetes but a long time from now, will get diabetes in the near future) and compared to other children (e.g. risk for diabetes is greater, less, or a about the same as other children). These questions are based on structured interviews to estimate parent’s cognitive understanding of infant’s risk (52).

2. *Parent’s distress (anxiety and depression) in response to the news that their child is at risk.*
   
   This will be assessed both immediately after the parent is informed of the infant’s genetic test results and on follow-up. The 20-item State portion of the State-Trait Anxiety Inventory (STAI) (53) is a reliable assessment instrument for assessing situation-specific anxiety. It has been used in several studies assessing anxiety in islet cell antibody (ICA) positive children and adults and their family members in Florida (54-56), in the German Nicotinamide Study (DENIS) (57; 58), and in the BABYDIAB (59) and BABYDIET studies. It has also been used to assess anxiety in mothers after they were told their infant was genetically at-risk for T1DM in PANDA (Prospective Assessment in Newborns of Diabetes Autoimmunity) (52).

   The Pre-POINT/POINT questionnaire will use a 6-item short form of STAI, which was evaluated in the PANDA study and is currently being used in the TEDDY protocol, and
a 6-item depression scale, which was used in several studies in a Well-Being Questionnaire (60) to measure depression in T1DM and T2DM.

Based on previous studies that have examined anxiety in parents of children at increased risk for diabetes (52; 54-58) it is expected:

a. Anxiety and diabetes worry will be elevated immediately after informing parents of their infant’s genetic risk.

b. Anxiety and diabetes worry will dissipate with time.

c. Although some mothers may report depression, depression will not be elevated in the group as a whole.

d. Elevated levels of depression will be associated with elevated levels of anxiety and diabetes worry over time.

3. Parent’s efforts to cope with the infant’s at-risk status and the participation in the study. This will be assessed using a modified version of the 68-item Ways of Coping Checklist (61; 62). This instrument has been extensively used in the U.S. and Europe and provides five coping scale scores: Problem Focused (e.g. “made a plan of action and followed it”); Seeks Social Support (e.g. “accepted sympathy and understanding from someone”); Wishful Thinking (e.g. “hoped a miracle would happen”); Avoidance (e.g. “tried to forget the whole thing”); and Blamed Self (e.g. “realized you brought the problem on yourself”). The instrument has been previously used with mothers of ICA+ children (56). Data using the instrument were also collected from 342 PANDA mothers with an infant at genetic risk for diabetes. Internal consistency estimates were adequate for most scales (Problem-Focused Coping $\alpha = .82$; Seeks Social Support $\alpha = .76$; Wishful Thinking $\alpha = .76$; Avoidance $\alpha = .44$; Blames Self $\alpha = .63$).

These data were used to shorten the measure in Pre-POINT/POINT to 48 items (including adding some new items to improve the reliability of Avoidance and Blames Self). Furthermore, three items of control are included, one assessing perceived control (“I can do something to reduce my child’s risk of diabetes”), one perception that the medical team can do something to prevent (“Medical professionals can do something to reduce my child’s risk for developing diabetes”) and one assessing perceptions of chance or fate (“It is up to chance or fate whether my child develops diabetes).

Based on previous studies with parents of ICA+ children (56) and mothers of genetically at-risk infants (63) it is expected:

a. High anxiety will be associated with use of more coping strategies.
b. Use of multiple coping strategies will decline over time.

c. Use of coping strategies will increase in response to any infant blood test result indicating possible progression toward diabetes.

d. Blamed self and avoidance coping strategies will be associated with maintenance of anxiety over time.

4. *Any changes in behavior that the family may make in an effort to prevent the disease.*

Based on previous studies (54; 64-66) it is expected that many parents will report changes in behaviour in an effort to prevent diabetes in the child, especially dietary changes, or changing to a healthier lifestyle. Parents who report greater perceived control will be more likely to report behaviour changes.

5. *Parental satisfaction with participation in the study.*

6. **Study Drug**

6.1 **Preparation and distribution of study drug**

Human insulin for oral administration is provided by Lilly Pharmaceuticals, Indianapolis, Indiana USA. It will be provided as bulk human insulin crystals. This insulin is sold by Lilly as an injectable formulation known as Humulin-R.
These insulin crystals will be prepared in a 0.5 mL capsule containing the study drug plus filling substance (cellulose). Placebo will be identical capsules containing 175 mg filling substance.

Families will be given their allocated study capsules on a 3-monthly basis.

6.2 Safety profile of drug

Safety profiles of the active insulin used in the study are available from the Investigator’s Brochure.

6.3 Study drug administration

Capsules will be administered with food. Capsules will be opened, the contents sprinkled on food such as cereal, yoghurt, fruit compotes, or bread, and the food containing the capsule contents consumed immediately. Administration will be performed daily with a recommendation that the medication is given at breakfast. The study drug should not be administered later than one hour before children go to bed. On days when blood glucose measurements are scheduled, the study drug can be given at any time that is convenient for the parents to allow blood glucose to be measured 1 hour after administration but not later than one hour before children go to bed. The study drug should not be administered if the child has an infection or injury in the mouth or throat. The capsule contents have no discernible taste, do not alter the taste of the food, and the study drug capsule and placebo capsule contents are indistinguishable by sight, taste, or smell.

6.4 Monitoring compliance

Parents will be asked to complete daily records of capsule intake (see Appendix 5 for daily compliance sheet) and submit these monthly to the study center. The study center will subsequently send these records to the DCC. The DCC will monitor the frequency and completeness of study drug intake as well as difficulties in study drug administration. The data will be compiled by the DCC and regularly summarized for the DSMB. Unused study drug will be collected from families and stored by the study center. The number of unused capsules will be used as a validation of family reported data. Compliance will be a composite of the family reported and the number of unused capsules.

6.5 Concomitant medications
The use of concomitant medications will be assessed at each study visit and recorded on the appropriate CRF. Participants are allowed to use all medications as advised by the investigator or the referring physician. Prohibited medications during study are oral steroids for longer than 7 days and/or other oral immunosuppressants.

6.6 Drug accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator is required to maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed. Records of receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed. All records regarding the disposition of the investigational product will be available for inspection by the clinical trial monitor.

7. Organization of the Study

7.1 Management Group

Principal Investigator and Study Coordinator: Ezio Bonifacio, Dresden
Deputy Coordinator: Anette-G. Ziegler, Munich
Project Administrators and Clinical Coordinators: Peter Achenbach, Munich
        Georgeanna Klingensmith, Denver
Data Coordinator and biostatistician: Joerg Hasford, Munich
Study advisor: Polly Bingley, Bristol

7.2 Steering Committee

The steering committee will be the body that will formally vote on amendments to the study protocol. The steering committee will consist of the study principal investigator, a senior representative from the data coordinating center, and a senior representative from each of the clinical centers.
7.3 Data Safety Monitoring Board

The DSMB will have five international members that include a Chair (Chantal Mathieu), two clinical paediatricians, an expert in immunology, and an expert in bio-epidemiology/-statistics. A JDRF representative (Teodora Staeva-Vieira) will be an *ex-officio* member of the DSMB.

Functions and responsibilities:

a. Safeguards confidentiality of and interests of individuals included in the study.

b. Monitors safety parameters during the study.

c. Advise the Study Group in the event of
   i. A positive result being found.
   ii. A negative result being found.

7.4 Dose-finding committee

The Study Principal Investigator, the Deputy Coordinator, the Data Coordinator and biostatistician, and the Study Advisor will form the committee that will decide on the dose that will be proposed for the POINT study. This committee will be advised by the DSMB.

Functions and responsibilities:

a. Review safety data obtained for each dose of the study drug during Pre-POINT.

b. Review immune response data at the completion of the Pre-POINT study.

c. Propose a dose of the study drug that would be tested for efficacy in the phase II/III POINT study.

7.5 Study Group

This will include the Management group, and up to 2 representatives of the national groups involved in the study.

Functions and responsibilities:

a. Assume responsibility for protocol development and execution of the project.

b. Allocates responsibility for data analyses arising from the study.

c. Monitors and advises on the activities of the Coordinating Center.

d. Safeguards the confidentiality of and interests of individuals included in the study.

7.6 Coordinating Centers

This will be the Institut für Diabetesforschung am Helmholtz Zentrum München/ Forschergruppe Diabetes, Technische Universität München, Munich, Germany and the
Clinical Trials Service and Research Unit at the Department of Medical Biometry and Epidemiology, Ludwig-Maximilians University, Munich, Germany.

Functions and responsibilities:

Clinical Coordinating Center
a. Practical administration of the study.
b. Communication with all interested parties.
c. Development of and provision of written guidelines (SOPs), and forms relating to the project.
d. Managing queries.
e. Supervision of Monitoring.
f. Reporting of ADR/AE according to the current laws and regulations.

Data Coordinating Center
a. Assistance in preparing quality control procedures.
b. Providing randomization lists.
c. Providing web-based randomization and registration.
d. Checking incoming data for completeness and plausibility.
e. Data entry.
f. Programming of data bank and loading of data.
g. Providing regularly reports about patient recruitment.
h. Providing every three months reports about recruitment and safety for the DSMB.
i. Preparing 3-monthly analyses.
j. Preparing the final analysis.
k. Writing the biostatistical parts of the final clinical study report.
l. Assistance for the preparation of presentations and publications.
m. Safeguarding integrity, data safety and appropriate storage of the data.

There will be a very close collaboration between the CCC and the DCC.

7.7 Core Laboratories
1. HLA testing will be performed centrally in the USA and in Europe.
2. Autoantibody outcome will be measured by the TEDDY autoantibody labs (Bristol, UK and Barbara Davis Center Denver Colorado). Using TEDDY thresholds for positivity, both laboratories had a specificity of 99% for GAD antibodies and 100% for IA-2
antibodies in the 2005 DASP workshop. Sensitivities for GAD antibodies were 78% and 76%, and for IA-2 antibodies were 72% and 66%.

2. Fasting blood glucose values, blood values and biochemistry will be measured locally.

3. Mechanistic autoantibody markers will be measured in Munich (Ezio Bonifacio and Peter Achenbach).

4. T cell ELISpot assays will be performed in Dresden and Munich, Germany for German and Austrian subjects, London (Mark Peakman) for UK subjects, and Denver (Peter Gottlieb) for US subjects using the TrialNet protocol.

5. IDDM2 genotyping will be performed in Denver.

7.8 Trial Monitors

Study monitors will be appointed. All study personnel will be trained for GCP with respect to this trial prior to commencement of the study. Personnel will be certified to understand and conform to GCP expectations and regulations for this study. Conformation to these regulations will be overviewed by the Data Coordinating Center.

8. Safety Monitoring

8.1 Definitions

8.1.1 General

Adverse events that are classified as serious according to the definition set forth by the health authorities must be reported promptly to the DSMB, health authorities, Pre-POINT investigators, and institutional review boards (IRBs). This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guideline for Good Clinical Practice*, and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events version 3.0* (June 10, 2003).

8.1.2 Adverse event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom (including an abnormal laboratory finding), or disease that is temporally associated
with the use of a medicinal product whether considered related to the medicinal product or not.

An adverse event will be followed until it is resolved or until 30 days after a participant terminates from the study, whichever comes first.

8.1.3 Serious adverse event (SAE)
An SAE is defined as “any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution.” This includes but is not limited to any of the following events:

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the patient or participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Regardless of the relationship of the adverse event to study drug, the event must be reported as an SAE if it meets any of the above definitions.

8.1.4 Unexpected adverse event
An adverse event is considered “unexpected” when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the investigational plan, or the investigator’s brochure.

8.2 Adverse events
8.2.1 Collecting procedure

Adverse events will be collected from the time the participant begins study treatment until the time the event is resolved or until 30 days after the participant completes study treatment, whichever comes first.

Adverse events may be discovered through any of these methods:
1. Observing the participant.
2. Questioning the participant or the guardian of the participant in an objective manner.
3. Receiving an unsolicited complaint from the participant or the guardian.

An abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the participant’s safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate laboratory evaluation form(s).

8.2.2 Recording procedure

Throughout the study the investigator will record all adverse events on the appropriate adverse event Clinical Report Form (CRF) regardless of their severity or relation to study medication or study procedure. The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.2.3 Grading and attribution

8.2.3.1 Grading criteria

The study site will grade the severity of adverse events experienced by study participants according to the criteria set forth in the National Cancer Institute’s Common Terminology Criteria for Adverse Events Version 3.0 (published June 10, 2003).

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:
Grade 1 = mild adverse event.
Grade 2 = moderate adverse event.
Grade 3 = severe and undesirable adverse event.
Grade 4 = life-threatening or disabling adverse event.
Grade 5 = death.
All adverse events will be reported and graded whether they are or are not related to disease progression or treatment.

8.2.3.2 Definition of attribution
The relationship, or attribution, of an adverse event to an investigational product will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE report form. The relationship of an adverse event to the study treatment will be defined according to the NCI-CTCAE attribution of adverse events provided below.

Category 1 = unrelated: The adverse event is clearly not related to the investigational agent(s).
Category 2 = unlikely: The adverse event is doubtfully related to the investigational agent(s).
Category 3 = possible: The adverse event may be related to the investigational agent(s).
Category 4 = probable: The adverse event is likely related to the investigational agent(s).
Category 5 = definite: The adverse event is clearly related to the investigational agent(s).

8.3 Serious adverse events (SAE)

8.3.1 Collecting procedure
Serious adverse events (see definition 8.1.3) will be collected from the time the participant begins study treatment until 30 days after he or she completes study treatment or until 30 days after he or she prematurely withdraws from the study.

8.3.2 Recording procedure
Serious adverse events will be recorded on the adverse event CRF and on the SAE form.

8.3.3 Reporting procedure
The following process for reporting a serious adverse event ensures compliance with the ICH guidelines and 21CFR §312.32.

8.3.3.1 Reporting criteria
After the SAE has been assessed (see definition 8.1.3), the event will be reported to the appropriate health authorities in the required manner based on the following criteria:

No reporting. This requirement applies if the adverse event is deemed not serious by the Clinical Coordinating Center as defined by the Pre-POINT Study Group.
**Standard reporting.** This requirement applies if the adverse event is classified as any of the following:

1. Serious, expected, and drug related.
2. Serious, expected, and *not* drug related.

**Expedited reporting.** This requirement applies if the adverse event is considered serious, *unexpected*, and *drug related*. This type of SAE must be reported by the National Coordinator to the appropriate health authorities within 15 days; fatal or life-threatening events must be reported within 7 days.

All serious adverse events (expected and unexpected) will be reported to local IRBs and the US Federal Drug Administration (FDA) within 48 hours, or sooner if mandated by the IRB, from the time the investigator learns of such events.

**8.3.3.2 Reporting timeline**

When an investigator identifies an SAE, he or she must notify the Sponsor/Clinical Coordinating Center (Technische Universität Dresden, Bonifacio – Center for Regenerative Therapies, Fetscherstrasse 105, 01307 Dresden, Germany; email: ezio.bonifacio@crt-dresden.de; Telephone: +49 351 458 82100) within 24 hours (up to maximum 48 hours) of discovering the event. In addition to telephone reporting, the investigator must ensure that these events are entered on the SAE report form (see Appendix 7) and the adverse event CRF. Both forms will be faxed to the Clinical Coordinating Center within 48 hours.

**8.3.3.3 Notifying the Study Group and health authorities**

The Clinical Coordinator is responsible for notifying the Study Group Principal Investigator. The Principal Investigator is responsible for disseminating reports to the health authorities, all investigators in the study, and the study drug manufacturer.

**8.3.3.4 Notifying the DSMB**

Listings of all SAEs will be provided to the DSMB on an ongoing basis. Furthermore, the DSMB will be informed of expedited reports of SAEs at the same time as health authorities.

**8.3.3.5 Notifying the IRB and Ethics Committee**

The investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB and Ethics Committee and the US FDA in accordance with applicable regulations and guidelines.
8.3.4 Updating source documentation

Documents describing the safety profile of a drug, such as the investigator’s brochure, will be amended as needed by the study drug manufacturer to ensure that the description of safety information adequately reflects any new clinical findings. Until these documents are updated, expedited reporting will be required for additional occurrences of a reaction.

8.4 Safety monitoring procedures

Safety of the trial will be overseen by an external Data Safety Monitoring Board. All case report forms information will be received by the CTSRU, the Data Coordinating Center in Munich, by a web-based CRF system. The data are checked for completeness and plausibility and transferred into a data base. Every three months a masked report by the DCC will provide relevant information on trial progress and safety for the DSMB. The DSMB may advise if an increase in the dose of study drug should be done (based on safety data) or if the trial should be terminated (based on positive or negative outcome or safety criteria).

The DSMB will be expeditiously informed of all adverse drug reactions that are serious or unexpected. Study Centers will be instructed to report serious adverse events to the Sponsor by fax or telephone (fax: +49 351 458 82109; phone: +49 351 458 82100) within 24 hours of the investigator becoming aware of the event. All investigators and centers will be informed of such events and of findings that could adversely affect the safety of subjects, impact the conduct of the trial or alter the IRBs opinion to continue the trial. Reports will comply with the ICH Guidance for Clinical Safety Data Management.

Prior to escalation to doses 3 and 4 the DSMB will review unblinded subject data in particular examining frequency of total and significant hypoglycaemic events. Subjects will be treated with the prior dose for a minimum of 3 months. Data will be compiled by the DCC and submitted to the DSMB for review prior to escalation to the next dose level. Escalation will only occur if no increase in definite or presumed hypoglycaemic events are noted.

Specific safety monitoring that will be performed are:

1. **Hypoglycemia.** A. Blood glucose measured before and 30, 60 and 120 minutes after drug has administered on the first day of treatment at each dose level. Venous (preferably) or capillary blood (if venous blood draw fails) will be used for measurements. Blood glucose measurements on the first day of administration will be performed at a clinical center using a hexokinase method on an analyzer. B. Capillary blood glucose measured 60 minutes after drug has been administered on day 2 to 7 and
It is recommended that, whenever possible, the child should be fasting between administration of the study drug and glucose measurement. Blood glucose will be measured on capillary blood obtained from a finger prick. These measurements will be performed at home by the families using a glucometer, which is provided by the Study Group. Electronically saved blood glucose measurements will be printed out at each monitoring visits and sent to the study center. Families will also be instructed to report suspected hypoglycaemic events. The families will be called at days when capillary blood glucose measurement is scheduled asking for the recent blood glucose levels and the child’s well-being including allergic reactions, infections and any disease or discomforts. At telephone interviews entries in the short monitoring questionnaire Compliance Control Sheet (see Appendix 5) that are filled out daily by the parents will also be discussed.

2. Monitoring visits to a study pediatrician or a study center will be conducted at the start of each dose, after two weeks (Day 15) of treatment at each dose, after 3 and 6 months of treatment (or end of treatment for children treated less than 6 months) at each dose, and then every 6 months until the trial is completed. Height and weight of the child will be recorded at start (Day 1) and every 6 months at the study visits and sent to the DCC. Blood samples will be taken for blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start (Day 1) and end of treatment with each dose. These will be sent to the DCC and a summary provided to the DSMB in a blinded manner.

3. Venous blood samples obtained at pediatric visits will be tested for diabetes-autoantibodies in the core laboratories. Results will be sent to the DCC. Clinical Sites will be notified if a participant is confirmed GAD and/or IA-2 autoantibody positive. Families will receive confirmed positive results of these autoantibody-tests communicated by telephone and in written form.

4. Total IgE and IgE antibodies to insulin will be measured at start of treatment at each dose, after two weeks (Day 15) of treatment at each dose, after 3 and 6 months of treatment at each dose, and then every 6 months until the trial is completed to check whether the child develops allergy to the study drug. Results will be sent to the DCC and a summary provided to the DSMB in a blinded manner.

5. Questionnaires to be completed by families at the start, after 3 months, and then every 6 months until the trial is completed (see Appendix 2).
6. HbA1c will be measured in GAD and/or IA-2 autoantibody positive children every 6 months.

7. Oral glucose tolerance tests will be measured every 6 months in children positive for GAD and/or IA-2 autoantibodies.

8. Psychosocial questionnaires completed by families prior to and after 3 and 9 months participation in the study (see Appendix 3).

Diabetes will be diagnosed by fasting or casual blood glucose measurements or OGTT using ADA/WHO criteria. An OGTT is not required for the diagnosis of diabetes in children when it is otherwise impractical to perform the test. Children who have a casual capillary blood glucose level equal or greater than 200 mg/dl [11.1 mmol/l] measured on two occasions with a blood glucose meter are required to verify the blood glucose level in a standardized laboratory measure. When symptoms are present, diabetes can be diagnosed if a single fasting blood glucose measure is equal or greater than 126 mg/dl [7.0 mmol/l] or a casual blood glucose measure is equal or greater than 200 mg/dl [11.1 mmol/l]. When no symptoms of diabetes are present, blood glucose criteria must be met on two separate samples.

9. Stopping Rules

9.1 Stopping rules for premature termination of individual treatment

The participant will no longer receive study medication if any one of the following is observed:

1. Two hypoglycemic events (Definite Hypoglycaemia; grade BI and BII; see 4.8.1) within a 1 month time frame that can be attributed to the administration of the insulin.

2. Allergic reactions or sufficient discomfort that can be attributed to the administration of the insulin.

3. Confirmed positive [positive on 2 samples] GAD autoantibodies or confirmed positive IA-2 autoantibodies.

4. Development of Type 1 Diabetes

Study treatment may also be discontinued if the investigator believes that the study treatment is no longer in the best interest of the participant. Participants who prematurely discontinue study treatment will remain in study and undergo all efficacy and safety assessments.

9.2 Stopping rules for premature termination of enrollment of participants
Enrollment in the study will be suspended if any one of the following occurs:
1. The DSMB requests termination of the study upon review of safety data.
2. Any death related to study therapy.
3. Two or more of the first six participants treated with oral insulin experience an adverse event resulting in the permanent discontinuation of study treatment.

The DSMB will be immediately notified of any such event. Resumption of enrollment is contingent upon a favorable DSMB review.

9.3 Stopping rules for premature termination of the study

The DSMB will evaluate safety parameters every three months. The trial will not continue if it is found that the administration of oral insulin increased the frequency of hypoglycaemia, altered growth, or increased concurrent diseases.

If the DSMB approves continuation of the trial, the trial may be stopped at any time if relevant hypoglycaemic events are significantly increased in the treatment group, or if there are sufficient adverse side effects that warrant stopping the trial.

The dose-finding committee will determine the end of all or part of Pre-POINT if or when:
1. The study drug is shown to be unsafe at the lowest dose.
2. A higher rate of GAD and/or IA-2 antibodies than expected is found in treatment groups. The appearance of persistent (two samples) GAD or IA-2 antibodies from three of six children at a single dose level will be considered as evidence of disease acceleration and will cause the trial to stop.
3. The study is no longer feasible or becomes very unlikely to provide information that will allow decisions pertaining to the dose to be used in POINT to be made. The study will be considered unfeasible if a. Recruitment is found to be too slow for the study to be conducted within the proposed study period; or b. The dropout rate before the 3 month evaluation visit is >33%; or c. Compliance to the protocol as determined by family reporting of administering medication or adherence to visits and specimen collection is <50%.
4. A safe dose of oral insulin that is shown to cause a change in the immune response to the study drug is identified.
5. All proposed doses of oral insulin have been tested.
6. Other information becomes available relating to the safety, immune bioavailability or efficacy of the study drug that no longer requires the completion of Pre-POINT in order to decide on the POINT protocol.

10. Statistical Consideration and Analytical Plan

10.1 Statistical Considerations for Pre-POINT

Pre-POINT will examine safety, feasibility and immune response. Safety will be monitored continuously over the entire study period. Safety data will be assessed before treatment with a new dose of oral insulin will be started.

The number of six individuals per group is typical in a dose-finding study of this sort. This number is based on the expected rate of development of IAA in untreated children with these genetic characteristics of whom up to 5% (i.e. 0 - 1 per group) are expected to develop IAA within 3 months (the time from study entry at which immune responses will be assessed). The appearance of IAA in two of the six individuals will be suggestive of an immune response but will require confirmation at the next dose level, and the appearance of IAA in five of six children at a single dose level will be considered as evidence of a response that could result in no further dose increases.

The DSMB will be presented with a summary of IAA results from both the subjects receiving the study drug and the subjects receiving placebo. If all doses are tested, the placebo group will be up to 10 individuals. The DSMB will have information from sequential doses and will use all information in their assessment of immune response efficacy. They will advise the Dose finding committee if IAA data are consistent with a drug related immune response (5 of 6 children).

With respect to safety (hypoglycemia and allergy), all adverse events will be reviewed by the DSMB assessment. Safety monitoring will be continued in phase II/III POINT study and safety will therefore eventually be assessed on larger numbers.

10.2 Outcome analysis in Pre-POINT

Parameters will be compared between the 6 children treated with one dose of oral insulin and the children who received placebo. The number of children on placebo will increase with increasing insulin doses. For the lowest dose, 4 placebo children will be available for comparison, increasing to 6, 8, and 10 children over the next 3 doses, respectively. Evaluation
will be undertaken by the dose-finding committee as soon as all data for individual doses are available (see Flow Chart above for expected time schedule; page 19). Data will be prepared by the DCC.

10.2.1 Analysis of safety data

All adverse events will be analysed with descriptive statistical methods.

1. **Hypoglycaemia.** Any hypoglycaemia (grade A, BI and BII; see 4.8.1) will be considered as an adverse event. Any two adverse events of grade BI in a single child should be investigated using laboratory measurements of glucose. The child’s physician in consultation with the Clinical Coordinator will determine action with respect to continued participation in the study.

2. **Total IgE and IgE-IAA.** Increases in total IgE above normal levels after commencement of treatment will be reported as an adverse event. IgE-IAA within 3 months after commencing treatment are very unlikely to be present in children who have not received the study drug. IgE-IAA without high total IAA titers will be considered evidence that the study drug can induce allergy (only data obtained within the first 3 months will be used for decision making with respect to moving to the next dose).

3. Other clinical and blood measurements will be described and assessed to determine whether the study drug causes unexpected changes or adverse events.

4. Any serious adverse event (including strong allergic responses) that can be attributed to the study drug will be sufficient to consider not using that dose.

5. An analysis of the impact of participation on the child and family will be performed and considered prior to proceeding to the phase II/III POINT study.

10.2.2 Analysis of immune response data

1. **Antibodies to insulin.** The risk of developing IAA in untreated children within 3 months is around 5%. Thus, the appearance of IAA (IgG or IgA) in the serum within 3 months of starting treatment in 2 of the 6 children receiving the study drug will be considered suggestive, and in >2 children as evidence that there is an immune response to the study drug.

Since the response may be restricted to the site of drug administration, IAA measurements will also be performed in saliva samples. These results will be described for children receiving the study drug and those receiving placebo. Evidence that there is an antibody response to the study drug will be indicated by increased signals in the IAA assay in treated compared to placebo subjects as well as between doses.
2. **T cell responses to insulin.** Cytokine responses in the ELISpot assays will be described for children who receive placebo or study drug. Significant quantitative differences (increase in treated children) to insulin or one or more of the four insulin peptides will be taken as evidence that there has been an immune response to the study drug at that dose.

**10.3 Considerations for planned POINT efficacy study**

The purpose of Pre-POINT is to identify a dose of insulin administration suitable to test for efficacy in the POINT study. The dose-finding committee will determine the dose of mucosal insulin for the phase II/III trial. One of two scenarios is likely. The first is that children will be randomized to one dose of oral insulin or placebo. The second is that the phase II/III trial will not proceed at the end of the dose escalation.

Pre-POINT will proceed to POINT if and when the dose-finding committee identifies a dose that can be tested for efficacy and determines that the efficacy study is feasible. The dose-finding committee is expected to choose a dose that is safe, feasible, and to which an immune response can be observed.

POINT will not proceed without approval by the funding agency and the IRBs.

Children still participating in Pre-POINT who have not become GAD or IA-2 antibody positive and have not developed diabetes will be moved to the dose chosen for POINT, remaining in their allocated treatment group (active or placebo) and take part in the POINT study. Children who have developed insulin antibodies but are GAD and IA-2 antibody negative will be considered eligible to continue to POINT if it is determined that the study drug could induce insulin antibodies at the administered dose.

POINT will continue as a randomized placebo-controlled, double-blinded/double-masked multi-center phase II/III primary intervention trial. The POINT study protocol will be developed considering the findings obtained in Pre-POINT or other relevant studies, as well as IRB requests. The study numbers will be those consistent with a trial aimed to determine 50% reduction in outcome with 80% power (two tailed alpha, 0.05).

Prior to initiating the POINT study, the investigators will conduct an end of Phase 1 meeting with the FDA. The investigators will compile safety data and data related to bioactivity/dose
response on immune marker into and clinical study report for FDA comment on study design and safety issues.

11. Identification and Access to Source Data

11.1 Identifying source data

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The results of all clinical and clinical laboratory evaluations will be maintained in the participant’s study records and the data will be transferred to clinical CRFs. Safety data will be recorded on CRFs specifically designed for this purpose. All the SAEs will be reported on an SAE report form (see Appendix 7) as well as on individual CRFs. All data will be reviewed periodically by the DSMB. The DSMB and/or the IRB have the authority to advise withdrawal any participants and/or termination of the study because of safety findings.

11.2 Permitting access to source data

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants in this clinical trial. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information will be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals. The investigational site will normally be notified before auditing visits occur.

12. Quality Control and Quality Assurance

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. Clinical Centers will be supervised by the DCC.
The DCC will review the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

Each Clinical Center is required to submit a 5% random sample of form sets to the DCC on a quarterly basis for a manual quality control review. A list of randomly generated ID numbers for each clinic is supplied by the DCC. The manual review of forms by staff of the DCC will entail a page-by-page review of the following items:

- form completion
- affixed Pre-POINT ID and quality control labels, when applicable
- interviewer IDs
- skip patterns observed
- ethnicity and study coding; and
- overall form consistency and preparedness for data entry.

A report of errors and/or recommendations for improvement will be sent to the Clinical Center and the Director of the DCC following each review.

There are quality assurance measures performed at the DCC for each type of data entry available to Clinical Centers, to capture data errors. Verification of the form data is done at the DCC after the form data has been entered onto the web-based system by the Clinical Center, and before the data is automatically inserted into the Pre-POINT database. This allows for system read errors to be corrected.

Another data quality assurance measure is the generation of quarterly compliance reports for each Clinical Center. These reports are used to assess the completeness, accuracy and timeliness of data entry. Information generated by these reports includes the percentage of forms that are considered complete, the percentage of forms that are completed by their due date, and a list of forms that are overdue. Pre-POINT is targeted to achieve levels of 95% each for compliance, timeliness and accuracy. If a particular level falls below 95%, the result is reported to the appropriate Clinical Center(s) and the Steering Committee. Each Clinical Center is then required to take commensurate remedial action.

The following are areas for quality control review of Clinical Centers:

a. Observation of HLA Screening Sample Collection

b. Review of Recruitment

Measures to be evaluated include: Goals; Changes to projections; Recruitment strategies and materials; Refusal records
c. Review of Data Entry/Edits
Measures to be evaluated include: Missing data; Errors; Data entry flow and timing from collection to entry

d. Review of Logs
This will include: Daily Freezer Temperature Log; Discarded ID Log; Participant and QC Selection Log/ Adequacy of QC Sampling; Data Editing Log; Other Clinical Center Logs

e. Storage of Forms
Review includes: Data Collection Forms; Shipping Forms from Laboratories; Layered Portion of Informed Consent Forms from Clinics; IRB Approvals/Informed Consents

f. Study Documents
Manual of Operations; Protocol

g. Miscellaneous
Communication with Laboratories; Communication with Coordinating Center; Reimbursement/ Invoicing; Adverse Events

Core laboratories will be reviewed using sample exchange and split duplicates. The DCC will implement methods to evaluate its management procedures and to assess the cost efficiency of various procedures for data handling and analysis.

13. Ethical Considerations and Compliance with GCP

13.1 Statement of compliance
This clinical study will be conducted using good clinical practice (GCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Ethics Committee or IRB. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

13.2 Potential benefits and risks to subjects

13.2.1 Benefits
The potential benefit for a participating child would be the prevention of T1DM. A benefit would also be a marked delay in the development of the destructive process against the beta-
cells. Because all participating children, including children who receive placebo, have a high risk of developing T1DM, testing blood samples in the study will allow early recognition of an immune response against the beta-cells, close monitoring and characterization of this response, and regular blood glucose testing. In children who develop islet autoantibodies, treatment to prevent the development of diabetes in autoantibody positive subjects could be offered as soon as this becomes available. If a participating child develops T1DM, the disease can be diagnosed very early, i.e. before the child shows the typical symptoms of T1DM of severe metabolic dysfunction, and an appropriate therapy could be started immediately. Early diagnosis and therapy of T1DM may help reduce complications both at onset of diabetes and later in life. Furthermore, information about available treatments and intervention studies that include children with new-onset T1DM in order to preserve the remaining beta-cells can be given to families.

13.2.2 Risks
The risks of blood sampling include the occurrence of discomfort and bruising. Discomfort for the child at blood draws will be minimized by the use of anesthetic creme at the puncture site. Oral insulin does not lower blood sugar, but unexpected hypoglycemia is theoretically possible. The insulin will be administered with food to avoid this risk. It is theoretically possible that an unexpected or allergic reaction to insulin or placebo may occur.

13.2.3 Why is participation of young children necessary?
Pre-POINT is a safety and dose-finding pilot study to a phase II/III primary intervention study (Diabetes POINT) aiming to prevent diabetes-associated islet autoimmunity and T1DM in islet autoantibody negative children at genetically high T1DM risk. The trial must be conducted in young children because most children who develop T1DM will have initiated the autoimmunity to islet beta-cells already early in childhood.

13.3 Informed consent and assent

13.3.1 General rules
The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representatives) must read, sign, and date a
consent form before entering the study, taking the study drug, or undergoing any study-specific procedures.

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

13.3.2 Consenting procedures

13.3.2.1 Consent for HLA screening
Children who are up to 7 years of age and who have a sibling OR two first degree relatives with T1DM are eligible for genetic screening. The diagnosis of T1DM in the child’s first degree relative(s) and an age of onset prior to 40 years are verified by patient records. Families of eligible children are informed that the genetic screening will identify children with increased risk for developing islet autoantibodies and T1DM, and that these will be requested to participate in a dose-finding primary intervention trial with oral insulin or placebo and frequent follow-up. Parents or legal guardians of the child will be required to provide written consent for the genetic typing prior to sample collection. In order to identify subjects that are identical by descent for HLA DR3/4 with their siblings who have T1DM, parents and the sibling with T1DM will also need to have HLA genotyping performed. This will require their informed consent.

13.3.2.2 Consent for intervention and follow-up
Signed consent by parents or legal guardians is required before children are included in the trial. Families are visited by a national trained study representative at the time of the first sample collection. During this visit, the parents are given a further detailed explanation of the study and are given the opportunity to ask any questions they may have regarding study issues. The family is also given detailed instructions on how to adhere to the study protocol. Families will be asked to confirm that they have been read the study information, that it has been explained to them and that they agree to their child taking part in the study. A sample for islet autoantibody measurement is collected and antibodies are measured not more than 3 months prior to commencement of treatment. Families of children found to be autoantibody
positive are informed that their child cannot participate in the study, but the children will be offered the opportunity to take part in follow-up studies monitoring diabetes risk.

13.3.2.3 Assent of minors
Children will be assented by their legal guardians. Their participation in the trial will be until a maximum age of 9 years. Children will be asked to assent their trial participation when they reach age 7.

13.4 Privacy and confidentiality protections
A participant’s privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number, and these numbers rather than names will be used to collect, store, and report participant information.

HLA screening
Families are informed of the results of genetic screening by phone and mail. Results will be classified as having high (genotypes eligible for participation), or moderate/low genetic susceptibility for T1DM. For children at high genetic susceptibility with one sibling with T1DM and no other first-degree relatives with T1DM, HLA typing of the affected sibling will be requested. Genotypes will not be linked to personal identifiers.

Intervention and follow-up
Participation in the Pre-POINT study will be offered to eligible children in the communication. For participants in the Pre-POINT study, information relating to child nutrition and health, the health of the child’s parents, siblings and other relatives will collected. Venous blood and saliva samples will be obtained from subjects before treatment, after two weeks (Day 15) of treatment at each dose, after 3 and 6 months of treatment at each dose, and then at 6-monthly intervals. These materials and information will be obtained specifically for research and will be used only for the purposes cited within the study protocol. The nature of the study requires retaining identifying data; however, confidentiality of study subjects and subject materials will be provided by saving it in the applicant’s institute. Information stored on computers will be accessible through passwords available only to study investigators. Hard copy data will be stored in locked filing cabinets kept in locked rooms. All data processing at the DCC in Munich will comply with the European regulations in their current version. As the DCC operates as part of the public administration, it is under the supervision of the Office for Data Confidentiality and Protection of the State of Bavaria, Germany.
The trial will may be audited by designated qualified auditors who are independent of the Pre-POINT clinical trial/data collection systems.

13.5 Publication policy

Any publications resulting from the Pre-POINT study (including meeting abstracts) will be agreed between the principal and collaborating investigators prior to submission. Patient names or other identifiers will not be disclosed.

13.6 Clinical Trials Insurance, incentives, and remuneration

Study participants within European countries will be insured for any adverse event occurring as a result of study participation.
Study participants will not receive any payment in this study but expenses for clinical visits will be reimbursed (up to the amount of €100) to the families and/or pediatrician.
In the event that a study participant experiences an injury due to procedures in the study, medical treatment will be made available through the investigators or staff.

13.7 Conflict of interest

George S. Eisenbarth, MD, PhD, former Deputy Coordinator of the Pre-POINT study, holds a patent for the use of oral insulin in inducing immunological tolerance in the prevention or treatment of type 1 diabetes. Otherwise, no other study personnel responsible for design, conduct, or reporting of this research has an economic interest in, or acts as an officer or a director of any outside entity whose financial interests would reasonably appear to be affected by, the research.
14. List of References


8. Schmid S, Buuck D, Knopff A, Bonifacio E, Ziegler AG: BABYDIET, a feasibility study to prevent the appearance of islet autoantibodies in relatives of patients with Type 1 diabetes by delaying exposure to gluten. *Diabetologia* 47:1130-1131, 2004


42. Monetini L, Cavallo MG, Sarugeri E, Sentinelli F, Stefanini L, Bosi E, Thorpe R, Pozzilli P: Cytokine profile and insulin antibody IgG subclasses in patients with recent onset Type 1 diabetes treated with oral insulin. *Diabetologia* 47:1795-1802, 2004


64. Hendrieckx C, De Smet F, Kristoffersen I, Bradley C: Risk assessment for developing Type 1 diabetes: Intentions of behavioral changes prior to risk notification. *Diabetes Metabolism Research and Reviews* 18:36-42, 2002

65. Johnson SB: Participant Experiences in the DPT-1: Preliminary Results. NIH Type 1 TrialNet Study Group, Bethesda, MD, 2002