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Supplementary appendix

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Supplement to: Ziegler A-G, Achenbach P, Weiß A, et al. Efficacy of once-daily, high-dose, oral insulin immunotherapy in children genetically at risk for type 1 diabetes (POInT): a European, randomised, placebo-controlled, primary prevention trial. *Lancet* 2025; published online Nov 11. https://doi.org/10.1016/S0140-6736(25)01726-X.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ziegler AG, Achenbach P, Weiß A, et al. Efficacy of once-daily high-dose oral insulin immunotherapy in children genetically at-risk for type 1 diabetes: a European randomised, placebo-controlled primary prevention trial

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Regulatory and ethical approvals
The study was approved by the local ethical committees of the Technical University Munich, Medical Faculty (326/17 Af), the Medical University of Warsaw (199/2017), the UK Health Research Authority (18/SC/0019), Onderzoek UZ/KU Leuven (S60711) and the Regionala etikprövningsnämnden i Lund (2017/918). It was approved by the regulatory authorities Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM, #4042411); Läkemedelsverket // Swedish Medical Products Agency (MPA, Dnr. 5·1-2017-56343), Urząd Rejestracji Produktów Leczniczych, Wyrobów Medycznych I Produktów Biobójczych (#UR.DBL.BLE.4500·03282·2017.EN1 (1587/17), MHRA - Medicines and Healthcare Products Regulatory Agency (#48912/0001/001-0001), FAMHP - Federal Agency for Medicines and Health Products (#1041436 5).

Timeline
First participant enrolled February 7, 2018; Last participant enrolled March 24, 2021; End of treatment December 3; End of follow-up and end of trial June 28, 2024; Data base lock October 23, 2024.

Eligibility for the POInT study
The inclusion criteria of the POInT study were:

1. Infant between the ages of 4 months and 7 months at the time of randomization.
2. A high genetic risk (>10%) to develop islet autoantibodies by age 6 years:
    a. For infants without a first-degree family history of type 1 diabetes, high genetic risk was defined as *DR3/DR4-DQ8* or *DR4-DQ8/DR4-DQ8* genotype, and a genetic risk score of >14·4.[1] These represent close to 1% of all newborns.
    b. For infants with a first-degree family history of type 1 diabetes, high genetic risk was defined as having HLA DR4 and DQ8, and none of the following protective alleles: *DRB1*1501, DQB1*0503*. These represent around one third of infants with a first-degree family history of T1D.
3. Solid foods introduced into diet of infant.
4. Written informed consent signed by the custodial parent(s).

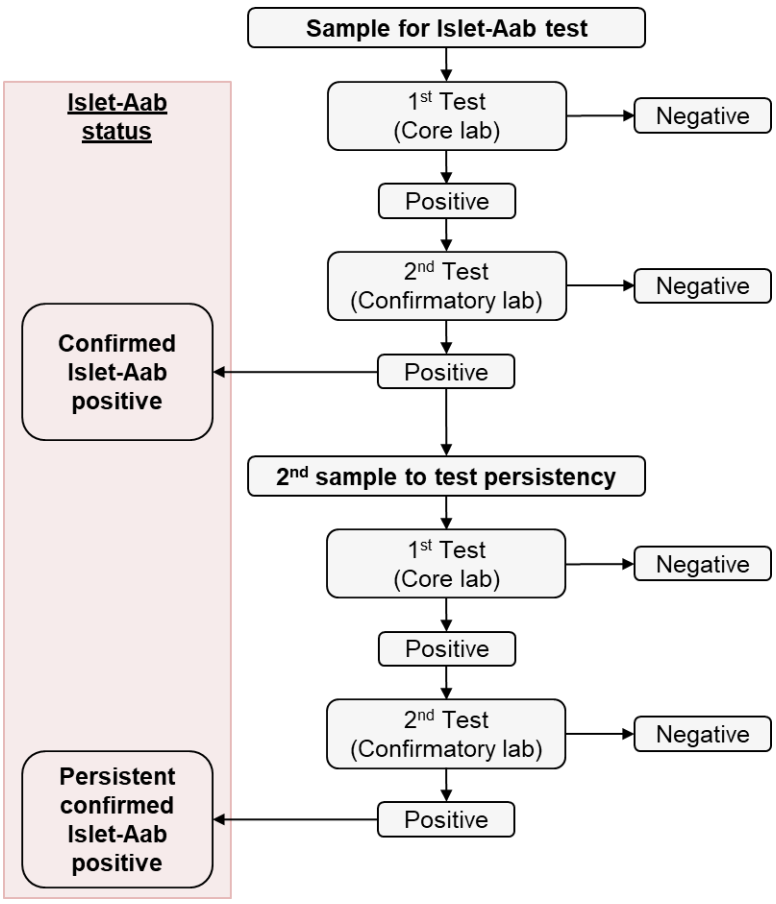
Exclusion criteria for the POInT study were:

1. Concomitant disease or treatment that may interfere with the assessments, as judged by the investigators.
2. Any condition that could be associated with poor compliance.
3. Any medical condition or medical condition coexisting, which, in the opinion of the investigator, may jeopardize the participant's safe participation in the study.
4. Diagnosis of diabetes at the time of recruitment.
5. Participation in another clinical trial.

Calculation of the genetic risk score
The genetic risk score was calculated as previously described,[2] by multiplying the number of risk alleles (i.e., 0, 1 or 2 for each single SNP) with the weight assigned to each SNP (Table S1) and then summing up the weighted contributions of all SNPs plus an additive constant for each of the two HLA class II categories: 3·15 for infants who have the *HLA DR4-DQ8/DR4-DQ8* genotype or 3·98 for infants who have the *HLA DR3/DR4-DQ8* genotype.

Laboratory measurements
DNA extraction from dried blood spots, genotyping for 46 single nucleotide polymorphisms, calculation of genetic risk scores, islet autoantibodies, and determination of glucose levels in oral glucose tolerance tests (OGTTs) were conducted centrally. The measurement of pre- and post-prandial glucose during investigational medicinal product intake, complete blood count and blood differential, and 25-hydroxyvitamin D3 (25-OH-vitamin-D3) were performed in a certified clinical laboratory at each study site. SNP typing was performed at LGC Group (https://www.lgcgroup.com).[1] Venous glucose concentrations were measured by enzymatic reference method with hexokinase (Belgium, Germany, Poland) or by HemoCue (Sweden, UK; HemoCue 201 system).

Islet autoantibody measurements and definition of positive islet autoantibodies
Islet autoantibody positive was defined as confirmed positive in two independent central laboratories and persistent in two consecutive samples.



Serum samples from each visit were measured for autoantibodies against insulin (IAA), glutamate decarboxylase-65 (GADA), insulinoma-associated antigen-2 (IA-2A), and zinc transporter-8 (ZnT8A) at the Core laboratory located at the Institute of Diabetes Research, Helmholtz Munich, Germany. IAA were measured by a competitive radiobinding assay (RBA) with protein A/G immunoprecipitation and ^{125}I-labeled recombinant human insulin.[3] GADA and IA-2A were measured by the National Institute of Diabetes and Digestive and Kidney Diseases harmonized assay protocol using ^{35}S-methionine-labeled N-terminally truncated GAD65 (amino acids 96-585) or IA-2ic (amino acids 606-979), as previously described.[4] For GADA, ELISA (RSR Ltd.) was used as the second test if the RBA result was positive. ZnT8A were measured by assays to separately detect autoantibodies to the arginine 325R and tryptophan 325W human ZnT8 variants (ZnT8RA and ZnT8WA, respectively) using ^{35}S-methionine-labeled recombinant ZnT8 (amino acids 268–369), as previously described.[5] Samples were classified as ZnT8A positive if they were positive for ZnT8RA and/or ZnT8WA. The assays had sensitivities and specificities of 54% and 99% for IAA, 66% and 99% for GADA, 76% and 100% for IA-2A, 56% and 99% for ZnT8RA, and 50% and 99% for ZnT8WA in the Islet Autoantibody Standardization Program (IASP) 2016 Workshop.[6] In addition, autoantibodies against tetraspanin 7 (TS7A) were measured by a luciferase immunoprecipitation system (LIPS) assay in the Core laboratory.[7]
Samples that were positive for IAA, GADA, IA-2A or ZnT8A at the Core laboratory were sent to the Confirmatory laboratory located at the University of Bristol Medical School, Diabetes and Metabolism, Learning and Research, Southmead Hospital, Bristol, United Kingdom for confirmatory testing. Here, IAA were measured by a competition RBA with ^{125}I-labeled human insulin, as previously described.[8,9] GADA and IA-2A were measured using the NIDDK harmonized assay protocol using ^{35}S-methionine–labelled recombinant full-length GAD65 or IA-2ic.[4] ZnT8RA and ZnT8WA were measured in separate RBAs based on the NIDDK harmonized assay protocol.[10] The assays had sensitivities and specificities of 54% and 99% for IAA, 74% and 97% for GADA, 70% and 100% for IA-2A, 60% and 100% for ZnT8RA, and 46% and 100% for ZnT8WA in the IASP 2015 Workshop.[6]
Samples that were positive for a specific autoantibody by both laboratories were considered confirmed autoantibody-positive. For GADA, positivity also required a positive result by ELISA and RBA on at least one occasion. Children

reached an islet autoantibody-positive status for an islet autoantibody when they were positive in two consecutive samples.

Maternally derived islet autoantibodies

In participants who were positive for any of the islet autoantibodies (IAA, GADA, IA-2A, ZnT8A) in their first sample taken, the status of the autoantibodies was classified as maternally derived islet autoantibodies if they had declining antibody titres on follow-up and subsequently became negative in a sample taken before age 3 years. Maternally derived islet autoantibodies were not a primary outcome endpoint and were not considered as a positive outcome in the statistical analysis.

Participants who were positive for an islet autoantibody from the start of sample collection without a subsequent decline in antibody titre and remained positive for the same autoantibody until age 3 years were classified as islet autoantibody positive from baseline.

Criteria for clinical diabetes and for dysglycaemia

Criteria for clinical diabetes were symptoms of diabetes and a random plasma glucose ≥200 mg/dL (≥11·1 mmol/L) or confirmed fasting blood glucose ≥126 mg/dL (≥7 mmol/L) or 2-h plasma glucose ≥200 mg/dL (≥11·1 mmol/L) in the OGTT.[11] The criteria for dysglycaemia were impaired fasting plasma glucose ≥110 mg/dL (≥6·1 mmol/L), or impaired 2-h glucose ≥140 mg/dL (≥7·8 mmol/L), or high glucose levels at intermediate timepoints during the OGTT (30, 60, and 90 min levels ≥200 mg/dL [≥11·1 mmol/L]) at two consecutive occasions or at one occasion followed by confirmation of clinical diabetes at the next contact. A trial committee evaluated and confirmed all cases of diabetes and dysglycaemia.

Measurements of IgG subclass-specific antibodies against insulin

IgG subclasses of IAA were determined by radiobinding assays as previously described,[12] using IgG subclass-specific biotin-labeled mouse anti-human monoclonal antibodies bound on Streptavidin Sepharose High Performance beads (GE Healthcare Life Sciences, Freiburg, Germany). Mouse anti-human IgG1 (clone G17-1; BD Biosciences, Heidelberg, Germany), IgG3 (clone HP6047; BioLegend, San Diego, USA), and IgG4 (clone JDC-14; BD Biosciences) monoclonal antibodies were used. Nonspecific binding was determined for each serum using biotin-labeled mouse anti-rat IgM monoclonal antibody (clone G53-238; BD Biosciences) bound on Streptavidin Sepharose beads. Briefly, sera (5 µl) were incubated with 1243 nU ^{125}I-labelled human recombinant insulin (2200 Ci/mmol; Revvity GmbH, Hamburg, Germany) in 25 µl 50 mmol/l Tris and 1% Tween-20 (pH 8; TBT buffer) at 4°C for 72 h before adding 50 µl IgG subclass-specific antibody-coated sepharose bead suspension, incubating for 2 h at 4°C under agitation, washing in cold TBT buffer and counting. Results for IAA IgG subclasses were expressed as nU bound insulin/ml, calculated as [(anti-IgG subclass counts − control anti-IgM counts)/(total counts per tube)] × 1243 × 200.

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Table S1. List of SNPs determined for the calculation of the genetic risk score.

SNP	Gene, Allele, or Haplotype	Score weight for genotype or per allele*
HLA class II		
rs17426593	HLA DR4-DQ8/DR4-DQ8	3·15
rs2187668 rs7454108	HLA DR3/DR4-DQ8	3·98
rs3129889	HLA *DRB1**1501	Exclusion criteria for first degree relatives
rs1794265	HLA *DQB1**0503	Exclusion criteria for first degree relatives
HLA class I		
rs1264813	HLA A 24	0·43
rs2395029	HLA B 5701	0·92
Non-HLA SNPs		
rs2476601	*PTPN22*	0·76
rs2816316	*RGS1*	0·16
rs3024505	*IL10*	0·22
rs1990760	*IFIH1*	0·16
rs3087243	*CTLA4*	0·16
rs10517086	*C4orf52*	0·19
rs2069763	*IL2*	0·11
rs6897932	*IL7R*	0·19
rs3757247	*BACH2*	0·19
rs9388489	*C6orf173*	0·14
rs6920220	*TNFAIP3*	0·15
rs1738074	*TAGAP*	0·05
rs7804356	*SCAP2*	0·15
rs4948088	*COBL*	0·17
rs7020673	*GLIS3*	0·23
rs12722495	*IL2RA*	0·47
rs947474	*PRKCQ*	0·15
rs10509540	*RNLS/C10orf59*	0·25
rs1004446	*INS*	0·65
rs4763879	*CD69*	0·06
rs2292239	*ERBB3*	0·36
rs3184504	*SH2B3*	0·24
rs1465788	*ZFP36L1*	0·13
rs17574546	*RASGRP1*	0·13
rs3825932	*CTSH*	0·15
rs12708716	*CLEC16A*	0·15
rs4788084	*IL27*	0·20
rs7202877	*CTRB2*	0·19
rs2290400	*ORMDL3*	0·25
rs7221109	*CCR7*	0·15
rs45450798	*PTPN2*	0·09
rs763361	*CD226*	0·12
rs425105	*PRKD2*	0·21
rs2281808	*SIRPG*	0·07
rs3788013	*UBASH3a*	0·16

rs5753037	*RPS3AP51*	0·15
rs229541	*IL2B*	0·18
rs5979785	*TLR8*	0·09
rs2664170	*GAB3*	0·14

*See supplementary methods and references #1 and #2

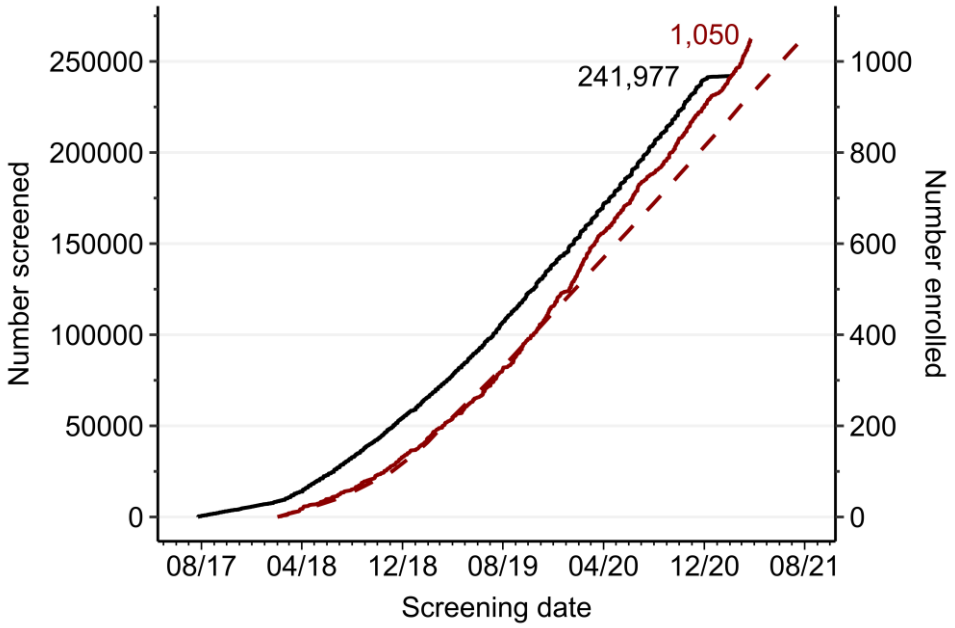


Figure S1. Screening for eligibility and enrolment of participants in the clinical trial. The cumulative number of newborns screened for genetic type 1 diabetes risk from July 24, 2017, to February 2, 2021, is shown as a solid black line. A total of 241,977 babies were screened, including 4165 who reported to have a first-degree relative with type 1 diabetes. Of these, 2750 were eligible, including 1304 who had a first-degree relative with type 1 diabetes. Of the 237,812 without a first-degree relative, there were 317 (0·13%) who had a genetic risk score >14·4 without a HLA DR4-DQ8 haplotype. The cumulative number of participants enrolled in the trial from February 7, 2018, to March 24, 2021, is shown as solid dark red line. In total, consent was obtained for 1050 infants, including 555 (53·7%) of 1034 who had a first-degree relative with type 1 diabetes and 495 (28·8%) of 1716 without a first-degree family history of type 1 diabetes ($p<0·0001$). The dotted dark red line represents the expected number of participants that were to be enrolled in the study over time.

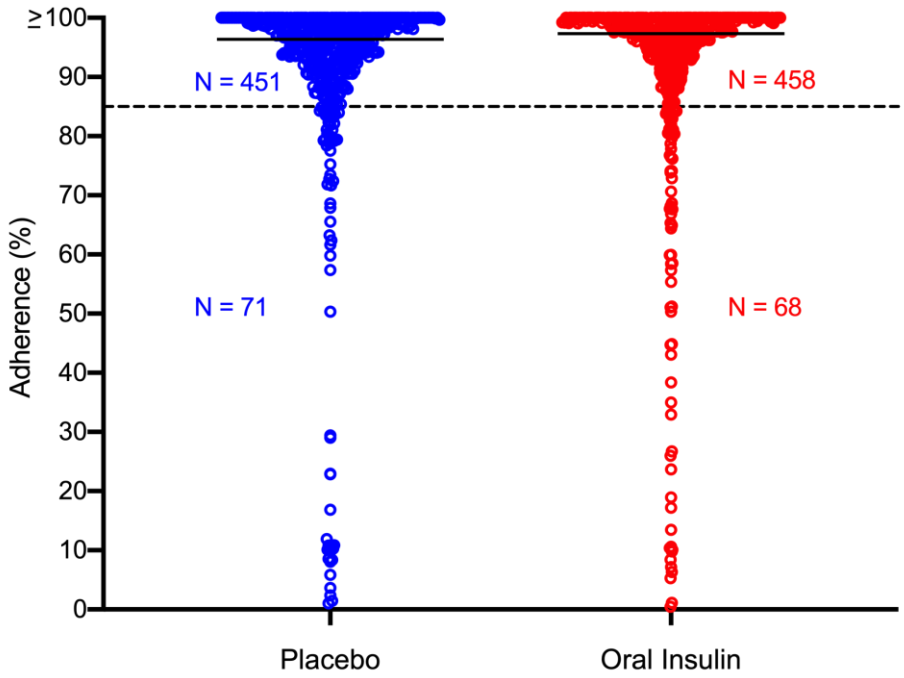


Figure S2. Shown is the absolute adherence to taking the study drug for each participant in both treatment groups (blue: placebo; red: oral insulin). Adherence was determined by counting the number of capsules returned. The number of participants who took at least 85% of the scheduled capsules is indicated above the dashed line. Median adherence is indicated by solid lines for both treatment groups and was 96·4% in the placebo and 97·3% in the insulin group.

Table S2. The cumulative incidences for developing the primary and secondary outcomes and the diabetes-free survival from the primary outcome to diabetes (exploratory outcome) for trial participants receiving oral insulin or placebo.

Outcome	Placebo	Oral Insulin	p-value
	5-year cumulative incidence, % (95% CI)	5-year cumulative incidence, % (95% CI)	(log-rank)
Two or more islet autoantibodies (primary outcome)	10·1 (7·2-13·1)	10·9 (8·0-13·7)	0·56
One or more islet autoantibodies	13·5 (9·6-17·4)	14·6 (11·0-18·1)	0·25
Insulin autoantibodies (IAA)	8·9 (6·4-11·5)	11·0 (8·2-13·8)	0·28
GAD autoantibodies (GADA)	9·9 (6·3-13·5)	10·2 (7·1-13·3)	0·33
Diabetes or dysglycaemia	6·3 (3·5-9·0)	4·2 (2·1-6·2)	0·34
Diabetes-free survival from primary outcome	3-year survival, % (95% CI)	3-year survival, % (95% CI)	
	35·5 (16·9-54·0)	63·2 (47·5-79·0)	0·048

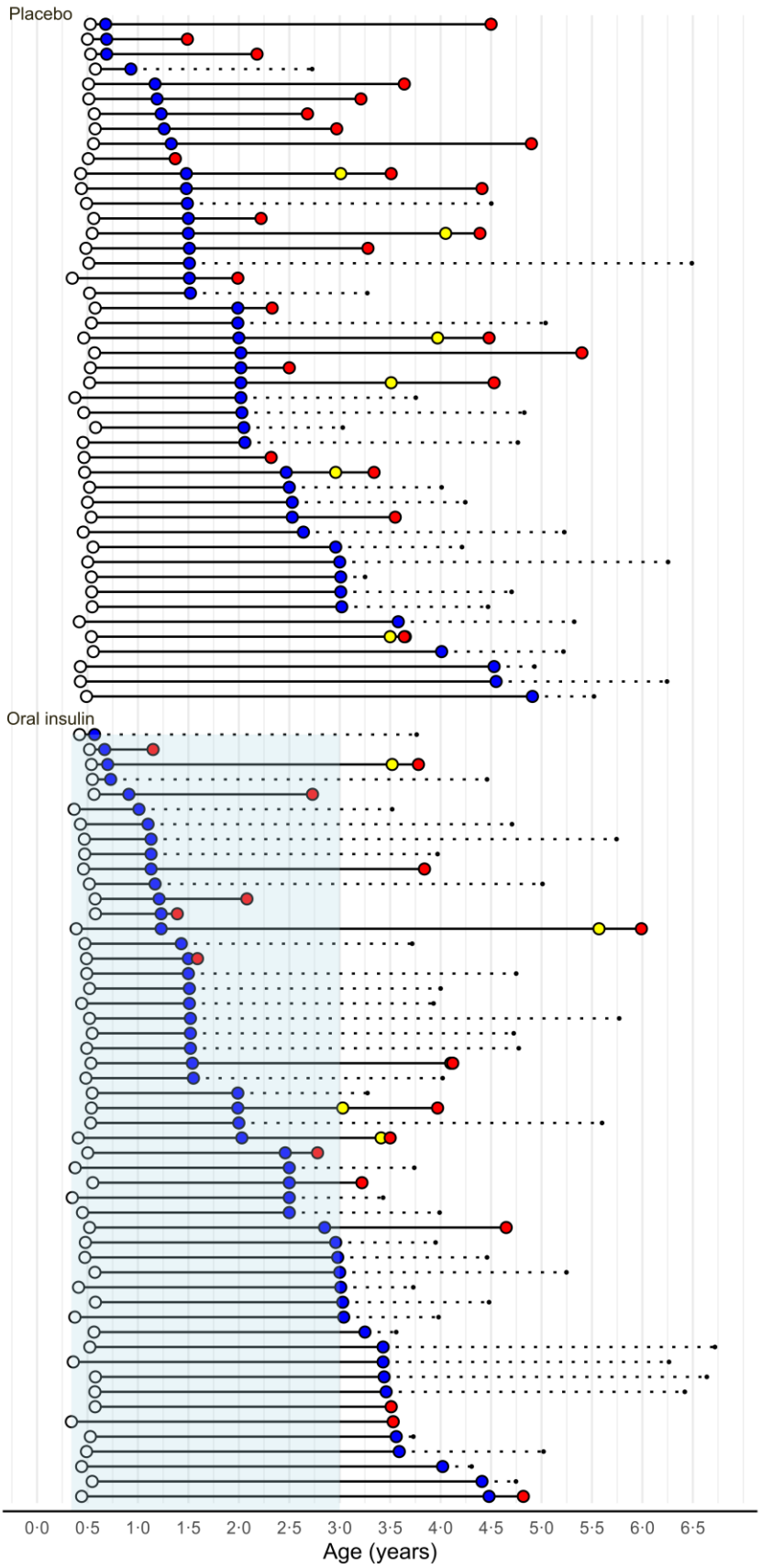


Figure S3. Shown are the 98 participants with primary outcome (46 Placebo, upper half and 52 oral insulin, lower half) and their course of events/outcomes by age (on the x-axis). Each participant is depicted by one line, showing white (baseline age), blue (age at primary outcome), yellow (age at dysglycaemia) and red dots (age at clinical T1D). Lines continue in dotted style if participants do not have further events after developing the primary outcome. The light blue background shows the treatment period in the oral insulin group (from baseline until third birthday).

Table S3. Overview of the 98 participants with a primary outcome (separated by treatment arm) and the combination of confirmed positive islet autoantibodies they had for at least one visit during the study.

Variable	Placebo	Oral Insulin
Participants with primary outcome, n	46	52
1 islet autoantibody (all IAA only), n (%)	3 (6·5)	2 (3·8)
2 islet autoantibodies, n (%)	11 (23·9)	12 (23·1)
IAA/GADA, n (%)	4 (8·7)	9 (17·3)
IAA/IA2, n (%)	4 (8·7)	2 (3·8)
IAA/ZnT8, n (%)	1 (2·2)	0
GAD/IA2, n (%)	1 (2·2)	1 (1·9)
IA2/ZnT8, n (%)	1 (2·2)	0
3 islet autoantibodies, n (%)	14 (30·4)	17 (32·7)
IAA/GAD/IA2, n (%)	9 (19·6)	10 (19·2)
IAA/GAD/ZnT8, n (%)	2 (4·3)	4 (7·7)
IAA/IA2/ZnT8, n (%)	2 (4·3)	2 (3·8)
GAD/IA2/ZnT8, n (%)	1 (2·2)	1 (1·9)
4 islet autoantibodies, n (%)	18 (39·1)	21 (40·4)

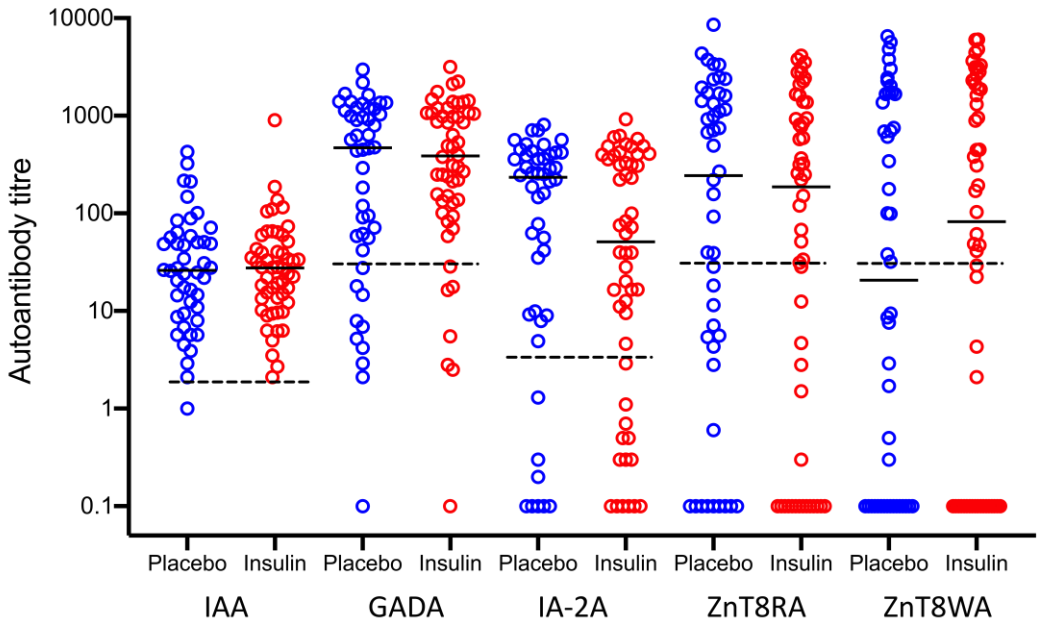


Figure S4. Maximum autoantibody titres for each islet autoantibody in participants who developed the primary outcome. The maximum titre in visits from islet autoantibody seroconversion is shown for each of autoantibodies to insulin (IAA), GAD65 (GADA), IA-2 (IA-2A) and the two variants of ZnT8 (ZnT8RA, ZnT8WA). The maximum titre is shown for measurements in the Munich central laboratory. Median values are indicated by unbroken black lines. Dashed lines represent the threshold for positivity for each antibody. No differences were observed between participants in the placebo and oral insulin groups.

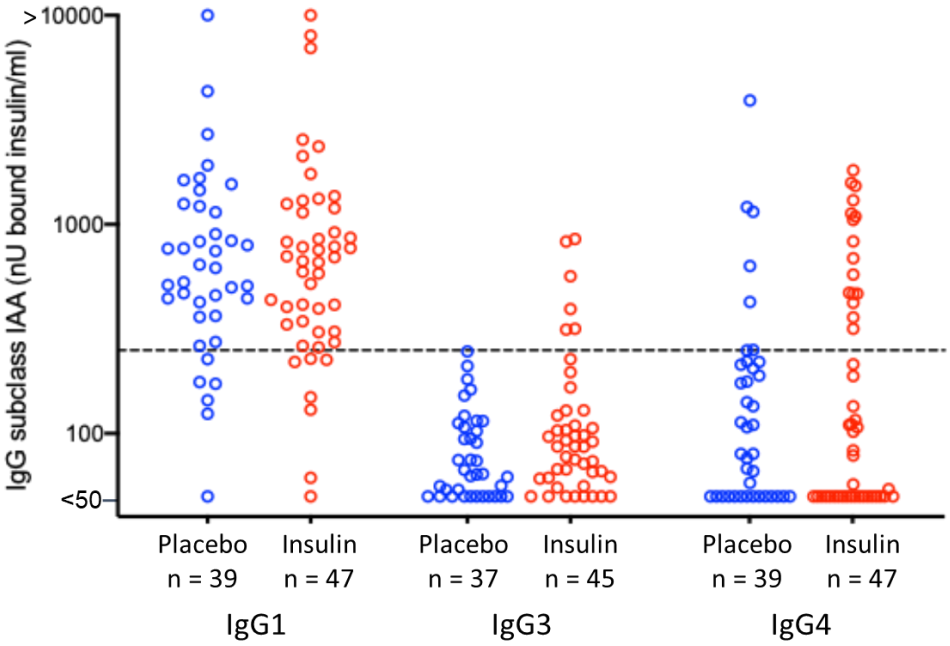
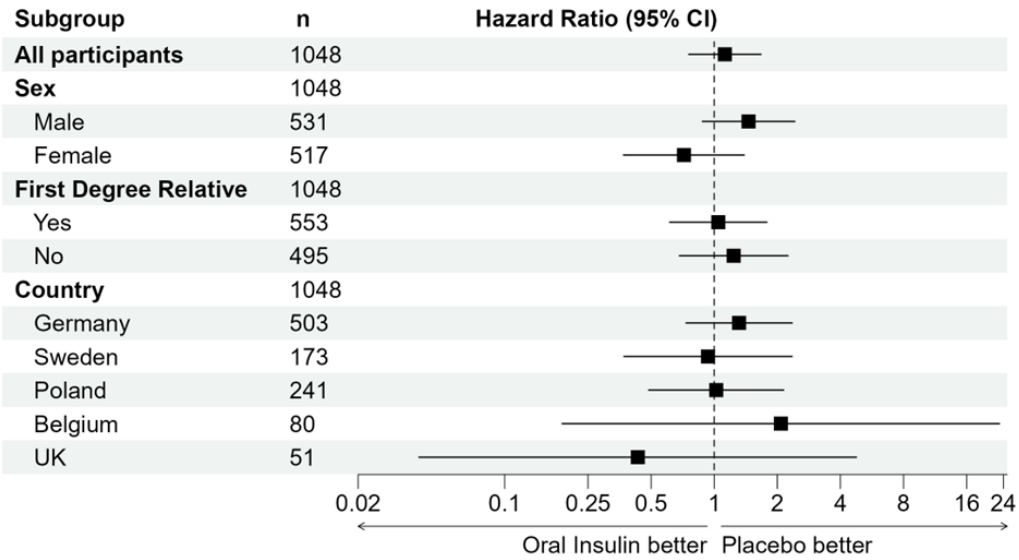


Figure S5. IgG1, IgG3, and IgG4 subclasses of autoantibodies to insulin (IAA) in participants who developed the primary outcome, including IAA. For each participant with remaining sample for measurement, a sample within 2 years of IAA seroconversion was measured (median time since seroconversion 0·78 years, IQR 0·5 to 1.0 years). Participants are grouped by treatment group (oral insulin or placebo). The dashed line at 250 nU bound insulin/ml represents the previously established threshold for positivity.[12] An increased proportion of participants with IgG3 IAA was observed in the oral insulin group (6 of 45; 13·3%) as compared to the placebo group (0 of 37; p=0·010, Fisher's exact test). IgG4 IAA was found in 16 of 47 (34%) and 6 of 39 (15%) samples in the insulin and placebo groups, respectively (p=0·081). See appendix p 4 for IAA IgG subclass method.

Table S4. The 5-year cumulative incidences for developing the primary outcome and secondary outcome diabetes or dysglycaemia for POInT study participants receiving oral insulin or placebo stratified by the type 1 diabetes-susceptible (CC) or non-susceptible (CT or TT) *INS* rs1004446 genotypes.

Outcome	*INS* susceptible			*INS* non-susceptible		
	Placebo	Oral Insulin		Placebo	Oral Insulin	
	5-year cumulative incidence, % (95% CI)	5-year cumulative incidence, % (95% CI)	p-value (log-rank)	5-year cumulative incidence, % (95% CI)	5-year cumulative incidence, % (95% CI)	p-value (log-rank)
Two or more islet autoantibodies (primary outcome)	12·6 (8·4-16·8)	9·6 (6·1-13·1)	0·28	7·1 (3·2-11·0)	12·8 (8·1-17·5)	0·025
Diabetes or dysglycaemia	10·1 (5·1-15·0)	3·5 (1·0-6·1)	0·016	2·0 (0·0-4·0)	5·0 (1·6-8·4)	0·094

A. Two or more islet autoantibodies (primary outcome)



B. Diabetes or dysglycaemia (secondary outcome)

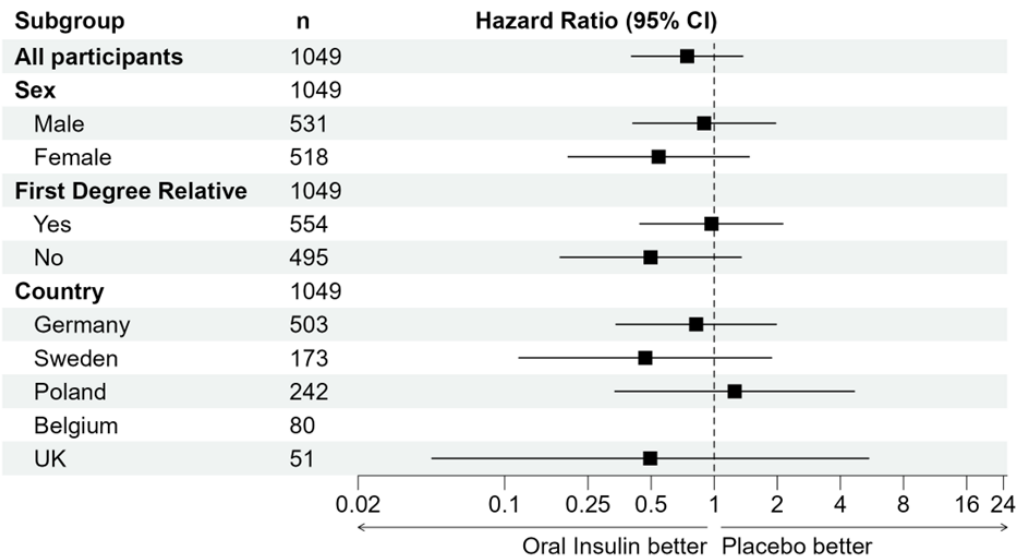


Figure S6. Forest plots showing the hazard ratios and 95% confidence intervals of main covariates for (A) the development of the primary outcome and (B) the development of the secondary outcome diabetes or dysglycaemia in the oral insulin group compared to the placebo group, calculated in univariate Cox proportional hazards models. Missing point estimates and confidence intervals resemble the absence of cases in the respective subgroup.

Table S5. Blood glucose values of the study participants before and after taking oral insulin or placebo at Visits 1 to 4.

Trial visit	Time (min)*	n	Placebo Blood glucose [mg/dl] median (IQR)	n	Oral Insulin Blood glucose [mg/dl] median (IQR)	*P* value
Visit 1	-10	515	88 (81-97)	523	88 (81-97)	0·82
	+30	497	100 (90-114)	493	101 (91-115)	0·17
	+60	478	96 (89-106)	496	97 (89-108)	0·22
	+120	491	92 (84-102)	496	93 (85-101)	0·37
Visit 2	-10	502	86 (80-94)	512	86 (79-96)	0·60
	+30	476	101 (92-114)	480	100 (91-112)	0·55
	+60	414	96 (87-107)	425	97 (89-108)	0·079
	+120	404	91 (84-101)	413	92 (84-102)	0·74
Visit 3	-10	497	84 (78-92)	507	85 (79-94)	0·30
	+30	465	99 (89-113)	498	97 (90-112)	0·73
	+60	402	95 (87-107)	415	94 (87-105)	0·48
	+120	393	92 (85-103)	397	92 (85-102)	0·53
Visit 4	-10	489	84 (78-92)	498	85 (78-94)	0·29
	+30	380	94 (85-106)	380	94 (86-106)	0·46
	+60	342	92 (85-103)	350	93 (86-103)	0·50
	+120	325	90 (83-99)	327	92 (85-101)	0·055

*Time relative to treatment application

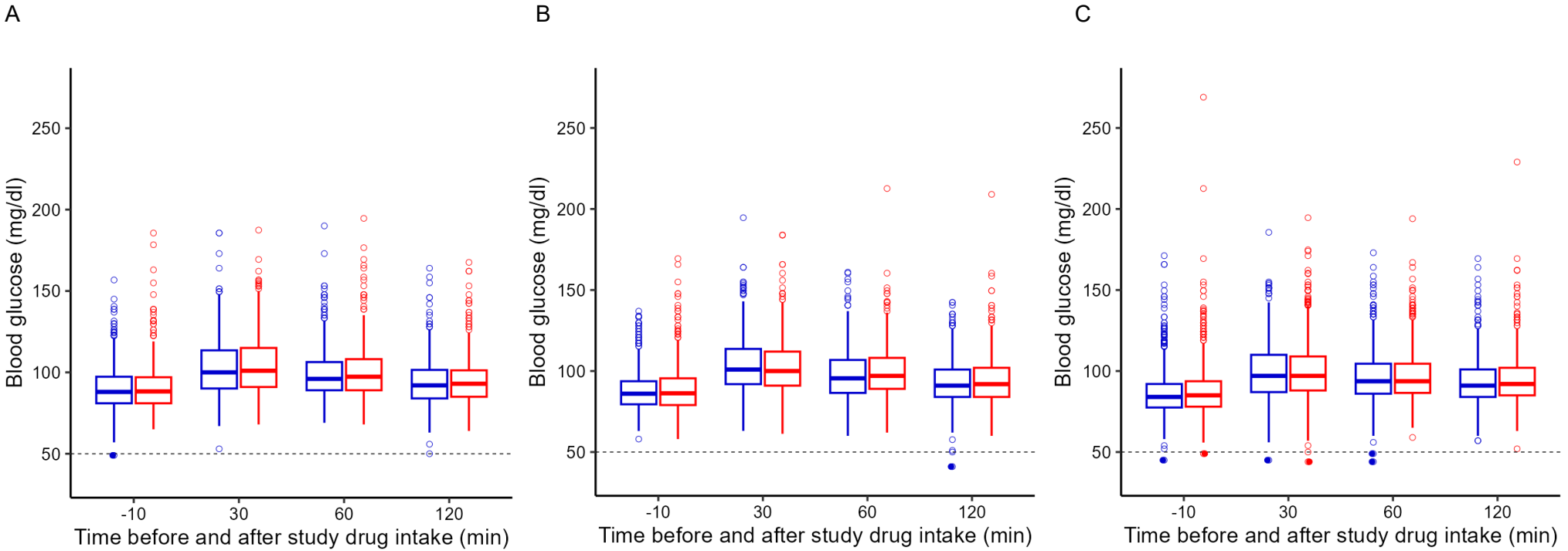


Figure S7. Comparison of blood glucose levels between the treatment groups (blue: placebo; red: oral insulin) before and after taking the study drug at different doses: (A) 7·5 mg oral insulin/placebo (Baseline, Visit 1); (B) 22·5 mg oral insulin/placebo (Visit 2); (C) 67·5 mg oral insulin/placebo (Visits 3 and 4). The dashed line indicates the threshold for hypoglycaemia (blood glucose values < 50 mg/dl). A total of 8 hypoglycaemic episodes occurred (solid dots), 2 in the group treated with oral insulin and 6 in the group treated with placebo. In the placebo group, one hypoglycaemia occurred before taking the study drug at visit 1 and at visit 4, and four after taking study drug (one after 120 minutes at visit 2, one after 30 minutes at visit 3, two after 60 minutes, i.e. one at visit 3 and one at visit 4). In the oral insulin group, two hypoglycaemias occurred (one before taking the study drug at visit 3, one after 30 minutes at visit 4).

Table S6. Full blood counts of trial participants at Visit 1 and Visit 8.

Parameter	Visit 1 (age 4-7 months)		Visit 8 (age 3 years)	
	Placebo	Oral Insulin	Placebo	Oral Insulin
	(N=522)	(N=528)	(N=477)	(N=486)
WBC [1000/µl]*	9·7 (8·0 - 11·6)	10·0 (8·3 - 11·9)	7·9 (6·7 - 9·9)	7·9 (6·8 - 9·5)
	(N=463)	(N=457)	(N=437)	(N=432)
RBC [10^6/µl]*	4·5 (4·3 - 4·8)	4·5 (4·2 - 4·7)	4·6 (4·4 - 4·9)	4·7 (4·5 - 4·9)
	(N=462)	(N=457)	(N=426)	(N=421)
Haemoglobin [g/dl]*	11·8 (11·2 - 12·3)	11·8 (11·2 - 12·3)	12·5 (11·9 - 12·9)	12·5 (11·9 - 13·1)
	(N=463)	(N=457)	(N=437)	(N=430)
Haematocrit [%]*	34·3 (33·0 - 36·0)	34·3 (32·8 - 36·0)	36·6 (35·0 - 38·0)	37·0 (35·0 - 38·1)
	(N=462)	(N=457)	(N=434)	(N=429)
MCV [fl]*	76·7 (74·0 - 79·0)	76·7 (74·0 - 79·0)	78·5 (76·5 - 81·0)	78·8 (76·6 - 80·8)
	(N=463)	(N=457)	(N=437)	(N=430)
MCH/HbE [pg/cell]*	26·2 (25·3 - 27·2)	26·2 (25·3 - 27·1)	26·9 (26·0 - 27·8)	26·9 (26·0 - 27·7)
	(N=464)	(N=457)	(N=435)	(N=428)
MCHC [g/dl]*	34·7 (33·9 - 35·6)	34·6 (33·8 - 35·7)	34·3 (33·5 - 35·4)	34·4 (33·5 - 35·4)
	(N=460)	(N=457)	(N=434)	(N=428)
Neutrophils [1000/µl]*	1·9 (1·3 - 2·7)	1·9 (1·4 - 2·7)	3·2 (2·3 - 4·5)	3·1 (2·3 - 4·1)
	(N=459)	(N=455)	(N=434)	(N=427)
Lymphocytes [1000/µl]*	6·6 (5·4 - 7·9)	6·7 (5·4 - 8·1)	3·6 (3·1 - 4·4)	3·7 (3·0 - 4·8)
	(N=459)	(N=455)	(N=434)	(N=423)
Monocytes [1000/µl]*	0·6 (0·5 - 0·8)	0·6 (0·5 - 0·8)	0·6 (0·5 - 0·8)	0·6 (0·5 - 0·8)
	(N=460)	(N=455)	(N=432)	(N=423)
Eosinophils [1000/µl]*	0·3 (0·2 - 0·5)	0·3 (0·2 - 0·5)	0·2 (0·1 - 0·4)	0·2 (0·1 - 0·3)
	(N=460)	(N=455)	(N=432)	(N=423)
Basophils [1000/µl]*	0·05 (0·03 - 0·09)	0·05 (0·02 - 0·09)	0·05 (0·03 - 0·09)	0·05 (0·03 - 0·08)
	(N=460)	(N=455)	(N=432)	(N=423)

* Values are shown as median (IQR).

Table S7. Number of adverse events reported, and number of participants affected, shown for oral insulin and placebo-treated participants, respectively, and grouped by system organ class (SOC). A total number of 10,252 adverse events were observed.

System Organ Class	Placebo		Oral Insulin		p-value
	No. of events	No. (%) of participants	No. of events	No. (%) of participants	(log-rank) *
Total	5176	500 (95·8)	5076	507 (96·0)	0·84
Blood and lymphatic system disorders	16	16 (3·1)	12	11 (2·1)	0·31
Cardiac disorders	6	4 (0·8)	2	2 (0·4)	0·40
Congenital, familial, and genetic disorders	9	9 (1·7)	5	4 (0·8)	0·15
Ear and labyrinth disorders [#]	6	3 (0·6)	15	15 (2·8)	0·0051
Endocrine disorders	1	1 (0·2)	0	0 (0·0)	-
Eye disorders	4	4 (0·8)	10	10 (1·9)	0·12
Gastrointestinal disorders	429	219 (42·0)	435	223 (42·2)	0·83
General disorders and administration site conditions	526	245 (46·9)	535	258 (48·9)	0·68
Hepatobiliary disorders	0	0 (0·0)	1	1 (0·2)	-
Immune system disorders	28	23 (4·4)	12	11 (2·1)	0·033
Infections and infestations	3677	488 (93·5)	3604	492 (93·2)	0·93
Injury, poisoning and procedural complications	166	99 (19·0)	142	88 (16·7)	0·27
Investigations	16	14 (2·7)	6	6 (1·1)	0·065
Metabolism and nutritional disorders	19	17 (3·3)	6	5 (0·9)	0·0084
Musculoskeletal and connective tissue disorders	6	5 (1·0)	8	7 (1·3)	0·59
Neoplasms benign, malignant, and unspecified (incl. cysts and polyps)	3	3 (0·6)	0	0 (0·0)	-
Nervous system disorders	16	15 (2·9)	23	17 (3·2)	0·77
Psychiatric disorders	5	5 (1·0)	6	6 (1·1)	0·80
Renal and urinary disorders	4	4 (0·8)	3	3 (0·6)	0·68
Reproductive system and breast disorders	6	5 (1·0)	5	5 (0·9)	0·98
Respiratory, thoracic, and mediastinal disorders	111	78 (14·9)	131	86 (16·3)	0·56
Skin and subcutaneous tissue disorders	108	78 (14·9)	87	75 (14·2)	0·70
Social circumstances	0	0 (0·0)	1	1 (0·2)	-
Surgical and medical procedures	12	11 (2·1)	25	22 (4·2)	0·058
Vascular disorders	2	2 (0·4)	2	2 (0·4)	0·99

* P-values from the log-rank test comparing the probabilities of a first record of the investigated SOC between the treatment arms. [#] One event in the oral insulin group was reported as moderate, all other events were reported as mild.

Table S8. The number of adverse events grouped according to severity and treatment arm.

	Placebo	Oral Insulin	Total
Mild	4736 (91·5%)	4596 (90·5%)	9332 (91·0%)
Moderate	397 (7·7%)	425 (8·4%)	822 (8·0%)
Severe	41 (0·8%)	54 (1·1%)	95 (0·9%)
Life-threatening or disabling	2 (0·04%) *	0 (0%)	2 (0·02%)
Death	0 (0%)	1 (0·02%) **	1 (0·01%)
Total	5176 (50·5%)	5076 (49·5%)	10252 (100·0%)

* Two children who received placebo developed life-threatening illnesses during their participation in the study. One child had acute respiratory distress and tachypnoea due to an infection, which later resolved completely. The other child had lymphoblastic B-cell lymphoma, withdrew from the study, and died two months later. This child's treatment group was unblinded and the case was assessed by the sponsor together with the investigator, pharmacovigilance, and the Data Safety Monitoring Board (DSMB) and deemed unrelated to the study.

** One child who received oral insulin died during his participation in the study. The cause of death was determined to be sudden infant death syndrome. The treatment group for this child was unblinded and the case was evaluated by the sponsor together with the investigator, pharmacovigilance, the DSMB, the local ethics board, and the respective regulatory authority. They all concluded that the death was not related to the study drug and agreed on the continuation of the clinical trial.

Table S9. Number of serious adverse events reported, and number of participants affected, shown for oral insulin and placebo-treated participants, respectively, and grouped by SOC. There were 250 serious adverse events reported, 130 events in 90 participants in the oral insulin arm and 120 events in 85 participants in the oral placebo arm. There were 245 serious adverse events that required admission to the hospital, 126 events in 87 participants in the oral insulin arm and 119 events in 84 participants in the oral placebo arm.

System Organ Class	Placebo		Oral Insulin		p-value
	No. of events	No. (%) of participants	No. of events	No. (%) of participants	(log-rank) *
Total	120	85 (16·3)	130	90 (17·0)	0·78
Congenital, familial, and genetic disorders	3	3 (0·6)	0	0 (0·0)	-
Gastrointestinal disorders	6	6 (1·1)	2	2 (0·4)	0·15
General disorders and administration site conditions	1	1 (0·2)	3	3 (0·6)	0·33
Immune system disorders	1	1 (0·2)	1	1 (0·2)	0·99
Infections and infestations	79	59 (11·3)	82	67 (12·7)	0·51
Injury, poisoning and procedural complications	11	11 (2·1)	15	14 (2·7)	0·58
Investigations	3	3 (0·6)	0	0 (0·0)	-
Metabolism and nutritional disorders	2	1 (0·2)	0	0 (0·0)	-
Neoplasms benign, malignant, and unspecified (incl. cysts and polyps)	1	1 (0·2)	0	0 (0·0)	-
Nervous system disorders	8	8 (1·5)	11	9 (1·7)	0·84
Reproductive system and breast disorders	0	0 (0·0)	1	1 (0·2)	-
Respiratory, thoracic, and mediastinal disorders	3	3 (0·4)	9	4 (0·8)	0·72
Skin and subcutaneous tissue disorders	0	0 (0·0)	1	1 (0·2)	-
Surgical and medical procedures	1	1 (0·2)	4	3 (0·6)	0·33
Vascular disorders	1	1 (0·2)	1	1 (0·2)	0·99

* P-values from the log-rank test comparing the probabilities of a first record of the investigated SOC between the treatment arm

Table S10. Protocol deviations by study arm. Overall, there were 468 minor (230 Oral insulin, 238 Placebo) and 23 major protocol deviations (12 Oral insulin, 11 Placebo). They were differentiated into several subcategories.

Protocol deviation category	Minor deviations		Major deviations	
	Placebo	Oral Insulin	Placebo	Oral Insulin
Total	238	230	11	12
Missed visits	1	0	0	0
Missing blood samples for measurement of islet autoantibodies	1	2	0	0
Errors in applying inclusion/exclusion criteria	0	0	0	1
Administration of expired IMP	0	0	11	10
Other study medication related issues	1	2	0	1
Visit time window not met	187	179	0	0
Missed phone call	20	20		
Single protocol assessments not performed or too many procedures performed	13	10	0	0
Psychological Impact Questionnaire not performed	15	15	0	0
Other	0	2	0	0

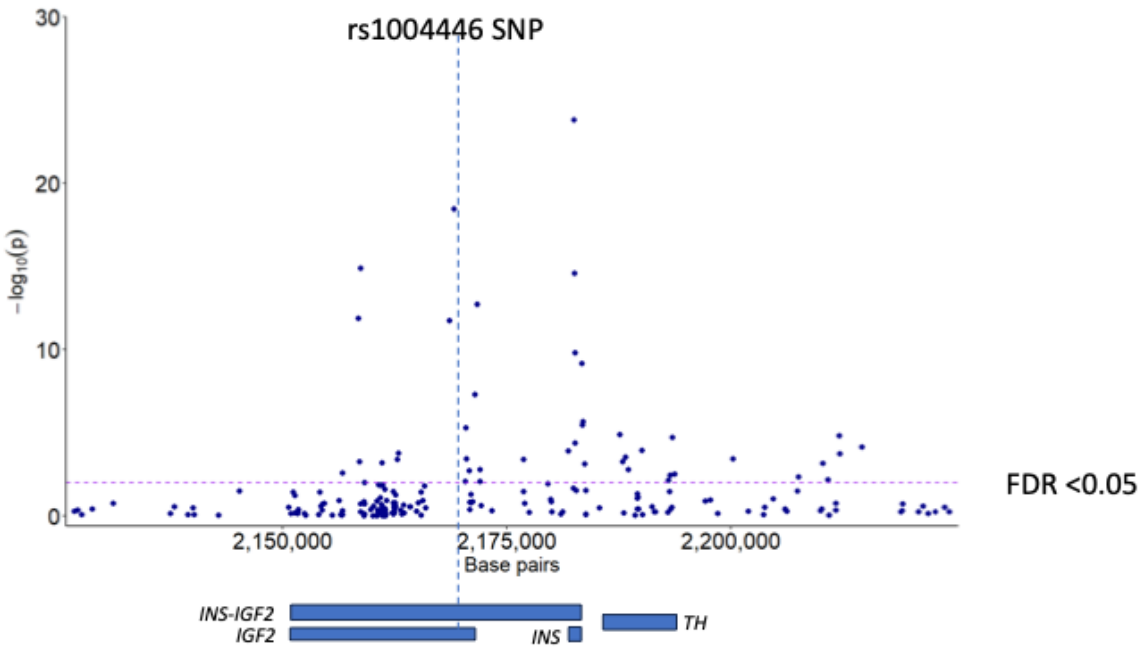


Figure S8. DNA methylation of CpG sites in the chromosome 11 region containing the *INS-IGF2* gene region. DNA was extracted from peripheral blood mononuclear cells collected from 794 participants of the POInT study a visit 5 (age 1·5 years), bisulphite treated and methylation at CpG sites quantified using the Infinium Methylation EPIC (850K) Bead-Chip array (Illumina, San Diego, CA, USA). DNA methylation at CpG sites was compared between participants with INS rs1004446 susceptible (n=450) and non-susceptible (n=344) genotypes by robust linear regression using the R package *limma*. Regression models were adjusted sex, the first three principal components, and the six estimated blood cell types. P values are given as $-\log_{10}$ on the y axis and the position of CpG sites on chromosome 11 are given on the x axis. The position of the rs1004446 SNP is indicated by the vertical dashed line. The positions of the *INS-IGF2* region, and the neighbouring *TH* gene are indicated by the blue boxes under the x axis. The horizonal dashed line represents a false discovery rate (FDR) of 0·05 and was used to define significance. A more detailed description of the methods is provided in reference 13 (appendix p 5).

Appendix documents:……..Page

GPPAD-03-POInT (Global Platform for the Prevention of Autoimmune Diabetes – Primary Oral Insulin Trial)

Oral Insulin Therapy for Prevention of Autoimmune Diabetes

A study of the Global Platform for the Prevention of Autoimmune Diabetes

Protocol No.: GPPAD-03-POInT (*path from GPPAD-02)
EudraCT-No.: 2017-003088-36
IMP: Insulin
Dose form: 7.5mg; 22.5mg; 67.5 mg insulin capsules

Version 2, 05.09.2017

Protocol Chair

Prof. Dr. Anette-G. Ziegler

Protocol Authors

Prof. Dr. Anette-G. Ziegler, Prof. Dr. Ezio Bonifacio, Prof. Dr. Helena Elding Larsson

Sponsor

Technische Universität München, School of Medicine

Site Principal Investigator:
Site Co-Investigator:

Supported by: The Leona M and Harry B Helmsley Charitable Trust
GPPAD-Coordinating Center:
Forschergruppe Diabetes, Klinikum rechts der Isar, TUM and Institute of Diabetes Research, Helmholtz Zentrum München
Statistical advice/ data analysis/ Trial Design: IBE, LMU Munich

PROTOCOL APPROVAL

My signature below confirms my agreement with the design of the study as outlined within this protocol

26.9.17

Prof. Dr. med. Peter Henningsen Date
Sponsor, Dean, Technische Universität München, School of Medicine, Munich, Germany

5.9.17

Prof. Dr. med. Anette-G. Ziegler Date
Protocol Chair, Forschergruppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Germany

26.9.17

Prof. Dr. Ezio Bonifacio Date
Protocol Committee Member, DFG-Center for Regenerative Therapies Dresden, Germany

27/9/17

Prof. Dr. Helena Elding Larsson Date
Protocol Committee Member, Lund University, Department of Clinical Sciences/Malmo, Skane University Hospital SUS, Malmo, Sweden

05/09/17

PD Dr. Markus Pfirrmann Prof. Dr. med. Joerg Hasford Date
Statisticians, IBE, Ludwig-Maximilians-Universität München, Munich, Germany

Version 2, 05.09.2017

SYNOPSIS

Sponsor	Investigator Initiated Trial, Technische Universität München, represented by the School of Medicine
Title	Oral Insulin Therapy for Prevention of Autoimmune Diabetes
Short title	GPPAD-POInT (Global Platform of Autoimmune Diabetes – Primary Oral Insulin Trial)
Study phase	Phase IIb
Protocol Chair / Committee	Anette-G. Ziegler, MD (chair) Ezio Bonifacio, PhD Helena Elding Larsson, MD, PhD
Population/ Indication	Infants genetically at-risk for type 1 diabetes, age 4 months – 7 months
Study Design	Investigator initiated, randomized, placebo-controlled, double-blind, multi-centre primary intervention study.
Accrual Objective	1040 (1:1 randomization to oral insulin and placebo arms)
Study Objective	To determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1 diabetes reduces the cumulative incidence of beta-cell autoantibodies and diabetes in childhood.
Intervention	Eligible subjects will be randomized either into 1. oral insulin group (dose escalation: 7.5 mg for 2 months, increasing to 22.5 mg for 2 months, increasing to 67.5 mg until age 3.00 years) or 2. placebo group. Guardians of participants will self-administer the Investigational Medicinal Product (oral insulin or oral placebo). Treatment will be administered daily preferably in the morning (7-10am).
End of treatment	Children will stop treatment the day of the 3rd birthday, or when they develop diabetes.
Primary Outcome	The primary outcome is the development of persistent confirmed multiple beta-cell autoantibodies (defined as confirmed IAA, confirmed GADA, confirmed IA-2A, or confirmed ZnT8A in two consecutive samples, AND a confirmed second antibody in one sample) or diabetes.
Secondary Outcome	1. Any persistent confirmed beta-cell autoantibody, defined as at least one confirmed autoantibody in two consecutive samples, including GADA, IA-2A, IAA, ZnT8A, or TS7A, or diabetes

	2. Persistent confirmed IAA. 3. Persistent confirmed GADA 4. Abnormal glucose tolerance (AGT) defined by dysglycemia or diabetes.
Timeline	Recruitment: 3.5 years intended start (FPFV): January 2018 Intervention period: 29 to 32 months per participant Follow-up after intervention: 6-54 months Intended End (LPLV): January 2025 Interim Analysis: ~4.5 years after first randomization
Inclusion criteria	1 Infant between the ages of 4 months and 7 months at the time of randomization. 2 A high genetic risk (>10%) to develop beta-cell autoantibodies by age 6 years: 1. For infants without a first degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4. 2. For infants with a first degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503. 3 Solid foods introduced into diet of infant 4 Written informed consent signed by the custodial parent(s).
Exclusion criteria	1. Concomitant disease or treatment that may interfere with the assessments, as judged by the investigators. 2. Any condition that could be associated with poor compliance. 3. Any medical condition or medical condition coexisting, which, in the opinion of the investigator, may jeopardize the participant's safe participation in the study. 4. Diagnosis of diabetes at the time of recruitment. 5. Participation in another clinical trial.
Investigational Product	Active ingredient: insulin provided as bulk human crystals filled in capsules. Formulation of 7.5 mg, 22.5 mg and 67.5 mg insulin and microcrystalline cellulose as filling substance. The reference placebo (filling substance only: microcrystalline cellulose) is identical in appearance to the active medication. Trade name: n.a. Manufacturer: NextPharma

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AGT	Abnormal Glucose Tolerance
AR	Adverse Reaction
CRF	Case Report Form
CTCAE	Common Toxicity Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
FBE	Full Blood Examination
FPFV	First patient first visit
GCP	Good Clinical Practice
GPPAD	The Global Platform for the Prevention of Autoimmune Diabetes
GPPAD CC	GPPAD Coordination Center
IASP	Islet Autoantibody Standardization Program
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
ITI	Immune Tolerance Induction
ISF	Investigator Site File
LPLV	Last patient last visit
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
RBQM	Risk-Based-Quality-Monitoring
RCT	Randomized controlled trial
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SDV	Source Data Verification
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1D	Type 1 diabetes mellitus
T1DGC	Type 1 Diabetes Genetic Consortia
TMF	Trial Master File
UAR	Unexpected Adverse Reaction
WTCCC	Wellcome Trust Case Control Consortium

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Data Management:	Dr. Florian Haupt Institute of Diabetes Research Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany and CenTrial GmbH Paul-Ehrlich-Straße 5 72076 Tübingen, Germany
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Randomisation:	Münchner Studienzentrum Klinikum rechts der Isar der Technischen Universität München Ismaninger Straße 22 81675 Munich, Germany
Monitoring supervision:	Münchner Studienzentrum Klinikum rechts der Isar der Technischen Universität München Ismaninger Straße 22 81675 Munich, Germany
AND European Monitoring acquisition and coordination:	CenTrial GmbH Paul-Ehrlich-Straße 5 72076 Tübingen, Germany

DECLARATION OF INVESTIGATOR

I have read the clinical study protocol and I confirm that it contains all information to accordingly conduct the clinical study. I know that the study will be done in agreement with GCP and I will cooperate with the respective Monitors and pledge the clinical study will be conducted at my study centre according to the protocol.

The first patient will be enrolled only after all ethical and regulatory requirements are fulfilled. I pledge that written informed consent for trial participation will be obtained from all patients.

I know the requirements for accurate notification of serious adverse events and I pledge to document and notify such events as described in the protocol.

I pledge to retain all trial-related documents and source data as described. All necessary documents will be provided before trial start. I agree that these documents will be submitted to the responsible Regulatory Authorities and Ethics Committees.

_____

Investigator of the site Date

GPPAD-POInT STUDY: VISIT –SCHEDULE (STUDY FLOW CHART)

POInT Trial	Trial												
	Screening Phase	Intervention											
Visits		baseline visit (age 4 - 7 months)	visit 2 months post baseline	visit 4 months post baseline	visit 8 months post baseline	call	visit at age 1.5 years	call	visit at age 2.0 years	call	visit at age 2.5 years	call	visit at age 3.0 years
Visit window	below 7 months of age		± 10d	± 10d	± 10d		± 10d		± 14d		± 14d		± 14d
Study visit	0	1	2	3	4		5		6		7		8
Study call						1		2		3		4	
Informed consent	X												
Review Incl./Excl. Criteria	X												
Randomization		X											
Medical History	X												
Psychological impact Questionnaire (mother&father)				X			X						X
Antibodies measurement [A] (IAA; GADA; IA-2A; ZnT8RA; ZnT8WA; TS7A)		X	X	X	X		X		X		X		X
Vitamin D (25OHD) [B]		X	X	X	X		X		X		X		X
Intervention													
dispense medication (+ compliance data sheet)		X	X	X	X		X		X		X		
Treatment		daily with 7,5 mg Insulin OR Placebo	daily with 22,5 mg Insulin OR Placebo	daily with 67,5 mg Insulin OR Placebo									
Investigations													
Physical examination (height, weight)		X	X	X	X		X		X		X		X
Assessment of AEs and SAEs [E]		X	X	X	X		X		X		X		X [E]
Blood glucose (0/30/60/120) [C]		X	X	X	X								
Blood glucose							X		X		X		X
Differential blood count		X											X
OGTT (0/30/60/90/120) [D]													X
Storage													
storage: serum samples		X	X	X	X		X		X		X		X
storage: plasma samples		X			X				X				X
PBMC		X			X				X				X
blood volumes for protocol parameters (mL):		5.0	3.8	3.8	3.8		3.8		3.8		3.8		5-11
blood volumes for protocol parameters (%):		0.8-1.0	0.5-0.6	0.5-0.6	0.4-0.5		0.4		0.4		0.3		0.4-0.9
additional biobank blood volumes (mL):		7.5	10.1	10.1	17.6		17.6		22.5		17.6		17.6-22.5
Total blood volumes (mL):		12.5	13.9	13.9	21.4		21.4		26.3		21.4		27.5-28.6
Total blood volume (%)**:		1.9-2.4	2.0-2.2	1.8-2.0	2.5-2.8		2.4		2.7		2.0		2.3-2.4

**Blood volumes are < 5% NIH/WHO allowance and in accorance to the Pre-POINT (Bonifacio et al.: Effects of high dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-1549, 2015), Pre-POINT Early (EudraCT number: 2014-005287-15, NCT02547519, BfArM no. 4040595), and Fr1da-Intervention (EudraCT number: 2015-003028-30, NCT02620072, BfArM no. 4040830) studies

[A] Tetraspanin 7 (TS7A) will be measured in children who are confirmed positive for GADA, IA-2A, IAA or ZnT8 (ZnT8A)

[B] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with 1000 IU vitamin D daily

[C] Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo)

[D] If the participant developed beta-cell-autoantibodies during trial. OGTT (0/30/60/90/120) will be performed and samples measured in laboratory
Children who seroconverted to beta-cell-autoantibodies should have a confirmation sample within 4 – 12 weeks (interim visit)

[E] AEs/SAEs/SUSARs will be noted and reported as under intervention phase for 60 days after end of treatment day

POInT Trial	Trial																	
	Follow-up																	
	minimum 6 months FU		variable with maximum up to 54 months FU															
Visits	call	visit at age 3.5 years	call	visit at age 4.0 years	call	visit at age 4.5 years	call	visit at age 5.0 years	call	visit at age 5.5 years	call	visit at age 6.0 years	call	visit at age 6.5 years	call	visit at age 7.0 years	call	visit at age 7.5 years
Visit window		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d
Study visit		9		10		11		12		13		14		15		16		17
Study call	5		6		7		8		9		10		11		12		13	
Informed consent																		
Review Incl./Excl. Criteria																		
Randomization																		
Medical History																		
Psychological impact Questionnaire (mother&father)		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]
Antibodies measurement [A] (IAA; GADA; IA-2A; ZnT8RA; ZnT8WA; TS7A)		X		X		X		X		X		X		X		X		X
Vitamin D (25OHD) [B]																		
Intervention																		
dispense medication (+ compliance data sheet)																		
Treatment																		
Investigations																		
Physical examination (height, weight)		X		X		X		X		X		X		X		X		X
Blood glucose (0/30/60/120) [C]																		
Blood glucose		X		X		X		X		X		X		X		X		X
Differential blood count																		
OGTT (0/30/60/90/120) [D]		X		X		X		X		X		X		X		X		X
Storage																		
storage: serum samples		X		X		X		X		X		X		X		X		X
storage: plasma samples				X				X				X				X		X
PBMC				X				X				X				X		X
blood volumes for protocol parameters (mL):		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8
blood volumes for protocol parameters (%):		0.3-0.8		0.3-0.7		0.3-0.7		0.3-0.6		0.2-0.6		0.2-0.6		0.2-0.5		0.2-0.5		0.2-0.5
additional biobank blood volumes (mL):		25.1		25.1		25.1		30		30		30		30		30		30
Total blood volumes (mL)*:		28.9-34.9		28.9-34.9		28.9-34.9		33.8-39.8		33.8-39.8		33.8-39.8		33.8-39.8		33.8-39.8		33.8-39.8
Total blood volumes (%)*:		2.5-3		2.1-2.6		2.1-2.6		2.2-2.6		2.2-2.6		2.0-2.3		2.0-2.3		1.7-2.0		1.7-2.0

**Blood volumes are < 5% NIH/WHO allowance and in accorance to the Pre-POINT (Bonifacio et al.: Effects of high dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-1549, 2015), Pre-POINT Early (EudraCT number: 2014-005287-15, NCT02547519, BfArM no. 4040595), and Fr1da-Intervention (EudraCT number: 2015-003028-30,

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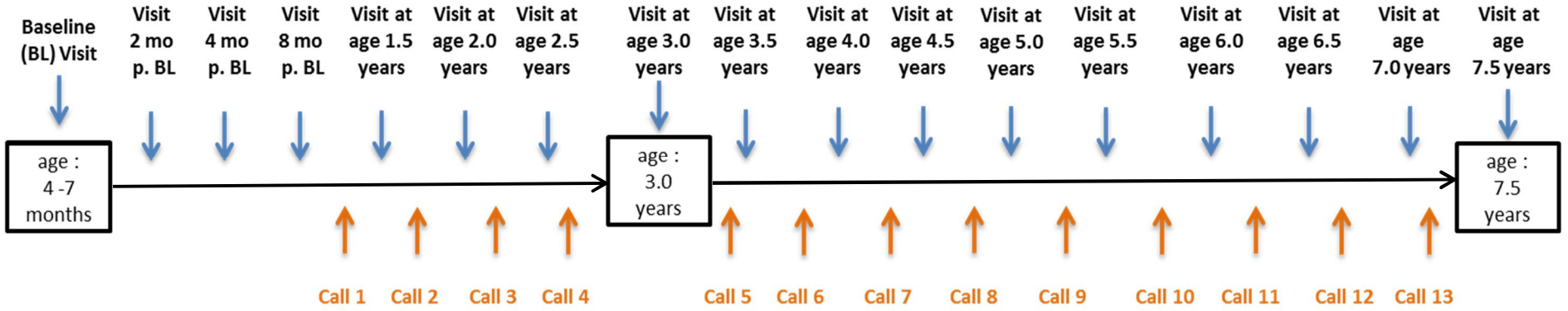
Children who seroconverted to beta-cell-autoantibodies should have a confirmation sample within 4 – 12 weeks (interim visit)

[F] hand out of the Psychological Impact Questionnaire only if this visit is childs last follow-up visit (i.e. only at End of Study Visit)

GPPAD-POInT: TIME SCHEDULE

Example for a participant with maximum follow-up of 54 months:



1. BACKGROUND AND SIGNIFICANCE

1.1 TYPE 1 DIABETES: DEFINITION AND METABOLIC CHARACTERISTICS

Type 1 diabetes (T1D) is an immune-mediated disease in which insulin-producing beta-cells are completely or near completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients diagnosed with T1D each year is increasing and is approaching an epidemic level in some countries that track this information (1, 2).

Compared to individuals with the more common form of diabetes, type 2 diabetes, (where individuals retain endogenous insulin production that is inadequate to maintain normal glucose and lipid metabolism), patients with T1D have a more severe metabolic impairment and a more complete loss of insulin production. At the time of diagnosis, many individuals, and children in particular, suffer significant morbidity frequently requiring ICU admission. Continuous exogenous insulin therapy is needed to prevent ketoacidosis and other catabolic effects of insulin deficiency and to promote anabolism and to maintain life. The Diabetes Control and Complications study (DCCT) showed that the long term complications could be reduced with near normal control of glucose levels but at the cost of an increased frequency of severe hypoglycemia (3). While there have been significant improvements in insulin analogs and insulin delivery systems, such as continuous subcutaneous insulin infusions with insulin pumps, normal glucose control, particularly in children, is rarely achieved. Therefore, individuals with T1D remain at risk for chronic secondary end-organ complications including visual impairment and blindness, renal failure, vascular disease and limb amputation, peripheral neuropathy, and stroke. They are also at high risk for acute complications such as severe hypoglycemia, recurrent ketoacidosis, and others.

Prevention of T1D would clearly represent a significant advancement.

1.2 NATURAL HISTORY OF TYPE 1 DIABETES

T1D results from an immune-mediated destruction of the pancreatic islet beta-cells resulting in insulin deficiency. This process is clinically silent and can be identified by circulating autoantibodies to beta-cell antigens (GADA, IA-2A, IAA and ZnT8A). Beta-cell autoantibody seroconversion has a clear peak incidence period between age 9 months and 3 years demonstrated in German (4), Finnish (5), and TEDDY studies (6) (Figure 1). In a recent combined analysis of over 13000 prospectively followed children form the BABYDIAB, DAISY, and DIPP studies, 80% of the children who developed T1D before the age of 20 years already developed beta-cell autoantibodies before the age of 5 years (median (IQR) age 2.1 (1.3-4.1) years) (7). On the basis of these findings, it is concluded that immune therapy given as a primary prevention strategy must be started early in life.

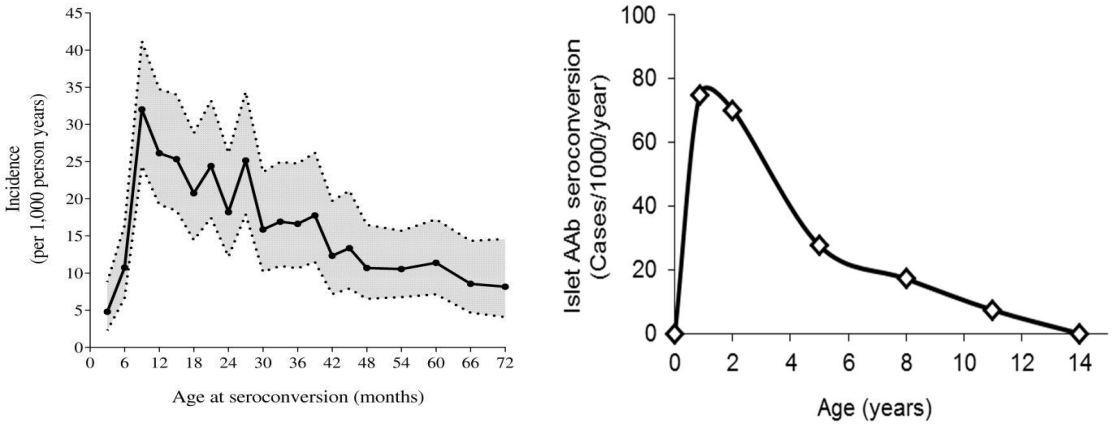


Figure 1: The incidence of beta-cell autoantibodies peaks in early childhood in children including general population at genetic risk for T1D (left) (8) and with a first degree relative with T1D (right) (9)

Almost all children who develop the stage of multiple beta-cell autoantibodies progress to clinical diabetes (Figure 2). The earlier the process of beta-cell autoimmunity is initiated, the more rapid is the progression to T1D (7).

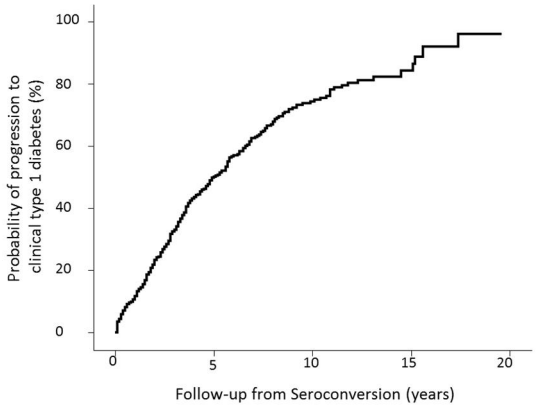


Figure 2: Children with multiple autoantibodies progress to symptomatic T1D

1.3 IDENTIFICATION OF SUBJECTS AT INCREASED RISK FOR BETA-CELL AUTOIMMUNITY AND T1D

T1D has a multifactorial etiology, which is determined by genetic and environmental factors (17). Risk in a European population is around 0.4%. A first degree family history of T1D is associated with a 5% risk for type 1 diabetes (18). There are also at least 50 known regions of the genome where genetic variation is associated with T1D risk (19). The most important of these is in the HLA DR-DQ region of chromosome 6. Certain HLA DR-DQ genotypes confer markedly elevated risk for T1D. Notably, infants who have the HLA DR3/DR4-DQ8 or the DR4-DQ8/DR4-DQ8 genotype have a risk of around 5% (20, 21). Typing at additional T1D susceptibility regions can identify infants with risks that are 10% or more (22). Thus, family history and genetic markers can be used to identify neonates or infants with 25-fold increased risk for T1D.

By analyzing data from the Type 1 Diabetes Genetic Consortia (T1DGC), it has been recently demonstrated that a genetic risk score generated from HLA class II genotypes and 40 SNPs of non-HLA genes associated with T1D predisposition can improve risk stratification for T1D over HLA alone (22). Similarly, by analyzing data from the Wellcome Trust Case Control Consortium (WTCCC), a genetic risk score of 30 SNPs was developed to estimate T1D risk (23). Both risk scores were now validated and applied to data from The Environmental Determinants of Diabetes in the Young (TEDDY) study (manuscript in preparation). In the TEDDY data, risk stratification using each genetic risk score was reproduced. Therefore, a score that merges the prior algorithms (22, 23) was calculated and used in the TEDDY children. Children with no family history of T1D who have the HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 genotype and a genetic risk score of >14.4 using the merged algorithm (corresponds to upper 75th percentile of HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 TEDDY population) had a risk of 15.9% for developing beta-cell autoantibodies by age 5 years and 11.4% for developing multiple beta-cell autoantibodies by age 6 years (figure 3 a, b). In first degree relatives of a patient with T1D, the presence of at least one HLA DR4-DQ8 haplotype and no protective HLA DR and DQB1 alleles is associated with a genetic risk of >10% for developing multiple beta-cell autoantibodies by age 6 years (18, 21).

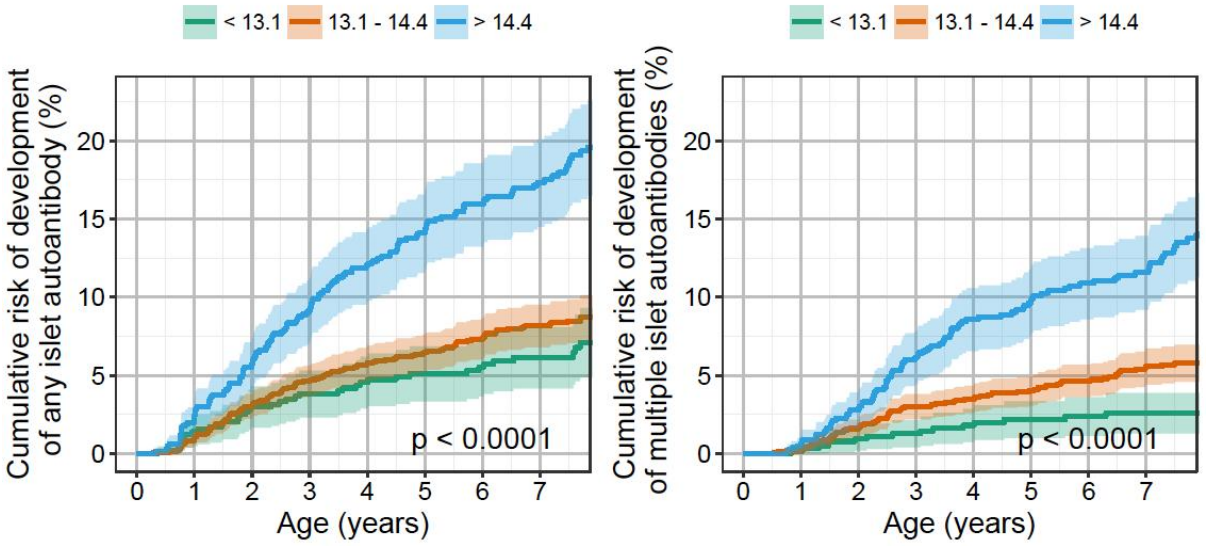


Figure 3: Risk of beta-cell autoimmunity (left, first autoantibody; right, multiple beta-cell autoantibodies) in TEDDY children with HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 genotype and a genetic risk score of > 14.4 (blue line) using the merged algorithm (corresponds to upper 75th percentile of HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 TEDDY population) compared to children with HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 genotype and a respective score between 13.1 and 14.4 (orange line), and below 13.1 (green line). P-values were derived from log-rank tests on differences in autoantibody risk between children in the three risk groups. Shaded areas represent the 95% confidence interval or risk estimates.

1.4 RATIONALE FOR USE OF ORAL INSULIN AS IMMUNE TOLERANCE INDUCTION THERAPY

Self-tolerance is achieved by T cell exposure to self-antigens in the thymus or in the periphery (i.e. outside the thymus or bone marrow, in secondary lymphoid tissues such as lymph nodes, gut and spleen) in a manner that deletes or anergizes autoreactive

effector T cells and induces regulatory T cells. Immunological tolerance can be achieved by administration of antigen under appropriate conditions (7, 17). Evidence is now emerging in humans that these approaches may be effective in chronic inflammatory diseases such as multiple sclerosis, allergy, and T1D (25-27).

Our goal is to introduce immune tolerance to autoantigen before the start of beta-cell autoimmunity as primary prevention for T1D (23, 28). Primary prevention of T1D has a strong rationale. There is clear evidence from man (29, 30) that insulin is the key early and primary autoantigen of childhood diabetes. There is also a strong genetic rationale for loss of tolerance against insulin as a primary cause of T1D. Allelic variation in the *insulin* gene is associated with T1D (31) and beta-cell autoimmunity (32) via an impaired mechanism of thymic T cell deletion (33). Polymorphisms in the *INSULIN (INS)* gene confer genetic risk for T1D by altering insulin expression in the thymus, thereby influencing immune tolerance to insulin and its precursors (32, 33). Children who have increased exposure to insulin in foetal and neonatal life as a result of having a mother with type 1 diabetes (34), have a reduced risk for developing beta cell autoantibodies (35). Moreover, insulin autoimmunity is closely linked to the HLA DR4-DQ8 haplotype present in the majority of children who develop T1D (36, 37).
There is a general consensus that turning back or undoing a full-fledged memory autoimmune response to multiple beta-cell autoantigens (secondary prevention) will require more aggressive therapies than preventing autoimmunity in the first place.
It is widely held that if infant tolerance to beta-cell autoantigens could be enhanced, this could prevent or delay the onset of pre- or asymptomatic T1D (defined as multiple beta-cell autoantibodies), and hence prevent or delay disease diagnosis. The key here is “infant”, the time when the natural mechanisms of immune tolerance are fully active as the child becomes tolerant to commensal microorganisms and dietary components. Currently, antigen-specific tolerance approaches are attempted in individuals in whom the immune system has matured and in whom an autoimmune memory response is well established. We, however, have laid the foundation for antigen-specific tolerance induction as primary prevention (initiated prior to an autoimmune response). We have identified a dose of insulin that, when administered orally on a daily basis to genetically at-risk children who are beta-cell autoantibody negative, is safe (does not affect plasma glucose levels) and appears to engage the immune system in a manner that is consistent with immune-mediated, tolerogenic protection (38).
Hence, we believe that there are two important pillars for primary prevention to move forward – knowledge of when diabetes inducing beta-cell autoimmunity starts and demonstration that insulin-specific protective ITI is feasible.

1.5 EVIDENCE FOR ANTIGEN BASED PREVENTION IN HUMAN ALLERGY

Supporting evidence of antigen based therapies has been recently shown in a large study, which aimed to prevent peanut allergy through active exposure to peanut antigen. Unlike previous attempts based on avoidance of peanuts, the consumption trial was successful. Relevant to primary prevention, the LEAP trial enrolled 542 infants who initially had no pre-existing sensitivity to peanuts, but who had an estimated 9% risk for developing peanut allergy by age 5 years (27). Children randomized to peanut consumption were instructed to eat at least three peanut-containing meals per week - starting at age 4 to 11 months - in order to consume at least 6 g of peanut protein per week until age 5 years. The prevalence of peanut allergy at 60 months of age was 13.7%

in children who avoided peanut consumption and, remarkably, only 1.9% in children who consumed peanuts (P < 0.001). The results are even more striking in a per protocol analysis where only 0.4% of non-sensitized children developed peanut allergy in the consumption group. Another impressive aspect of the LEAP trial was that only 12 (2.2%) of the 542 enrolled children did not complete the study at age 5 years. Overall, the primary prevention part of the LEAP trial is encouraging for GPPAD attempts to introduce autoantigen-based primary prevention for T1D.

1.6 SIGNIFICANCE OF THE GPPAD-POINT STUDY

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.
Additionally, antigen-based therapy in T1D could also serve as a model for other childhood conditions and illnesses, with a major underlying goal of the promotion of better health outcomes early in life based on improved understanding of the human immune system.

2. CLINICAL AND PRE-CLINICAL DATA

2.1 PREVIOUS CLINICAL TRIALS USING ORAL INSULIN

Primary Prevention
The Pre-POINT (Primary Oral Insulin Trial)-Study (Protocol number: 80804002, BfArM number: 4034919, EudraCT number: 2005-001621-29, ISRCTN76104595).
We have conducted and completed a primary autoantigen immune tolerance dose-finding study in which children with high genetic risk for T1D were administered insulin orally daily (38). The objective of this pilot Pre-POINT study was to identify a dose of oral insulin that was safe and could engage the immune system when administered as a primary intervention to children without beta-cell autoimmunity. Pre-POINT was performed as a double-blind placebo controlled dose increasing phase I/II clinical trial. Children aged two to seven years with a family history of T1D and T1D susceptible HLA class II genotypes and without beta-cell autoantibodies (n=25) were randomized to receive placebo (n=10) or insulin (n=15) orally once a day for 3 to 12 months. The design included dose escalation so that six children were included in each of the 2.5 mg, 7.5 mg, 22.5 mg, and 67.5 mg insulin dose groups. Safety was assessed by blood glucose measurements following administration of medication, serum IgE concentrations, serum IgE against insulin, and measurement of autoantibodies to glutamic acid decarboxylase and IA-2. Activation of the immune system by the study drug was measured by insulin autoantibodies, IgG- and IgA-binding to insulin in serum and saliva, and CD4+ T cell proliferative responses /gene expression to study drug. Oral insulin at all tested doses in Pre-POINT was considered safe: None of the children who received study drug or placebo experienced hypoglycaemic episodes after administration of medication, and no allergic reactions were observed. Adverse events were similar between study drug and placebo groups. No child developed autoantibodies to glutamic acid decarboxylase or IA-2 or diabetes during the reported observation period of 6 months to maximum 3.5 years (see Appendix 1: Synopsis of Final Study Report). Important for the current

protocol, five of six children exposed to a dose of 67.5 mg insulin had evidence of an antibody or T cell response to insulin (see Table 1). The response differed to the typical responses seen in children who develop diabetes in that the antibody responses were of weak affinity (not inhibitable with low concentrations of cold insulin in reference methods used to measure insulin autoantibodies) and the T cell responses had a preponderance of cells with regulatory T cell phenotypes. These results are encouraging from a safety viewpoint and indicate that oral exposure to insulin at doses that are approximately equivalent to efficacious doses in rodents may promote tolerance in children.

Table 1: Summary of immune responses to study drug (insulin) in Pre-POINT children

Response measure against insulin	Placebo	2.5 mg insulin	7.5 mg insulin	22.5 mg insulin	67.5 mg insulin
Serum IgG-IAA	1/10	0/6	1/6	1/6	3/6
Salivary IgA-IAA	0/10	0/6	0/6	1/6	0/6
$CD4^+$ T cell	1/7	1/6	0/5	1/5	2/4
Antibody or $CD4^+$ T cell response	2/10	1/6	1/6	2/6	5/6

The Pre-POINT-Early Study (Protocol number: 80804017, BfArM number: 4040595, EudraCT number: 2014-005287-15, NCT02547519).

Pre-POINT-Early is a study using oral insulin at early age for safety and immune efficacy. The aim of this Phase II Study is to determine whether daily administration of up to 67.5 mg insulin to young children aged 6 months to 2 years with a high genetic and familial risk for T1D is safe and induces immune responses to insulin with features of immune regulation. Autoantibody negative children at high genetic risk for T1D, age 6 months – 2 years are randomized either into 1. Oral insulin (dose escalation: 7.5 mg for 3 months; increased to 22.5 mg for 3 months; increased to 67.5 mg for 6 months) or 2. placebo. Currently, all 44 subjects are enrolled. There have been no safety issues observed thus far (no hypoglycemia, 215 AEs, 5 SAE). All blood glucose measurements and all SAE and AE that have occurred in participants of the Pre-POINT-Early study are provided in appendix D.2 and D.6 of the Investigator Brochure. A regularly updated AE list is available on demand from GPPADregulatory@helmholtz-muenchen.de. The safety data for Pre-POINT-Early without unblinding is provided in appendix D4.7 of the Investigator Brochure. Thus, far 2 (4.5%, 95%CI, 1.3% to 15%) of the 44 participating children have developed autoantibodies to GAD, IA-2 or ZnT8, which is not more than the expected frequency in at risk children of similar age and follow-up time. Also consistent with Pre-POINT, insulin autoantibody data has demonstrated the development of high affinity insulin autoantibodies in only one of the participants so far; some participants have developed low or moderate affinity insulin autoantibodies (without developing autoantibodies to GAD, IA.2 or ZnT8) as a potential sign of treatment response – ie, responses which are not unintended).

Secondary Prevention

The TN07 study (Oral Insulin for Prevention of Diabetes in Relatives at Risk for Type 1 Diabetes Mellitus) (Protocol number: TN07, 80804005, EudraCT number: 2006-006550-96, NCT00419562).

A secondary prevention multicentre study using 7.5 mg oral insulin administered daily is conducted by the TrialNet Study Group. This study included multiple sites in Europe such

as the Forschergruppe Diabetes, Klinikum rechts der Isar der Technischen Universität München, and the Lund University, Skåne University Hospital SUS. Autoantibody, normoglycemic subjects aged 3 to 45 years are treated with oral insulin. There are over 500 subjects enrolled (479 children <18 years and 102 subjects <5 years respectively). No safety issues have been observed thus far (a regularly updated AE list is available on the internal TrialNet TN07 website and available upon request at trialnetinfo@epi.usf.edu). The TN07 results were presented orally at the American Diabetes Association Conference (San Diego) in June 2017. There was no evidence that treatment with 7.5 mg oral insulin accelerated disease in any of the pre-specific participant Strata. In the secondary stratum with first phase insulin response to glucose below threshold (n=55), time to diabetes was significantly longer with oral insulin: HR=0.45 (95% CI 0.22, 0.91), p=0.01, indicating protection. In the other secondary stratum (n=116), and the entire cohort (n=560), there was no significant difference between groups: HR=1.03 (95% CI 0.44, 2.42), p=0.95 and HR= 0.79 (95% CI: 0.58, 1.06) p=0.10. The most common adverse event was infection (n=255) but there were no significant study related adverse events.

The Fr1da-Insulin-Intervention Study (Mechanistic study using oral insulin for immune efficacy in secondary prevention of type 1 diabetes) (Protocol number: 808040019, EudraCT number: 2015-003028-30, NCT02620072).

The objective of this phase II study is to determine the bioavailability and immune efficacy of high dose oral insulin in children with multiple beta-cell autoantibodies in a secondary intervention study. Immune efficacy is defined as a change in the immune response to the treatment that is associated with a reduction in the progression to dysglycemia. Children in the oral insulin group receive increasing dose of daily oral insulin: 7.5 mg for a duration of 3 months and increasing to 67.5 mg for 9 months of intervention. Children in the placebo group will receive 12 months of daily oral placebo. The study aims to recruit 220 participants. As of February 2017, there are 68 children enrolled in the trial. No safety concerns have been observed thus far (129 AEs, 3 SAEs). A regularly updated AE list is available on demand from GPPADregulatory@helmholtz-muenchen.de.

Table 2: Cumulative exposure to study drug in Pre-POINT-Early and Fr1da-Insulin-Intervention (as of 17.02.2017)

RCT	Status		7.5 mg or Placebo	22.5 mg or Placebo	67.5 mg or Placebo
Pre-POINT	• Study completed • Exposure unblinded	Age 2-7 yrs	54 month	48 months	48 months
Pre-POINT-Early	• Study active, recruitment completed • Cumulative exposure numbers blinded • Treatment allocation 1:1 ratio	Age 6 mo - 2 yrs	134.8 months	127.6 months	195.8 months

Fr1da-Insulin-Intervention	• Study active and recruiting • Cumulative exposure numbers blinded • Treatment allocation 1:1 ratio	Age 2-12 yrs	248.1months	n.a.	538.4 months
Total			436.9months	175.6 months	782.2 months

2.2 PRECLINICAL DATA AND OTHER HUMAN STUDIES

Previous studies in rodents had indicated that mucosal administration of insulin is effective in inducing regulatory immune responses that can prevent autoimmune diabetes (39-42). Mouse studies indicated that the dose of oral insulin is important (41). For Pre-POINT we had reasoned that doses above 50 mg per day were required in children in order to match efficacious doses in mouse models of autoimmune diabetes (41), and the results shown in table 1 support this reasoning.
Other previous human studies had given oral insulin at doses between 2.5 mg and 15 mg to diabetic or non-diabetic subjects without side effects (43-45). The studies demonstrated no obvious benefit in diabetic subjects with respect to preservation of residual beta-cell function. The administration of oral insulin (7.5 mg per day) to prediabetic ICA and IAA positive first degree relatives of T1D patients in the DPT-1 study showed no significant beneficial effect in the intention to treat analysis. A sub-analysis of the data, however, showed significant benefit in those relatives with higher titer IAA (43), that was persistent (46).

3. STUDY DESIGN

3.1 OVERVIEW

The GPPAD-POInT Study is designed as a randomized, placebo-controlled, double blind, multicentre, multinational primary prevention phase IIb study aiming to induce immune tolerance to beta-cell autoantigens through regular exposure to oral insulin for a period of 29 to 32 months.

3.2 HYPOTHESIS

We hypothesize that regular exposure to oral insulin throughout the period in life where beta-cell autoimmunity usually initiates will tolerize against insulin and train the body's immune system to recognize the treatment product without reacting adversely to it in a manner seen in children who develop T1D. This immune tolerance induction therapy would reduce the likelihood of beta-cell autoimmunity.

3.3 OBJECTIVES

The study objective is to determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1

diabetes reduces the cumulative incidence of beta-cell autoantibodies and diabetes in childhood.

3.4 SUMMARY OF INCLUSION / EXCLUSION CRITERIA

Participants must meet all entry criteria for the protocol as outlined below.

3.4.1 Inclusion criteria

Participants must meet all entry criteria for the protocol as outlined below.

1. Infant between the ages of 4 months and 7 months at the time of randomization.
2. A high genetic risk (>10%) to develop beta-cell autoantibodies by age 6 years:
    a. For infants without a first degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4[1]. These represent close to 1% of all newborns.
    b. For infants with a first degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503. These represent around one third of infants with a first degree family history of T1D.
3. Solid foods introduced into diet of infant
4. Written informed consent signed by the custodial parent(s)

3.4.2 Exclusion criteria

Participants meeting any of the following criteria will NOT be eligible for inclusion into the study:

1. Concomitant disease or treatment that may interfere with the assessments, as judged by the investigators. Any condition that could be associated with poor compliance.
2. Any medical condition or medical condition coexisting, which, in the opinion of the investigator, may jeopardize the participant's safe participation in the study.
3. Diagnosis of diabetes at the time of recruitment.
4. Participation in another clinical trial.

3.5 ENROLLMENT

Potential study subjects will be identified through the GPPAD-02- (Freder1k-) study or through similar studies testing for type 1 diabetes risk in infancy. In the Freder1k-study, testing for genetic risk of T1D is offered either at delivery (cord blood), together with the regular newborn screening, or at a pediatric baby-visit before the age of 3 months, with collection of blood using Freder1k filter paper cards. Infants are tested for genetic risk of

[1] The genetic risk score is calculated by multiplying the number of risk alleles (i. e. 0, 1 or 2 for each single SNP) with a specific weight assigned to each SNP (see Table 3 on page 60) and then summing up the weighted contributions of all SNPs plus an additive constant for each HLA category. All 47 SNPs as listed in Table 3 will be determined in each child.

T1D based on risk scores derived from SNPs that define HLA DR3, HLA DR4, and HLA DQ8 alleles as well as SNPs from HLA class I, and non-HLA T1D susceptibility genes, and from HLA class II protective alleles. Infants with a predicted risk for T1D of >10% to develop beta-cell autoimmunity by age 6 years and who fulfill the inclusion criteria as stated above will be asked to participate in the GPPAD-POInT Study.

3.6 DESCRIPTION OF TREATMENT GROUPS

The intervention will be conducted only in children for whom consent to participate has been provided. Eligibility will be verified by the study physician shortly before randomization and at the baseline visit. Infants will be randomized to receive either oral insulin or placebo. Treatment will be provided at approved GPPAD clinical sites with appropriate facilities. Blood and serum samples for the primary and secondary outcome determinations will be sent to central laboratories for analysis. Clinical safety parameters may be done at the local sites.

Participants will be randomly assigned in a 1:1 ratio to the following two groups:

- to receive daily oral insulin 7.5 mg for 2 months, followed by 22.5 mg for 2 months, followed by 67.5 mg until age 3.0 years. Close monitoring for beta-cell autoantibodies, dysglycemia, and diabetes will occur through the duration of study.
- to receive daily oral placebo until age 3.0 years. Close monitoring for beta-cell autoantibodies, dysglycemia, and diabetes will occur through the duration of study.

3.7 DOSAGE FORM

The IMP used in the GPPAD-POInT Study will be essentially identical to the IMP used in the Pre-POINT [2] (36), Pre-POINT-Early[3], and Fr1da-Insulin-Intervention[4] studies. Human insulin for oral administration is provided by Lilly Pharmaceuticals, Indianapolis, Indiana USA. It is provided as bulk human insulin crystals. This insulin is sold by Lilly as an injectable formulation known as Humulin-R. In the GPPAD-POInT Study three doses are given: Dose 1 is 7.5 mg rH-insulin crystals; dose 2 is 22.5 mg rH-insulin crystals; dose 3 is 67.5 mg rH-insulin crystals. The insulin crystals are formulated together with filling substance (microcrystalline cellulose to a total weight of 200 mg) and contained in hard gelatin capsules. The dose was chosen based on demonstrated immune efficacy in children in the Pre-POINT study, and demonstrated safety in children participating in the Pre-POINT, Pre-POINT-Early, and Fr1da-Insulin-Intervention study (see also part 2.1).
The conversion of the mg unit into IU for the 7.5 mg of the oral insulin results in 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.
For further information about the IMPs please also refer to the Investigator's brochure.

[2] Protocol ID: 80804002; EudraCT-No.: 2005-001621-29; CurrentControlledtrials ID: ISRCTN76104595
[3] Protocol ID: 808040017; EudraCT-no.: 2014-005287-15; Clinicaltrials.gov ID: NCT02547519
[4] Protocol ID: 808040019; EudraCT-no.: 2015-003028-30; Clinicaltrials.gov ID: NCT02620072

3.8 APPLICATION, DOSE AND DOSAGE REGIMEN

The study treatment will be given orally as a powder spread on a small quantity meal serving e.g. with infant formula, tea spoon of water, commercial baby food or yogurt. The insulin will be provided in a capsule box à 32 hard gelatin capsules containing rH-insulin crystals or placebo.
The investigational product (oral insulin or placebo) will be self-administered by the child's parents as content of one capsule per day. Treatment will be administered preferably in the morning (7-10am). Parent(s) will be instructed in the administration and storage of study drug at their baseline visit.
Participants will be observed for 2 hours after administration of the study drug at visits 1, 2, 3, and 4. They are advised to immediately report any adverse events experienced following treatment.

3.9 DOSE WITHHOLDING, WITHDRAWAL OR DROP OUT OF PARTICIPANTS

Withdrawal Criteria

Participants will be withdrawn from study treatment if they:

- develop diabetes (study endpoint)
- report moderate to severe intolerance of study treatments
- develop an intercurrent illness deemed incompatible with the study, as judged by the investigators
- have consent withdrawn by custodial parent(s)

The participant and/or his or her parent(s) will be informed that being in the trial is voluntary and that he or she may withdraw consent from the study at any time, for any reason. 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". The reason for discontinuation of study treatment and/or withdrawal from the study will be captured on the Case Report Form. In case custodial parent(s) withdraw consent re treatment but agree to continue the observation of the infant as specified in the trial protocol, this will be done. Every effort will be made to follow all participants enrolled in the study (including those that do not complete the treatment period).

3.10 TREATMENT ASSIGNMENT

Trial inclusion and registration will take place at the baseline visit. Infants will be included and registered if they meet the inclusion criteria and none of the exclusion criteria and after written consent has been obtained by the custodial parent(s). As this trial is double-blind, the trial pharmacy will provide the medication packages sequentially numbered. The study drug packages and thus the participants too will be randomized in a 1:1 ratio to each arm. The Münchner Studienzentrum will generate a unique randomization list for each trial centre centrally and provide these lists to the trial pharmacy only.

3.11 PROCEDURES FOR UNMASKING

Emergency unmasking will occur upon notification of the POInT Medical Monitor and the GPPAD CC via the 24 hour emergency number and approval by POInT Protocol Chair.

Regular (non-emergency) unblinding of study drug assignment is planned to be conducted upon completion and verification, closure of database and completion of all parameter analyses. It will occur upon notification of the GPPAD CC and approval of the POInT Protocol Chair. There are special provisions for the DSMB.

3.12 STUDY ASSESSMENT

During the course of the study, participants will be tested for beta-cell autoantibodies, glucose levels, and will be assessed for their overall health and well-being. In children with beta-cell autoantibodies, OGTTs will be performed at six month intervals starting from age 3.0 years. Individuals in both of the study arms will have examinations performed as detailed in the Flow Table (see page 13).

As an ancillary component of the trial, Biobank repository samples will be drawn for storage in local biobanks at each study site and for storage of aliquots at a central biobank for future research related to T1D. Samples will be pseudonymised before transferred to the biobank. Drawing and storage of samples that are assigned for the biobank is not considered a trial violation.

3.13 QUALITY ASSURANCE

Investigational sites have a clinical study centre with adequate medical space and equipment. Study physicians and study nurses have experience in the conduct of clinical trials and are trained according to GCP Guidelines. Study medication will be stored in a closed, limited access area at the clinical study centre. Approval from the competent ethics committees will be obtained. Medical and research records are maintained at the clinical study centre in the strictest confidence.

Beta-cell autoantibodies as the primary outcome marker are assessed in two central GPPAD autoantibody laboratories. The performance of both laboratories in the Islet Autoantibody Standardization Program (IASP) will be documented and monitored. Additionally, quality assurance of local routine laboratories is guaranteed by choosing accredited laboratories which must present valid ring test certificates or equivalent certificates throughout the study. Study centres must keep documentation of all laboratory certificates.

3.14 STUDY TIMELINE

Study Duration

It is estimated that the enrollment period will last approximately 3.5 years and a total study duration of 7.0 years will provide a sufficient number of events to detect the assumed treatment difference.

Enrolled participants will start treatment from earliest age of 4 months to latest 7 months for a period of 29 up to 32 months (until 3.00 years of age). Participants will be followed until completion of the trial for a period of 6 to 54 months at last follow-up visit.

Note that the accrual period and the study sample are only projections since the actual accrual rate and the lost to follow-up rate are unknown. Participants who discontinue treatment or withdraw from the study will not be replaced.

Participants who develop beta-cell autoantibodies will remain on study and will be followed according to protocol to assess the secondary outcomes until completion of the trial or until diabetes development.
Individuals who develop diabetes may be eligible for interventional studies available through other consortia such as INNODIA.

4. PARTICIPANT MANAGEMENT

4.1 IDENTIFICATION OF ELIGIBLE SUBJECTS FOR POINT

This study will mainly draw participants from the Freder1k-Study (GPPAD 02) that tests the genetic risk for beta-cell autoantibodies in newborns and infants.
Potential participants in the oral trial will have a high genetic risk (>10%) to develop beta-cell autoantibodies by age 6 years.
Inclusion- and exclusion criteria are listed in chapter 3.4.

4.2 SCREENING AND INFORMED CONSENT PROCESS FOR POINT

Prior to randomization, in appropriate time before the baseline visit a screening visit will be conducted. During the screening visit, inclusion and exclusion criteria will be assessed and medical history information will be obtained. Furthermore, the GPPAD-POInT Study will be described to the custodial parent(s) of potential participants by a GPPAD-POInT Study physician. After reading the informed consent form and clarifying all questions to satisfaction with the study physician/investigator(s), all eligible participants' custodial parent(s) will be invited to provide written consent. They will have the opportunity to read the consent document and to discuss any questions concerning the consent or trial participation. If participation in the clinical trial is considered by the family, they may take the consent document home to discuss with family, friend or advocate. The families will be given enough time to consider whether or not to participate. The custodial parent(s) will then be asked to sign and date an informed consent document describing the purpose, risks, and benefits of the trial prior to or at the baseline visit. The signature of the custodial parent(s) indicates that he/she understands the potential risks and benefits of study participation.

The assessment at screening therefore include the following procedures:

1) Assessment of inclusion and exclusion criteria
2) Assessment of medical history
3) Informed consent process

4.3 BASELINE VISIT (BETWEEN 4 AND 7 MONTHS OF AGE)

After written informed consent has been properly obtained, the participant will be randomized to a treatment group (see part 3.10 Treatment Assignment). Participant eligibility must be reviewed and confirmed once more by the study physician immediately prior to randomization.

The clinical assessment at baseline includes the following procedures:
Before first study drug intake:

1) Collection of venous blood for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) 25-OH-Vitamin D3
   c) FBE (differential blood count)
   d) As ancillary storage (subject to ethical approval and separate informed consent): Serum, plasma, PBMC
2) General physical examination (weight, height)
3) Documentation and assessment of AEs and SAEs
4) Administration and dispensation of blinded study drug (7.5 mg insulin OR placebo) will be performed after collecting baseline biological samples (at time point 0 minutes)
5) Monitoring of blood glucose over 2 hours from when blinded study drug is administered:

   Collection of venous blood or capillary blood after study drug intake (30min, 60min, 120min) for blood glucose

4.4 INTERVENTION - VISITS 2, 3 AND 4

The study visits 2 (2 months post baseline visit ± 10 days), visit 3 (4 months post baseline visit ± 10 days), visit 4 (8 months post baseline visit ± 10 days), follow the same schedule. At visit 2, a dose escalation to 22.5 mg Insulin and at visit 3, a dose escalation to the final dose of 67.5 mg Insulin are designated.

The following procedures are scheduled:

1) Collection of venous blood for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) 25-OH-Vitamin D3
   c) As ancillary storage (subject to ethical approval and separate informed consent): serum, plasma, PBMC (at visit 4 only)
2) Physical examination (weight, height)
3) Documentation and assessment of AEs and SAEs
4) <u>At visit 3 only:</u> Psychological Impact Questionnaire completed by mother and father (Appendix 5)
5) Collection of information on medication compliance
6) Administration and dispensation of blinded study drug will be performed after collecting baseline biological samples (at time point 0 minutes). Return of opened/ broken study medication.
7) Monitoring of blood glucose over 2 hours from when blinded study drug is administered:

Collection of venous blood or capillary blood after study drug intake (30min, 60min, 120min) for blood glucose

4.5 INTERVENTION - VISITS 5, 6 AND 7

The study visit 5 (visit at age 18 months ± 10 days), visit 6 (visit at age 24 months ± 14 days) and visit 7 (visit at age 30 months ± 14 days) follow the same schedule.

The following procedures are scheduled:

1) Collection of venous blood for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) blood glucose by glucose meter and laboratory assessment
   c) 25-OH-Vitamin D3
   d) As ancillary storage (subject to ethical approval and separate informed consent): Serum, plasma, PBMC
2) Physical examination (weight, height)
3) Documentation and assessment of AEs and SAEs
4) Collection of information on medication compliance
5) Administration and dispensation of blinded study drug will be performed after collecting baseline biological samples (at time point 0 minutes). Return of opened/ broken study medication.
6) Psychological Impact Questionnaire completed by mother and father (Appendix 5) (only at visit 5)

4.6 TELEPHONE CALLS BETWEEN VISITS

Regular telephone calls between visits will be made to keep closely in touch with the participants and their families. These will be at age 15 months ± 14 days, 21 months ± 14 days, 27 months ± 14 days, and 33 months ± 14 days.
During the telephone call, the following will be made:

1) Assessment of general compliance
2) Documentation and assessment of AEs and SAEs

4.7 INTERVENTION VISIT 8 (END OF TREATMENT)

Study visit 8 will be conducted at age 3.00 years (± 14 days), intake of study medication ends the day before this visit.
The following procedures are scheduled for this visit:

1) Collection of venous blood for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) blood glucose

c) 25-OH-Vitamin D3
d) FBE (differential blood count)
e) As ancillary storage (subject to ethical approval and separate informed consent): Serum, plasma, PBMC

2) Physical examination (weight, height)
3) Documentation and assessment of AEs and SAEs (record and reporting until 60 days after End of Treatment visit)
4) Collection of information on medication compliance
5) Psychological Impact Questionnaire (Appendix 5) completed by mother and father
6) Return of study medication and return of opened/broken study medication.

4.8 FOLLOW-UP VISITS 9, 10, 11, 12, 13, 14, 15, 16 AND 17

The minimum number of follow-up visits is 1 visit in the study centre and 1 telephone calls; the maximum number of follow-up visits is 9 visits in the study centre and 9 telephone calls.
The study visit 9 will be conducted at age 42 months (± 30 days), the study visit 10 will be conducted at age 48 months (± 30 days), visit 11 at age 54 months (± 30 days), visit 12 at age 60 months (± 30 days), visit 13 at age 66 months (± 30 days), visit 14 at age 72 months (± 30 days), visit 15 at age 78 months (± 30 days), visit 16 at age 84 months (± 30 days), and visit 17 at age 90 months (± 30 days). Visit 17 will be the last visit (maximum).
The following procedures are scheduled:

1) Collection of venous blood for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) blood glucose
   c) As ancillary storage (subject to ethical approval and separate informed consent): Serum; at visits 10, 12, 14, 16 and 17 additionally storage of plasma and PBMC
2) Physical examination (weight, height)
3) If this visit is the End of Study Visit for the child, Psychological Impact Questionnaire (Appendix 5) completed by mother and father

A missed visit during the follow-up period is not a protocol violation.

4.9 TELEPHONE CALL BETWEEN VISITS

Regular telephone call visits will be made to keep closely in touch with the participants and their families. These will be at age 39 months ± 14 days, 45 months ± 14 days, 51 months ± 14 days, 57 months ± 14 days, 63 months ± 14 days, 69 months ± 14 days, 75 months ± 14 days, 81 months ± 14 days, and 87 months ± 14 days.
During the telephone visit, the following will be made:

- inquiry on well-being of the participant

4.10 FOR PARTICIPANTS WHO DEVELOPED POSITIVE BETA-CELL AUTOANTIBODIES

If the participant develops one or more beta-cell autoantibodies during the study, the beta-cell autoantibody positive status will be confirmed in the same blood sample by a second central autoantibody laboratory. Study sites will be informed about a positive result.
If the sample is confirmed autoantibody positive by both trial central autoantibody laboratories, the participant should have a confirmation sample drawn within 4-12 weeks. The collection of venous blood for the confirmation sample can be obtained by a local physician and shipped to the study centre.
Additionally, the participant will have a fasting glucose and OGTT evaluation at the regular scheduled study visits from age 3.00 years or from when the child has a confirmed persistent beta-cell autoantibody status, whichever is last. OGTT (0/30/60/90/120)
For the OGTT, the participant needs to be fasting for at least 8 hours. Venous blood samples will be taken immediately prior to the start of intake of 1.75 g/kg glucose solution (time point 0 minutes) at the study centre. The child must drink the entire glucose solution consistently and within 5 minutes.[5] Additional venous blood samples for OGTT are taken 30, 60, 90 and 120 minutes after start of drinking the glucose solution. Blood glucose is measured at the central laboratory on each sample. Capillary blood glucose will be tested immediately at these time points with a glucose meter.

Custodial parent(s) will be informed when a child has developed persistent confirmed beta-cell autoantibodies (early pre-symptomatic stage of T1D). The child will remain in the study and continues to be treated or followed as planned until the child has developed T1D. The parents will be asked to participate in an educational program informing about the diagnosis of beta-cell autoantibody positivity. Contents of the education will be:

- Information what the diagnosis “beta-cell-autoantibodies” means
- How to recognize clinical symptoms of T1D
- How to self-monitor blood glucose in case of diagnosed early pre-symptomatic stage of T1D

4.11 VITAMIN D SUPPLEMENTATION

Vitamin D levels are often low in children who develop T1D. Vitamin D is also considered important for a healthy immune system. Therefore, 25-OH-Vitamin D3 concentrations will be monitored at every visit of the GPPAD-POInT Study. If concentrations are below 75 nmol/l, the family pediatrician will be notified and the family and pediatrician advised to introduce a daily vitamin D supplement or increase the dose of supplementation.

[5] Time point ‘0 minutes ist he time the child starts drinking the glucose solution. If a child needs more than 5 minutes, this must be documented in the participant chart and eCRF accordingly.

4.12 LABORATORY ASSESSMENT

Safety clinical laboratory assessments will be determined locally at the sites qualified laboratory (as described in chapter 3.13) and include the following parameters:
WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH/HbE, MCHC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils.

Beta-cell autoantibodies will be determined centrally at the Institute of Diabetes Research in Munich, Germany. In case of a positive test result, a sample will be shipped from the first GPPAD Central autoantibody laboratory to the second GPPAD Central autoantibody laboratory for confirmation. The second central autoantibody laboratory is Southmead Hospital, Department of Diabetes and Metabolism in Bristol, UK.

25-OH-vitamin D3 concentrations will be determined locally.
Fasting blood glucose, and blood glucose after OGTT will be determined centrally in participants with beta-cell autoantibodies.

5. SAFETY AND EFFICACY ASSESSMENT

5.1 MEASUREMENT OF SAFETY PARAMETERS

Local and systemic adverse effects will be elicited by direct questioning of the participant (if already feasible) and/or parent. Systemic effects will be sought by questioning about any untoward symptoms or signs, and graded as mild, moderate, or severe according to level of intervention required.

5.1.1 Hypoglycemia

Blood glucose measured before and 30, 60, and 120 minutes after drug has been administered during study visits at baseline (placebo or 7.5 mg oral insulin), 2 months (dose increase to placebo or 22.5 mg oral insulin) and 4 months (dose increase to 67.5 mg oral insulin). Capillary blood will be used for measurements. Families will also be instructed to report suspected hypoglycaemic events. Families will be instructed to monitor their participating child for symptoms or hypoglycemia after study drug intake. In case of a suspected hypoglycaemic event, with symptoms such as trembling, sweating and impaired consciousness, parents are instructed to immediately give their child something to drink or eat that contains fast absorbable carbohydrates e.g. juice or dextrose. Additionally, parents will be provided with an emergency number and can contact a study physician at any time. Families will also be instructed to report suspected hypoglycaemic events.

5.1.2 Clinical examination and laboratory tests

A physical examination including measurement of height, weight and oral inspection will be performed. Blood samples will be taken for differential blood count at visit 1 and visit 8.

5.1.3 Signs of allergy to study drug

No allergy to orally administered insulin has been reported. IgE anti insulin levels have been measured in Pre-POINT and in Pre-POINT-Early and no child has developed an IgE response to insulin. Nevertheless, families are asked to report any adverse reaction such as wheezing seen within 2 hours of taking study medication that may be considered as indicative of a hypersensitive response to study drug. Families will report these to the study physician. The study physician will discuss these with the protocol chairs in order to determine whether administration of study drug should be continued or temporarily ceased in such cases.

5.2 MEASUREMENT OF EFFICACY MARKERS

Beta-cell autoantibodies

Venous blood samples obtained at all visits will be tested for antibodies to insulin (IAA), GAD (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8RA; ZnT8WA) and tetraspanin 7 (TS7A). IAA will be measured by competitive immuno-precipitation of ^{125}I-insulin (47), antibodies to IA-2 and ZnT8 by RBA (48), antibodies to GAD by RBA, LIPS, or ELISA, and TS7A by LIPS (49). The clinical study site will be notified if a participant is beta-cell autoantibody positive (see chapter 4.10). The clinical study site will communicate persistent confirmed positive autoantibody results to families by telephone and in written form.

5.3 STORED SAMPLES

As an ancillary component to the study and with the participant's custodial parent(s) consent, serum, plasma, and peripheral blood mononuclear cell (PBMC) samples will be stored at a biobank for further analyses and assays valuable to test drug efficacy for prevention of T1D or investigate T1D pathogenesis. Analyses performed on the samples may include (but will not necessarily be limited to) expression of RNA and its protein products, T cell functional assessments including cytokine expression, and beta-cell autoantibody isotypes.

5.4 FOLLOW-UP OF PARTICIPANTS AFTER END OF STUDY

Participants will be contacted regularly for up to 10 years to determine whether the child has developed diabetes. Children will also be invited to participate in an available follow-up study for the detection of beta-cell autoantibodies. Follow-up will be organized by each clinical study centre by separate consents outside of the GPPAD-POInT Study.

Participants who reach the study end point and develop T1D during the trial will pass over to the regular diabetes care centre and their local physician for management of their diabetes.

6. ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1 DEFINITIONS

Adverse Event Reporting
The investigators are responsible for the conduct of the study in accordance with GCP regulations, which includes the recording and reporting of adverse events observed during and 60 days after the study.

Adverse Event
An adverse event is any untoward medical occurrence in a clinical investigational subject, which may or may not be related to the administered pharmaceutical product regardless of the causal relationship with treatment. An event can therefore be an unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the investigational product. Events such as misuse of study medication and medication errors are deemed adverse events and will be recorded as such.

Serious Adverse Event
Each of the following events is defined as a 'serious adverse event' for the purposes of this protocol, to meet or exceed the requirements of the ICH Guideline for Good Clinical Practice:

- Results in death.
- Life-threatening event. A 'life-threatening event' is present when the participant was, in the view of the investigator, at immediate risk of death from the event as it occurred. Note that this definition does not include an event that, had it occurred in a more serious form, might have caused death.
- Events, which develop during the study and require inpatient hospitalization or prolongation of existing hospitalization.
- Events, which are permanently disabling or incapacitating or cause a severe or permanent disruption of one's ability to carry out normal life functions or daily activities.
- Any congenital anomaly.

Note: Hospitalization for elective treatment of a pre-existing condition that did not worsen beyond the natural course of the pre-existing condition during the study is NOT considered a serious adverse event unless a complication occurs during the hospitalization.
Furthermore, hospitalization due to dysglycemia or the onset of T1D is not considered as an adverse event or serious adverse event, but the frequency of these events will be monitored by the Medical Monitor and the DSMB.

Assessment of Causality
The relationship, or attribution, of an adverse event to an investigational medicinal product will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate eCRF 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).

Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:

· _A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
· _One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the populations exposed to the drug
· _An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Assessment of Severity

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 4.03 (published June 14, 2010).

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.

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

6.2 HYPOGLYCEMIA DEFINITION

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

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

- 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,
  or
- symptoms commonly associated with severe hypoglycaemia (e.g. loss of consciousness, convulsion, stupor) that are reversed by treatment with intravenous glucose or subcutaneous glucagon.

6.3 EXPEDITED REPORTING

This requirement applies if the adverse event is considered serious, unexpected, and drug related (SUSAR). This type of SAE must be reported by the Sponsor to the appropriate health authorities and Ethics committees within 15 days; fatal or life-threatening events must be reported within 7 days.

6.4 POST-STUDY ADVERSE EVENTS

The investigator should notify the Medical Monitor, Ethics Committee and regulatory authorities of any death or adverse event occurring at any time after a participant has been signed out of a clinical study but no longer than 3 months, when such death or adverse event may reasonably be related to the investigational product. However, the investigator is not obligated to seek adverse events in former study participants.
Investigators should notify the Medical Monitor, Ethics Committee and regulatory authorities if they become aware of a former study participant who has developed cancer.

6.5 REPORTING OF UNDESIRABLE EVENTS AND ADVERSE REACTIONS

6.5.1 Reporting obligation of the investigator (§ 13 (1) – (6) GCP Guideline)

The investigator shall report any serious adverse event (SAE), which occurs in a subject immediately to the sponsor.
When an investigator identifies a SAE, he or she must notify the Sponsor/ Pharmacovigilance and the POInT Protocol Committee within 24 hours of discovering the event.
This will be done via Fax: +49 89 4140-6480, addressed to the *Münchner Studienzentrum (MSZ), Klinikum rechts der Isar, Technische Universität München, Ismaninger Straße 22, 81675 München, Germany (Tel: +49 89 4140-6477).*
For documentation purposes it is recommended to the study centre to note date and time of learning of the event. In addition to telephone reporting, the investigator must ensure that these events are entered on the SAE report form (see Appendix 2) and the adverse event eCRF. The SAE report form must be faxed to the Sponsor/Pharmacovigilance within 24 hours.

Adverse Events (AEs)
Throughout the study the investigator will record all adverse events on the appropriate adverse event Clinical Report Form (eCRF) 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.
Where the event reported consists of, or results in, the death of a subject, the investigator shall supply the sponsor with any additional information requested by the sponsor. Where the death has been reported to the relevant ethics committee, the investigator shall supply any additional information requested by that committee.

6.5.2 Reporting obligation of the Sponsor (§ 13 (1) – (6) GCP Guideline)

The sponsor shall keep detailed records of all adverse events relating to a clinical trial, which are reported to him by the investigators for that trial. The Competent Authority may require the sponsor to provide those records.
The sponsor shall ensure that all relevant information about a SUSAR, which occurs during the course of a clinical trial and is fatal or life-threatening is reported as soon as possible to the Competent Authority in which the trial is being conducted, and the relevant ethics committee.
This needs to be done not later than seven days after the sponsor was first aware of the reaction. Any additional relevant information should be sent within eight days of the report.
A sponsor shall ensure that a SUSAR which is not fatal or life-threatening is reported as soon as possible, and in any event not later than 15 days after the sponsor is first aware of the reaction to the competent authorities of any EEA State, in which the trial is being conducted and the relevant ethics committee. SUSARs will be reported to the national competent authority of the Member State concerned directly or indirectly through the EudraVigilance Clinical Trial Module for electronic reporting of SUSARs as required by Directive 2001/20/EC.

Updating source documentation
Documents describing the safety profile of a drug, such as the investigator's brochure, will be amended as needed by the sponsor or delegated responsible person or principal investigator 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.

Annual Safety Report (DSUR)
In addition to the expedited reporting required for SUSAR, the sponsor will submit a safety report to the Competent Authority and Ethics Committee, once a year throughout the clinical trial or upon request. The annual safety report should take into account all new available safety information received during the reporting period. Serious Adverse Events and Adverse Events will be reported with the DSUR on a yearly basis or upon request.

Final Study Report
According to point 5.1.4 of the ICH-GCP, a final study report will be prepared and provided by the sponsor. The report will be prepared within 12 months after completion of the clinical study.

7. PARTICIPANT SAFETY

7.1 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS

7.1.1 Benefits

The potential benefit for a participating child would be the prevention of emerging beta-cell autoantibodies. A benefit would also be a marked delay in the development of beta-cell autoantibodies, dysglycemia, or diabetes. Because all participating children, including children who receive placebo, have a high risk (>10%) of developing beta-cell autoantibodies and diabetes, testing blood samples in the study will allow early recognition of an immune response against the beta-cells, close monitoring and regular blood glucose testing. Children identified as beta-cell autoantibody positive, will be invited to receive education and teaching to learn about the risk of hyperglycemia and means to prevent diabetic ketoacidosis. Participation in ongoing prevention trials aiming to prevent disease progression may be possible (TrialNet studies, or others, separate consents, https://www.diabetestrialnet.org/). If a participating child develops T1D during the study, the disease can be diagnosed very early, i.e. before the child shows the typical symptoms of severe metabolic dysfunction, and an appropriate therapy could be started immediately. Early diagnosis and therapy of T1D reduces complications at onset of diabetes (50, 51) and potentially later in life. Furthermore, information about available treatments and intervention studies that include children with new-onset T1D in order to preserve the remaining beta-cells can be given to families.

7.1.2 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 anaesthetic cream at the puncture site. The volume of blood drawn at each visit that is strictly for the study protocol is $\leq$1% of the total blood volume and within the suggested limits from the European guidelines for a paediatric population. Additional blood volumes are requested for ancillary purposes and storage. These require separate informed consent and are subject to local ethical approval. The total blood volume for study protocol and ancillary purposes is less than 3%, which is within the limits of NIH guidelines for a paediatric population. A volume of up to 3% of total blood volume has been collected at 3 month intervals from children in three studies conducted in Munich with no clinically relevant reductions in blood counts or haemoglobin concentrations and no reported adverse events that suggested anaemia. Thus, we consider the proposed blood draw volumes in the study to pose no added risk to the participant safety.
Oral insulin does not lower blood sugar. Unexpected hypoglycemia is theoretically possible, but hasn't been shown in preceding studies (Pre-POINT Study, TrialNet Oral Insulin Study, Pre-POINT-Early, Fr1da-Insulin-Intervention).
There were also no reported allergic reactions or alterations in routine chemistry laboratory values in individuals receiving oral insulin. IgE anti insulin levels have been measured in Pre-POINT and in Pre-POINT-Early and no child has developed an IgE response to insulin. Detailed safety data from the Pre-POINT-Study and Pre-POINT-Early Study and Fr1da-Insulin-Intervention Study are discussed in chapter 1 and are shown in the Investigator's Brochure.

Oral insulin has not been shown to increase the risk of beta-cell autoimmunity or diabetes. The DSMB will monitor the development of beta-cell autoantibodies and diabetes in study participants and can request unblinding of data if there is reasonable concern that the frequency of beta cell autoantibodies or diabetes development in participants exceeds expectations. Parents of participating children will be soundly informed about the likelihood of their child to develop beta-cell autoantibodies, dysglycemia and T1D.

8. PSYCHOLOGICAL IMPACT OF STUDY PARTICIPATION ON FAMILIES

The psychological effect of study participation will be monitored by a questionnaire at visit 3 (appendix 5; 52) and visit 5, visit 8 and at the last visit at the end of participation (appendix 5; 53). A similar questionnaire has been used in the Pre-POINT Study (38) as well as the TEDDY Study (54,55).
When a parent is identified with high levels of anxiety and/or distress (PHQ-D/A; diabetes-specific items), a structured concept of psychological care will be provided (see also Figure 4). 1) direct (phone) contact and structured assessment of burden and need of support; 2) if the psychological burden is a consequence of study participation psychological measures that were proven in the care of families with newly diagnosed children with type 1 diabetes are provided by psychologists (experienced in the care of children with diabetes and their parents), e. g. personalized information focusing on diabetes specific fears and feelings of guilt, elements of cognitive behavioral therapy (CBT) focusing on negative thoughts, support to diabetes-specific parenting; family discussion; if necessary referral to psychotherapy.

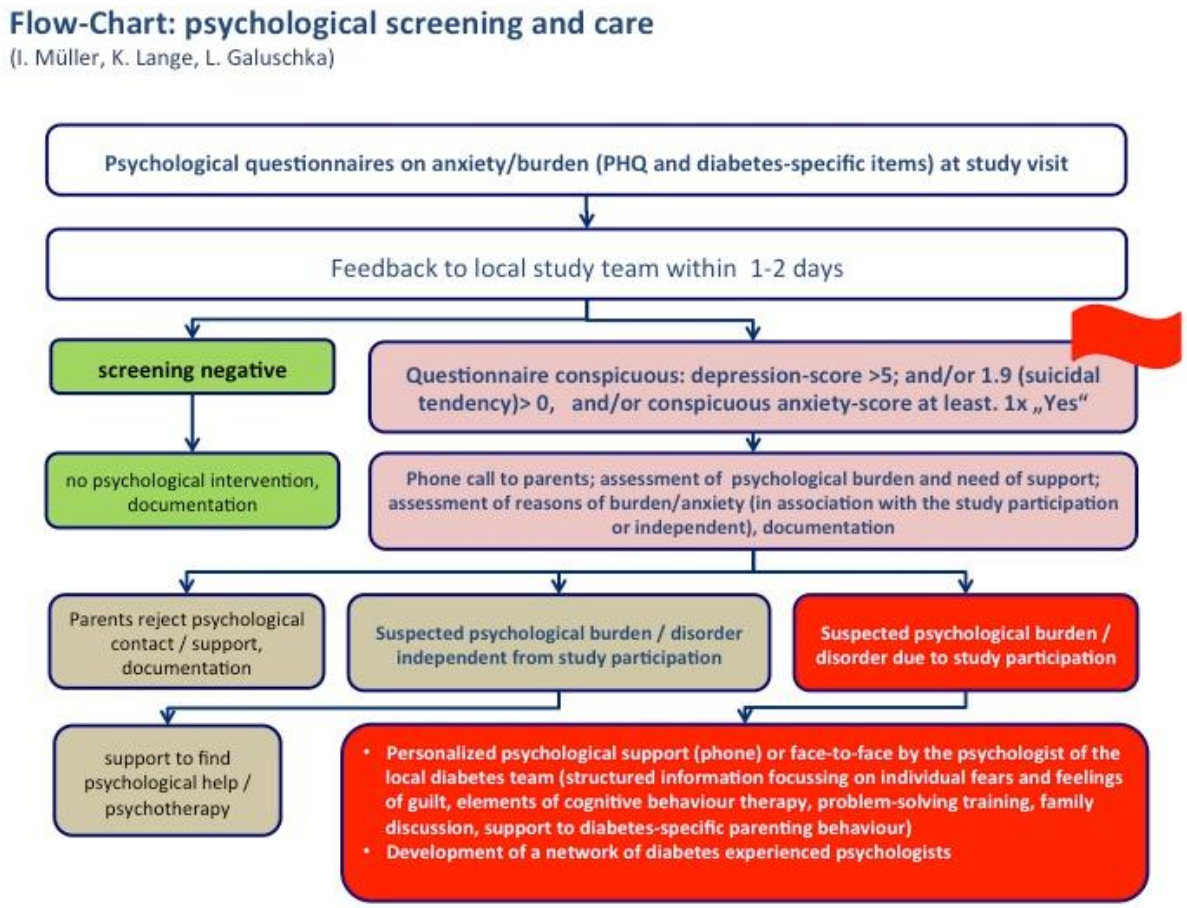


Figure 4: Psychological screening and care

9. PARTICIPANT, STUDY AND SITE DISCONTINUATION

9.1 PARTICIPANT DISCONTINUATION

A participant has the right to voluntarily withdraw from the study at any time. In addition, the investigator has the right to withdraw a participant from the study at any time.
Reasons for withdrawal from the study may include but are not limited to the following:

- Withdrawal of consent for participant at any time
- Any medical condition that the investigator or Sponsor determines may jeopardize the participant's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the participant

Every effort should be made to obtain information on participants who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF.

9.2 STUDY AND SITE DISCONTINUATION

The Sponsor has the right to terminate this study due to different reasons. Reasons for terminating the study may include but are not limited to the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to participants
- Unsatisfactory participant enrolment
- There is convincing evidence regarding the superiority of the investigational treatment
- The continuation of study is unethical or it has been proven that the therapy has a clearly negative influence;
- Unforeseen complications arise that no longer justify a continuation of the study;
- The results of the pre-planned interim analysis (see 11.4) fulfil the criteria to stop the trial prematurely

The Sponsor and POInT Protocol Committee will be advised by the DSMB with respect to study termination for any of these reasons. The DSMB will receive all and complete data for safety parameters, including the islet autoantibodies and diabetes in all participants, along with the expected frequencies for each of these outcomes in the placebo group in each 6 monthly DSMB report. If the DSMB has concerns of over-morbidity, the DSMB will be able to ask for unblinding at any stage of the trial and will advise the POInT Protocol Committee and the Sponsor as to whether the study should be continued, modified, suspended, or terminated.

Guidelines for considering suspending and/or stopping the study include:

- A >1.7-fold and significant increase in the frequency multiple beta cell autoantibody (10% expected cumulative frequency at the end of the study) in the participant group that receives the study drug as compared to the participant group receiving placebo.
- A >2-fold and significant increase in the frequency of diabetes (<5% expected cumulative frequency at the end of the study) in the participant group that receives the study drug as compared to the participant group receiving placebo.
- A statistically significant effect is observed.
- The results of the pre-planned interim analysis (see 11.4) fulfil the criteria to stop the trial prematurely.

The Sponsor will notify the investigator of a decision to discontinue the study.

The Sponsor has the right to close a site at any time. Reasons for closing a site may include, but are not limited to the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice

- No further study activity (i.e., all participants have completed and all obligations have been fulfilled)

The investigator may discontinue the clinical study at his site if he no longer considers the continuation of the study, for example because of ethical and/or medical concerns.

10. DOCUMENTATION

It is the investigator's responsibility to ensure the conduct of the study in accordance with the GCP Guidelines as well as with the national regulatory requirements and the study protocol and that all data is correctly documented. All collected data must be noted and filed in the corresponding eCRF's by authorized personnel. This also applies to data that refers to subjects that have been excluded from the study.
The investigator keeps a record of all participating subjects on a specific subject identification list. This list contains subject ID, full name, date of birth, date of enrolment into the trial (i.e. randomization date) and serves as a possibility for later identification of the participants. This list will be kept at a secured place in the clinical centre throughout the study and after the study according to local laws and regulations.
Additionally it has to be ensured that each personnel that is responsible for the documentation of eCRF's can be identified. Therefore a staff signature log with signature and acronym of authorized personnel will be filed and kept updated. The log will be filed in the investigator site file (ISF) and trial master file (TMF).

10.1 CASE REPORT FORMS

All study relevant subject data and laboratory results must be documented in corresponding eCRFs.
Corrections on the source documents must be made so that the initial record is still readable (i.e. whiteout is not permitted!). Corrections on eCRFs will be recorded and tracked through an approved audit trail database system. Furthermore, corrections have to be dated and signed. Missing data or data that was not collected has to be indicated as such (i.e. n.a. or n.d.), where appropriate reasons for missing data should be documented.
The investigator must ensure that study source data of participating subjects are documented instantly, readable, complete, and transferred correctly from patient's records on the eCRFs.

Source documents and the respective data entries in the eCRF as specified in the monitoring manual will be reviewed by a monitor for correctness, plausibility and completeness and will be archived for 10 years after completion of the study. CRF data will be entered into the study database.

10.2 INVESTIGATOR SITE FILE

An Investigator Site File (ISF) will be kept at the clinical centre. All essential and required information according to GCP documents will be filed in the ISF.
Upon completion or termination of the study, the ISF must be archived for 10 years. The investigator is responsible for the completeness and archiving of the ISF.

10.3 ARCHIVING OF DATA

10.3.1 Archiving obligations of the sponsor

Following closure of the study, the sponsor must maintain all study related records in a safe and secure location for at least 10 years.

10.3.2 Archiving obligations of the investigator

Following closure of the study, the investigator must maintain all study related records and source documents in a safe and secure location for at least 10 years.

11. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address the primary, secondary and exploratory objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

All efficacy analyses will be conducted under the intention-to-treat principle whereby all outcome data in all randomized subjects who have received at least one dose of study drug or placebo will be included in all analyses as appropriate. Subjects who drop-out of the study will not be replaced. All data acquired prior to termination will be included in the primary analysis unless a participant withdraws consent. Every effort will be made to conduct a final study visit with the participant who drops out of the study and participants will be followed clinically until, if applicable, all adverse events resolve.

11.1 PRIMARY OUTCOME

The primary outcome is the elapsed time from random treatment assignment to the development of persistent confirmed multiple beta-cell autoantibodies or diabetes among those enrolled in the primary analysis cohort consisting of subjects with an elevated genetic risk. For subjects who developed persistent confirmed multiple beta-cell autoantibodies, the elapsed time will be from the random treatment assignment to the first confirmed positive sample used in defining persistent beta-cell autoantibodies. It is expected that beta-cell autoantibodies will be detected prior to diabetes onset; however, the presence of diabetes in the absence of beta-cell autoantibodies is also considered as a primary outcome endpoint, and in this case situation, the date of diagnosis is the time of the end point.
The study end point is realized with either persistent confirmed multiple beta-cell autoantibodies or OGTT criteria for diabetes or clinical criteria for diabetes.
Although children who develop persistent confirmed multiple beta-cell autoantibodies will have reached the primary study endpoint, these children will continue to receive assigned treatment and will be followed in the study for continued monitoring of glucose tolerance and diabetes development and safety assessments.

Criteria for persistent confirmed beta-cell autoantibodies:
Criteria are based on the measurement of beta-cell autoantibodies against insulin (IAA), GAD65 (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8A) tested in the GPPAD central autoantibody laboratory and, if positive, confirmed in the GPPAD confirmatory laboratory. Children who are IAA positive in both laboratories will be further tested by methods that provide an assessment of affinity of insulin autoantibodies. Autoantibodies to tetraspanin 7 (TS7A) will be measured, but will not be considered in the primary outcome.

- Confirmed IAA is defined as sample positive for IAA in both the GPPAD central and confirmatory laboratories. Positivity for IAA in two laboratories is a feature of high affinity insulin autoantibodies. High affinity insulin autoantibodies are not expected to be induced by the study drug.
- Confirmed GADA is defined as sample positive for GADA in both the GPPAD central and confirmatory laboratories.
- Confirmed IA-2A is defined as sample positive for IA-2A in both the GPPAD central and confirmatory laboratories.
- Confirmed ZnT8A is defined as sample positive for ZnT8RA or ZnT8WA in both the GPPAD central and confirmatory laboratories.

Persistent confirmed multiple beta-cell autoantibodies (primary outcome) is defined as confirmed IAA, confirmed GADA, confirmed IA-2A, or confirmed ZnT8A in two consecutive samples, AND a confirmed second antibody from these four antibodies in one sample. Persistent confirmed beta-cell autoantibodies that are considered maternally derived GADA or IA-2A are NOT included as positive for the primary outcome. Maternally derived autoantibodies are defined as follows:
In children who are positive for any of the four beta-cell autoantibodies in the first sample taken and where there is no negative sample prior to this sample, the likelihood they have maternally derived autoantibodies will be considered. The status of the autoantibodies will be classified as maternally derived beta cell autoantibodies if they become negative in a subsequent sample taken before age 3 years. Maternally derived beta cell autoantibodies are not a primary outcome endpoint and are not considered as a positive outcome in the statistical analysis.
If children are positive for beta-cell autoantibodies at their first sample and therefore potentially have maternally derived beta cell autoantibodies, they are still eligible for randomization and treatment. The elapsed time from randomization to primary outcome in children with maternally derived beta-cell autoantibodies will be determined as:
For children who become beta-cell autoantibody negative before age 3 years, the primary outcome is defined as the first confirmed beta-cell autoantibody positive sample after the negative sample.
Children who are positive for a beta-cell autoantibody from the start of sample collection and remain positive for the same autoantibody until age 3 years will be classified as beta-cell autoantibody positive and outcome positive from baseline.
Children who are positive for two or more beta-cell autoantibodies from the start of sample collection and remain positive for only one of these autoantibodies until age 3 years will be classified as multiple beta-cell autoantibody positive from when they subsequently develop multiple beta cell autoantibodies.
Children who are positive for multiple beta-cell autoantibodies from the start of sample

collection and remain positive for the same autoantibodies until age 3 years will be classified as multiple beta-cell autoantibody positive from baseline. These are expected to be rare cases*.

*Maternal autoantibodies are expected in a minority of infants at baseline and expected to be randomly distributed between treatment groups. The presence of masking maternal autoantibodies decreases rapidly over the first year of life and is only present at 12 months in a minority of those who have maternally transferred autoantibodies at birth. Thus, the majority of these antibodies will be lost by the time the child starts the highest dose of study drug (between age 8 and 11 months). We assume that at most, 10% of randomized infants with have a mother with type 1 diabetes, we expect that around 50% of these will have antibodies at baseline and that less than 1% of the other recruited infants will have maternally derived antibodies at baseline (age 4 to 7 months). The majority (>50%) of these have only one islet autoantibody and not confound the definition of the primary outcome of the study. Thus, 3% of children in each treatment group may have maternally transferred multiple beta-cell autoantibodies that may mask a primary outcome. By age 3 years, the expected frequency of the primary outcome is around 5% in the placebo group. Thus, at most we may expect a maximum of 5% of 3% (ie 0.15%) with maternally transferred multiple beta-cell autoantibodies at baseline who could reach 3 years without losing their maternally derived multiple islet autoantibody state. Even this is highly inflated expectation, however, since we also expect that the majority of the children with maternally derived multiple islet autoantibody positive children to have lost at least one of their antibodies prior to the development of multiple islet autoantibody development. Hence, we expect maternally transferred islet autoantibodies to have no significant effect on the primary outcome of the study. A larger potential bias may affect some of the secondary analysis such as single islet autoantibodies and the potential effect of the bias will be considered. Evidence for bias will be assessed by considering the frequency of islet autoantibody positivity assigned to baseline samples in insulin and placebo-treated groups. If evidence of bias is found, then an analysis will also be performed in children who are negative for islet autoantibodies (including maternally acquired autoantibodies) at baseline.

Criteria for T1D onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis).

One of the following criteria must be met on two occasions as soon as possible but no less than 1 day apart for diabetes to be defined:

1. Symptoms of diabetes and a casual plasma glucose ≥200 mg/dL (11.1mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

OR

2. Fasting plasma glucose (FPG) ≥126 mg/dL (7 mmol/L), fasting is defined as no caloric intake for at least 8 hours

OR

3. Two-hour plasma glucose (PG) ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75g anhydrous glucose dissolved in water.

It is preferred that at least one of the two testing occasions involve an OGTT. Cases identified will be confirmed as having diabetes if the glucose values to make these determinations were obtained in a GPPAD central autoantibody laboratory as part of an OGTT. Cases diagnosed with diabetes by symptoms and casual glucose ≥ 200mg/dL or by other criteria than the above will be adjudicated by the GPPAD Endpoint Adjudication Committee. Trial treatment will be terminated if T1D is reached.

Primary Analysis
The study objective is to determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1 diabetes reduces the cumulative incidence of multiple beta-cell autoantibodies and diabetes in childhood.

The cumulative incidence of beta-cell autoantibodies over time since randomization within each treatment group will be estimated from a Kaplan-Meier estimate of the "beta-cell autoantibody-free" survival function. The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.05 level, two-sided, using the Cox regression including site as covariate. The estimates of cumulative incidence and the test will adjust for periodic outcome assessment visits to assess beta-cell autoantibody status. The critical value for the test statistic, and confidence intervals in this primary analysis will be determined by the group-sequential procedure.

In case the assumptions of the sample size estimation in section 11.3 hold, it will be possible to reject the null hypothesis of equal hazard rates with the power of 80%, if 832 children will be uniformly randomized over 3.5 years and afterwards, all 832 patients will be followed up for another 3.5 years. We have assumed a drop-out rate of 20%, and therefore we need to randomize 1040 children to support an 80% power by a complete follow-up of 832 children ranging from 3.5 to 7 years. All randomized children will be included in the analysis. Those children meeting the inclusion criteria and randomized who drop out of the study before their last study visit will also be considered for the analyses of the primary endpoint. Their observation time until the point of drop-out will add to the Wald statistic of the Cox model and increase the power of the study.

11.2 SECONDARY OUTCOMES AND ANALYSES

In addition to the primary outcome of multiple islet autoantibodies, four secondary outcomes will be included for analysis.

1. Any persistent confirmed beta-cell autoantibody, defined as at least one confirmed autoantibody, in two consecutive samples, including GADA, IA-2A, IAA, ZnT8A, or TS7A, or diabetes.
2. Persistent confirmed IAA.
3. Persistent confirmed GADA
4. Abnormal glucose tolerance (AGT) defined by dysglycemia or diabetes.

Dysglycemia is defined as:
• impaired fasting plasma glucose of ≥110 mg/dL (6.1 mmol/L), or

• impaired 2-hour glucose of ≥140 mg/dL (7.8 mmol/L), or
• high glucose levels at intermediate time points on OGTT (30, 60, 90 min levels of ≥200mg/dL (11.1 mmol/L))

Diabetes is defined as described in chapter 11.1.

The treatment arms will be compared on the corresponding incidence rates of each secondary outcome using the log rank statistic.

A variety of secondary analyses are planned after completion of the trial. These include the following.

Subgroup analyses will be conducted comparing the effects of oral insulin versus placebo on the risk of multiple beta-cell autoantibodies with a test of the group by subgroup factor interaction in a Cox proportional hazard (PH) Model. Subgroups of the population classified by sex, race/ethnicity (if appropriate), first degree relative status, beta-cell autoantibody status at baseline, maternally transferred beta cell autoantibody status at baseline, genetic risk score tertiles, and INS genotype. Differences in the treatment effect between subgroups will be tested using a covariate by treatment group effect in a Cox PH model.

Similar analyses will be conducted using the values of quantitative baseline factors including weight (z score), height (z score), BMI (z score), and genetic risk score. The dependence of the treatment effect on the quantitative levels of a covariate will also be assessed by a covariate of treatment group interaction in a PH model.

Analysis will also be performed considering outcomes that occur during the treatment period vs the non-treatment period. Incidence rates will be determined for each of these periods within the oral insulin and placebo groups and compared in a PH model.

Additional factors may be defined before unmasking of the study data to the investigators. The analyses will distinguish between factors specified prior to unmasking, and those identified post-hoc during analysis. If the assumption of proportional hazard is not appropriate, the data will be examined to determine the cause of non-proportional hazard, such as the presence of a decaying, diverging, or crossing effect of hazard ratios over time. Based on the cause of the non-proportional hazard, post-hoc analyses such as frailty models, parametric models, or models with interactions and time-dependent covariates may be employed.

Longitudinal analyses will assess the effects of oral insulin versus placebo treatment on immunologic and metabolic markers over time up to the onset of beta-cell autoantibodies. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors if the assumption of multivariate normal random errors is violated.

Immunologic and metabolic markers will be modelled to determine the effects of oral insulin versus placebo treatment while adjusting for subject characteristics for each follow-up time point of interest. For continuous endpoints that lend themselves to normal error linear models, ANOVA and ANCOVA models will be employed. Generalized linear modelling will be employed for dichotomous and categorical endpoints by using the most appropriate link functions. Longitudinal analyses maybe employed in order to characterize the relationship among the repeated measures during the treatment period and possibly beyond. Due to the exploratory nature of the longitudinal modelling, treatment effect hypothesis testing will not conducted.

The association of demographic, genetic, immunologic, metabolic, and other factors, both at baseline and over time, with the risk of beta-cell autoantibodies onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on beta-cell autoantibodies risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the oral insulin and placebo groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Models will then be assessed within the two groups combined, taking account of any group by covariate interactions.

Subgroup analyses analogous to those described for the beta-cell autoantibodies endpoint will be conducted on the secondary outcome endpoints.

11.3 STUDY POWER AND ACCRUAL TARGET

The study has been designed to provide 80% power to detect a 50% risk reduction in the rate of beta-cell autoantibodies using a two-sided test at the 0.05 level after 7.0 years of study duration. A total of approximately 1040 infants will be allocated in a 1:1 ratio to the two groups.

For the sample size estimation, the following scenario was chosen:
Overall alpha level = 0.05 (two-sided).
Overall beta level = 0.2, i.e. power = 0.8.
In the placebo group, at 3.5 years (approximate age of participants, 4 years), an event probability of 7.5% was assumed. Based on the exponential distribution, this leads to a hazard of 0.02227.
It is expected that the hazard is halved by the treatment.
Accrual time is 3.5 years.
Follow-up time is 3.5 years.
A dropout rate of 20% was expected.
An interim analysis will be performed when 53% of the total information is obtained.

11.4 INTERIM ANALYSIS PLAN

After 53% of the total information expected is obtained, an interim analysis on primary endpoint data is planned. According to our assumptions this will be around 4.5 years.
Using above scenario, without considering dropouts, and choosing the adaptive design of O'Brien and Fleming (56), n=832 patients should be randomized between the two groups. It is expected, that n=70 events are observed in total. After 37 events are observed (53% information is obtained around 4.5 years after first randomization), the

interim analysis will be performed.
At the interim analysis, it will be possible to reject the null hypothesis, if the standardized normal-distributed Z-statistic (which can be directly calculated from the Wald test statistic of the Cox model) is either below the boundary -2.65176 or above the boundary 2.65176.
Futility will also be considered. At the interim analysis, the study will be stopped due to futility, if the standardized normal-distributed Z-statistic lies between the boundaries -0.80692 and 0.80692.

The interim analysis report will be reviewed by the DSMB. Based on the interim analysis results, the DSMB will make recommendations to the sponsor and GPPAD-POInT protocol committee on how to procede the study.

At the final analysis, it will be possible to reject the null hypothesis, if the standardized normal-distributed Z-statistic is either below the boundary -1.93657 or above the boundary 1.93657.
With 20% drop-outs, n=1040 children will need to be randomized. All randomized children, including those who drop out of the study before their last visit will be included in the primary and secondary analyses where appropriate and possible.

All sample size calculations were performed with SAS 9.4.

12. DATAMANAGEMENT, MONITORING AND AUDIT

12.1 QUALITY ASSURANCE

The sponsor will implement study specific quality risk management processes in order to manage quality during the planning and implementation phase.

For quality assurance purposes monitoring will be conducted throughout the clinical study (RBQM).
The Investigator(s)/institution(s) will permit trial related monitoring, audits, Ethics Committee review and regulatory inspection, providing direct access to source data/documents.
A representative of the sponsor will visit the investigator periodically to monitor the progress of the study in accordance with national and ICH-GCP guidelines. A monitoring manual describing the scope of the monitoring activities in detail will be prepared. All data pertaining to a subject's participation in this study must be made available to the monitor during these visits. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.
A monitoring visit report is prepared for each visit describing the progress of the clinical trial and all identified problems.

Designated personnel may perform an audit at any time during or after completion of the study. All study-related documentation must be made available to the designated auditor. A regulatory authority may also audit the study. Access to case report forms, source documents and study files must be made available for monitoring and audit

purposes, at reasonable times, during the course of the study and after completion.

12.2 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 eCRFs.
Safety data will be recorded on eCRFs specifically designed for this purpose. All the SAEs will be reported on an SAE report form as well as on individual eCRFs. The data forms will be checked by the clinical study centre and families called if necessary (incomplete or incorrectly completed forms). The clinical study centre will report the data from the forms into the GPPAD-POInT Study database system. The clinical study centre will store all data forms electronically or as paper versions, depending on national requirements.

12.3 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 in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the clinical site must permit authorized representatives of the sponsor and health authorities to examine (and where required by applicable law) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. 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.

13. REPORTS

13.1 STUDY REPORT

Confidentiality and assurance of security of confidential documents such as the protocol, eCRF, Investigator's Brochure, final study reports, manuscript drafts, unpublished data, correspondence, etc. will be maintained throughout the study.
The statistical report and compilation of the final study report will be provided by the PI in cooperation with the data management and will be evaluated and signed by the PI, co-Investigator(s) and other responsible persons. All information and data of the report will be kept confidential.

13.2 PUBLICATIONS

Any publications resulting from the GPPAD-POINT Study (including meeting abstracts) will be agreed between the principal investigators and co-authors prior to submission. Patient names or other identifiers will not be disclosed. The major publication will provide registration details to www.clinicaltrials.gov.

14. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GCP

14.1 RESPONSIBILITIES OF SPONSOR AND INVESTIGATORS

The sponsor of the clinical study (*Technische Universtät München represented by the Fakultät für Medizin*) has the responsibility for commissioning, organisation and financing of the clinical study being undertaken (ICH-GCP 1.5.3). Sponsor and investigator must ensure that the conduct of the clinical study is in accordance with current laws and regulations, corresponding to ICH-GCP Guidelines, Declaration of Helsinki and the national Pharmaceutical Act and GCP regulation (2004).
The investigator accepts the requirements of the signed study protocol.

The investigators responsibilities are inter alia:

- Comprehension of the IMP's characteristics as outlined in the Investigator's Brochure,
- Comprehension for the implementation of the intervention plan,
- Securing that there is sufficient amount of time and capacity for the conduct of the study,
- Correct collection and documentation of the data, reporting,
- Supply all data for the sponsor, monitor and relevant regulatory authorities for audits and/or inspections,
- Securing that information of participants as well as all information from the sponsor of all persons involved in the trial are treated strictly confidentially
- Where applicable, statement for the involvement of persons dependent on sponsor or investigator
- Statement of conflicts of interest of investigator(s) in relation to the IMP

According to ICH-GCP 4.1.1 the investigator has the responsibility for the correct conduct of the clinical study at the clinical centre.

14.2 ETHICAL APPROVAL AND REPORTING TO REGULATORY AUTHORITIES

Prior to study initiation, the protocol will be reviewed and approved by an appropriate Ethics Committee and regulatory authority. Also, the informed consent documents will be approved by the leading Ethics Committee. Any amendments to the protocol or consent materials must also be approved by the study DSMB and where appropriate, the relevant competent authorities and ethics committees before they are implemented. The participating GPPAD clinical sites will obtain approval from their corresponding ethics committee in accordance with their local procedures and institutional requirements.
Application for regulatory approval will be submitted by the sponsor or sponsor delegate in accordance with § 7 GCP-Guideline.

14.3 PARTICIPATING CENTRES

Participating GPPAD clinical sites must have sufficiently qualified staff and time, as well as visit room- and laboratory space (sample processing) and equipment in order to conduct the trial.
The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

14.4 INFORMED CONSENT

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. The custodial parent(s) must read, sign, and date a consent form before entering the study, taking the study drug, or undergoing any study-specific procedures. As statement of the information process and assurance that they have understood all obligations and trial procedures, date and signature of the study investigator will also be obtained on the consent form.

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 handed out. The custodial parent(s) of the prospective participant will be told that being in the trial is voluntary and that the participant may withdraw from the study at any time, for any reason.
Once the participant infant is able to understand that he/she is participating in a clinical trial it has to be informed in an appropriate way and assent should be asked for.

14.5 CLINICAL TRIAL INSURANCE

On behalf of the sponsor, obligatory clinical trial insurance according to ICH-GCP point 5.8. is being set up for all study participants (before the first participant is recruited) with the following insurance company:

HDI Global SE

Germany

HDI-Gerling Industrie Versicherung AG
Ganghoferstrasse 37-39
80339 München, Germany
Phone: 089 92431 - 87000

UK and Sweden

HDI Global SE
10 Fenchurch Street
London EC3M 3BE, United Kingdom
Phone: +44 20 7696 8099

Poland

TUIR WARTA S.A.
ul. Chmielna 85/87
00-805 Warszawa, Poland
Phone: +48 22 581 01 00

Belgium

HDI Global SE
Avenue de Tervuren 273 bte 1
1150 Bruxelles, Belgium
Phone: +32 2 773 08 11

Study participants will be insured for any adverse event occurring as a result of study participation covering max. 500.000 Euro per participant. The insurance covers all direct or indirect damages that participants have experienced in the course of intervention with the study drug or by any study related test and examination procedures.

14.6 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 and not names will be used to collect, store, and report participant information.

The nature of the study requires retaining identifying data; however, confidentiality of study subjects and subject materials will be provided by filing and storage at the clinical centres. Information stored on computers will be accessible through passwords available only to authorized study personnel and -investigators. Hard copy data will be stored in locked filing cabinets kept in locked rooms. All data processing will comply with the European regulations in their current version.

The trial may be audited by designated qualified auditors who are independent of the GPPAD-POINT Study clinical trial/data collection systems.

15. STUDY ADMINISTRATION

15.1 ORGANIZATIONAL STRUCTURE

GPPAD is a network of collaborating investigators from European countries. The network was created to allow for a coordinated, multi-disciplinary approach to prevent T1D by early intervention.
The following organizational structures will be relevant for POInT:

15.1.1 GPPAD Coordination Centre

The GPPAD Coordination Centre (GPPAD CC) is part of the GPPAD platform and provides support for any trial conducted under GPPAD. The GPPAD CC will provide communication and coordination among the POInT clinical study centres, and manage the collection,

analysis and storage of clinical data. The GPPAD CC will work together with external partners in order to establish and maintain the data acquisition, transfer, and management system; provide procedures for ensuring subject confidentiality and safety; provide procedures for quality control, and supervise the orderly collection and transmission of data. The GPPAD CC will overview monitoring activities, CRO activities, the pharmacy, and the central laboratories.

The GPPAD CC puts in place a work system for a well-functioning trial conduct and logistic. It will meet on a regular basis to discuss current status of the trial, protocol compliance issues, and current topics in the trial conduct. The GPPAD CC will work closely with the protocol committee and provide status updates to the Sponsor, POInT Protocol Committee, DSMB, POInT Medical Monitor, and the competent authorities.
The GPPAD CC will organize telephone conferences and meetings between the POInT Clinical site PIs, the POInT Protocol Committee, the POInT Medical Monitor, The POInT DSMB and between the local POInT clinical study centres as required.

15.1.2 GPPAD-POInT Protocol Committee

The GPPAD POInT Protocol Committee (GPPAD PC) consists of the protocol authors Prof. Dr. Anette-G. Ziegler (chair), Prof. Dr. Ezio Bonifacio and Associate Prof. Dr. Helena Elding Larsson.
Significant changes that occur to this protocol during the course of the trial require the formal approval of the Protocol Committee.
The GPPAD PC committee members will receive periodic reports from the GPPAD CC; these will include accrual rates and baseline demographic characteristics, and AE and SAE reports and protocol compliance.

If required, further functions of the committee may include e.g.:

- the review of protocol deviations on a regular basis
- address and work out protocol amendments as they become necessary
- review of inclusion and exclusion criteria upon request by clinical site investigator or study physician
- assessment of new clinical study centres on cooperation with GPPAD CC

The committee will make decision on endpoint on the basis of protocol definitions. Consultation of the committee's opinion will also be available on demand in case of ambiguous cases.

15.1.3 POInT Medical Monitor

The medical monitor will be selected by the GPPAD CC in agreement with the protocol committee. The functions of the medical monitor are

- periodic review all adverse event reports, masked to treatment assignment
- periodic review and second assessment of SAEs
- provide updates on the assessment of the overall safety of the trial where they become necessary
- periodic review of multiple beta cell autoantibody and diabetes outcome reports, masked to treatment assignment
- periodic review of laboratory parameters during intervention and follow-up of participants throughout the study

- source data verification
- periodic review of protocol and GCP compliance

The GPPAD CC will provide periodic reports to the Medical Monitor.

15.1.4 POInT DSMB

The Data and Safety Monitoring Board (DSMB) is an independent group of experts that advises the sponsor. The major responsibility of the DSMB is to safeguard the well-being and safety of the trial participants and to provide pertinent advice to the sponsor and the POInT Protocol Committee. The DSMB will review each protocol amendment for any major concern prior to implementation.

Functions and responsibilities:

a. Monitors safety parameters during the study, e.g. the DSMB will receive each case of a serious AE or SUSAR after receipt within 96 hours for assessment
b. Monitors the proper conduct of the trial, e.g. recruitment rate, rate of drop outs and lost to follow ups, compliance with the trial protocol;
c. Reviews the 6-monthly DSMB reports and the report of the interim analysis and advises pertinent recommendations.
d. Safeguards confidentiality of and interests of individuals included in the study

Data as defined in the DSMB Charta will be presented to and reviewed by the DSMB. The DSMB will meet six-monthly during the conduct of the study. Conference calls are permitted.

At six-monthly intervals, the DSMB will receive a report with all relevant data for safety and efficacy, including the islet autoantibodies and diabetes data for each participant, along with the expected frequencies for each of these outcomes in the placebo group. The DSMB will be asked to examine this data and comment on this data with respect to safety, and at later stages also for efficacy. If the DSMB has concerns of over-morbidity, the DSMB will be able to ask for unblinding at any stage of the trial.

The DSMB will conclude each review with their recommendations to the sponsor and the POInT Protocol Committee as to whether the study should continue without change, be modified, suspended, or terminated.

15.2 THE PARTICIPATING POINT CLINICAL STUDY CENTRES

The participating clinical study centres are responsible to obtain ethical approval and regulatory approval with support of the GPPAD CC and protocol committee. Any amendments to the protocol or consent materials must also be approved before they are implemented. The participating clinical study centres are responsible for coordinating the collection and validation of clinical data. They will adhere to SOPs/MOO to support the study protocol.

15.3 THE GPPAD-POINT STUDY GROUP

This includes all site PIs and sub-investigators, as well as study nurses and further involved study personnel that contribute to the conduct of POInT.

15.4 ROLE OF INDUSTRY

The Insulin Crystals used for manufacture of the IMP will be provided by Eli Lilly and Company.

15.5 FINANCING OF RESEARCH STUDY

The GPPAD-POInT Study is financed by research grants from the Leona M. and Harry B. Helmsley Charitable Trust. There will be no cost to the participating families.

16. AMENDMENTS TO THE PROTOCOL

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. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.
In exceptional cases where an amendment becomes necessary the reason must be outlined in writing and must be signed by all responsible parties. All amendments will be reviewed by the DSMB prior to getting into force. Amendments will only be implemented in agreement with the Protocol Committee. If an amendment is considered substantial (according to GCP-Guidance §10) the amended protocol and other concerned study documents have to be approved by the respective ethics committee and regulatory authority.

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18. APPENDIX

18.1 APPENDIX 1: SYNOPSIS OF FINAL STUDY REPORT (PRE-POINT)

Name of Sponsor/Company: Technische Universität Dresden	**Individual Study Table Referring to Part of the Dossier** Not applicable	*(For National Authority Use only)*
Name of Finished Product: Oral Insulin capsules at doses 2.5mg, 7.5mg, 22.5 mg and 67.5mg	**Volume:** Not applicable	
Name of Active Ingredient **Human recombinant insulin**	**Page:** Not applicable	

Title of Study:

Pre-POINT (*P*rimary *O*ral *IN*sulin *T*rial) - 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

Investigators:

Bonifacio, Ezio, PhD, Ziegler, Anette-G., MD, Klingensmith, Georgeanna, MD, Bingley, Polly, MD, Schober, Edith, MD, Hasford, Joerg, MD, Achenbach, Peter, MD, Roth, Roswith, PhD,

Study centre(s):

Germany/Munich:	Forschergruppe Diabetes, Technische Universität München
USA/Denver:	Barbara Davis Center for Childhood Diabetes, University of Colorado
Austria/Vienna:	Universitätsklinik für Kinder und Jugendheilkunde
UK/ Bristol:	Diabetes and Metabolism, University of Bristol, Southmead Hospital

Publication (reference):

Achenbach P, Barker J, Bonifacio E. Modulating the natural history of type 1 diabetes in children at high genetic risk by mucosal insulin immunization. Curr Diab Rep 2008; 8:87–93.

Studied period (years):	4	**Phase of development:**
date of first enrolment:	23.10.2009	Phase II Pilot-Study
date of last completed:	15.09.2013	

Objectives:

To determine the feasibility, safety and bioavailability of oral insulin in children with high genetic risk for type 1 diabetes (T1D) in a dose escalation primary intervention pilot study.

Methodology:

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 daily

Children were treated for the duration of 2.5 months to 20 months. Study visits were scheduled for each dose at baseline, day 15, at 3 months, and 6 months and semi-annually thereafter. Follow-up until the 3 month visit was sufficient for study completion.

Safety assessment included monitoring of hypoglycemia through capillary blood glucose measurement at 30, 60 and 120 minutes after drug had been administered on the first day of treatment at each dose; and home capillary blood glucose measurements 60 minutes after drug administration.

Laboratory tests included blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start and end of treatment for each dose. Allergy to the study drug was monitored by total IgE and IgE antibodies to insulin, and family self-reporting. Diabetes, GAD and IA-2 autoantibodies were measured to assess possible increase in diabetes risk.

Bioavailability was measured by antibody and T cell response to study drug.

Psychosocial effects of study participation were assessed through questionnaires completed by families prior to and after 3 and 9 months participation in the study.

Number of subjects (planned and analysed):

Twenty-five children were enrolled into the study. Subjects were randomized in a 3:2 ratio to either insulin or placebo. Children were allowed to increase the dose of the study drug once during the dose-finding Pre-POINT study. In this way, each dose was given to 6 children, and 10 children received placebo. All 25 children were analysed.

Diagnosis and main criteria for inclusion:

1. Children aged 2 years to 7 years who:

A. have a multiplex first degree family history of T1D (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 T1D;

and are identical by descent for the HLA DR3/DR4-DQ8 genotype with their diabetic sibling;

2. Islet autoantibody negative at time of recruitment.

Test product, dose and mode of administration, batch number:

Daily intake of insulin capsules administered orally:

2.5 mg:	80804002/032010-25; 80804002/252010-25; 80804002/042011-25
7.5 mg:	80804002/402010-75; 80804002/072011-75; 80804002/412011-75; 100715-1; 100815-1
22.5 mg:	80804002/042011-225; 80804002/272011-225; 80804002/062012-225; 80804002/422012-225; 80804002/032013-225; 101905-8
67.5 mg:	80804002/062012-675; 80804002/282012-675; 80804002/422012-675; 80804002/052013-675; 100815-2; 100815-3

Duration of treatment:

The treatment duration per subject per dose varied between 2.5 months and 12 months.

Cumulative treatment duration per dose is 36 months for 2.5 mg, 54 months for 7.5 mg, 50 months for 22.5 mg and 48 for 67.5 mg insulin capsules.

Reference therapy, dose and mode of administration, batch number:

Daily intake of placebo capsules administered orally:

Placebo:	80804002/032010-25; 80804002/252010-25; 80804002/042011-25; 80804002/402010-75; 80804002/072011-75; 80804002/382012-75; 80804002/422012-75; 80804002/272011-225; 80804002/062012-225; 80804002/052013-675; 090819-37; 090724-14; 100613-1; 100615-2; 090819-90; 100615-3; 101908-3; 101908-4; 102013-2; 090819-37

Criteria for evaluation:

Safety: 1. Potential risk of hypoglycaemia was assessed by blood glucose after administration. Bood glucose values below 50 mg/dl were considered an adverse event and reportable. 2. General effects were assessed by blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start and end of treatment for each dose. 3. Allergy was assessed by family reports, total IgE concentrations and IgE against insulin. 4. A potential increase in diabetes risk caused by study drug was assessed by measuring autoantibodies to GAD65 and to IA-2.

Efficacy: Immune bioavailability of the study drug was assessed by islet autoantibody titer, IgG- and IgA-binding to insulin in serum and saliva, and by CD4+ T cell responses to insulin.

Statistical methods:

The number of hypoglycaemic events at each dose were compared by Kruskall-Wallis test, and the blood glucose concentrations after taking study drug at each dose were compared to placebo using ANOVA.

IgE concentrations were compared to baseline at each study dose using students t test.

The number of adverse events (total and separately for event type) in each dose and for all doses were compared to the placebo group using Fischer's exact test.

The number of children with antibody and/or T cells responses to study drug at each dose were compared to placebo using Fischer's exact test and Chi squared test for trend.

SUMMARY – CONCLUSIONS

EFFICACY RESULTS:

Increases in IgG-binding to insulin, salivary IgA-binding to insulin, and/or $CD4^+$ T cell proliferative responses to insulin were observed in two of ten placebo-treated children, one treated with 2.5 mg insulin, one 7.5 mg-treated, two 22.5 mg-treated, and five of six 67.5 mg-treated children (p=0.017). FOXP3/IFNg signature ratios of insulin-responsive cells increased (p=0.02) in children who received oral insulin treatment.

SAFETY RESULTS:

None of the children who received oral insulin or placebo experienced hypoglycaemia after administration of medication. There were no increases in IgE concentration or IgE responses to insulin. Adverse events were similar between insulin and placebo groups. No child developed autoantibodies to glutamic acid decarboxylase or IA-2 or diabetes.

CONCLUSION:

This pilot study indicates that daily oral administration of insulin to islet autoantibody negative children who are genetically at risk for T1D appears safe, and at a daily 67.5 mg dose can actively engage the immune system with features of immune regulation.

Date of the report: 15. September 2014

18.2 APPENDIX 2: SAE REPORT FORM

GPPAD-POInT Study
-Oral Insulin Therapy for Prevention of Autoimmune Diabetes-

SERIOUS ADVERSE EVENT REPORT FORM

Subject ID: __ __ __ __ __
Staff initials: __ __

EudraCT No.: 2017-003088-36
Sponsor: Technische Universität München

EUDRACT No. : 2017-003088-36					
PROTOCOL IDENTIFICATION: GPPAD-03-POInT INDICATION: Diabetes prevention *SPONSOR*: *Technische Universität München*					
Patient No.	Sex ☐ M ☐ F	Age (years)	Height (cm)	Weight (kg)	SAE No.
REPORT INFORMATION ☐ INITIAL REPORT Date: ☐ FOLLOW-UP REPORT Date:	NAME OF INVESTIGATOR: SITE NO.: INSTITUTION: Date investigator became aware of the SAE: Date: (DD/MM/YYYY)		COUNTRY: TELEPHON: FAX: E-MAIL:		
SERIOUSNESS CRITERIA OR REPORTABLE REASON					
☐ results in death ☐ life-threatening ☐ requires inpatient hospitalization / or prolongation	☐ results in persistent or significant disability/incapacity ☐ congenital anomaly / birth defect ☐ other medically important condition				
SERIOUS ADVERSE EVENT (SAE)					
SAE: Diagnosis (if possible) including symptoms _____ _____ _____ _____ _____ _____	Onset date of SAE (DD/MM/YYYY) _____ Date of resolution *(DD/MM/YYYY)* _____ Date of death (if applicable) *(DD/MM/YYYY)* _____				
SEVERITY *or* CTC-GRADE					
CTC-grade: ☐ CTC grade 1 ☐ CTC grade 2 ☐ CTC grade 3 ☐ CTC grade 4 ☐ CTC grade 5					
INVESTIGATIONAL DRUG(S)	UNBLINDING: ☐ not applicable ☐ no ☐ yes				

Active Substance Name Batch No.	Date of first administration	Time interval (incl. unit) between last drug administration and start of event	Daily dose, unit, route of administration
1. Batch No.:			
Causality: ☐ definite ☐ probable ☐ possible ☐ unlikely ☐ unrelated			
2. Batch No.:			
Causality: ☐ definite ☐ probable ☐ possible ☐ unlikely ☐ unrelated			
3. Batch No.:			
Causality: ☐ definite ☐ probable ☐ possible ☐ unlikely ☐ unrelated			

PATIENT INFORMATION

Patient- No.	Age (years)	SAE No.	☐ INITIAL REPORT Date: ☐ FOLLOW-UP Date:

RELEVANT MEDICAL HISTORY (pre-existing / concurrent conditions)	Start date	Stop date
1.		
2.		
3.		

RELEVANT CONCOMITANT MEDICATION

	Indication	Daily dose, unit, route of administration	Date of first administration	Date of last administration
1.				
2.				
3.				

RELEVANT LAB FINDINGS OR INVESTIGATIONS			
	Normal range	Date	Result
1.			
2.			
3.			
4.			

TREATMENT OF SAE	ACTION TAKEN WITH TRIAL MEDICATION	OUTCOME OF SAE
☐ none ☐ drug treatment ☐ others Specify: _____ _____ _____ _____	☐ dose not changed ☐ dose reduced ☐ dose increased ☐ drug withdrawn, date: _____ Has a re-challenge been done? ☐ yes ☐ no ☐ unknown Did reaction recur on re-administration? ☐ yes ☐ no ☐ unknown ☐ unknown ☐ not applicable	☐ recovered / resolved ☐ recovering / resolving ☐ not recovered / not resolved ☐ recovered / resolved with sequelae ☐ fatal cause of death: _____ autopsy? ☐ yes ☐ no ☐ unknown

COMMENT:

INVESTIGATOR SIGNATURE

Name Signature Date (DD/MM/YYYY)

INFORM PHARMACOVIGILANCE IMMEDIATELY:

Fax: +49 89 4140-6480

at the

Münchner Studienzentrum (MSZ), Klinikum rechts der Isar, Technische Universität München, Ismaninger Straße 22, 81675 München, Germany

Tel: +49 89 4140-6477

18.3 APPENDIX 3: RISK SCORE

Table 3: List of SNPs determined in the GPPAD-02 Study

	SNP	Gene, Allele, Haplotype	Merged Score Weight
	HLA class II		
1	rs17426593	HLA DR4-DQ8/DR4-DQ8	3.15
2	rs2187668		3.98
3	rs7454108	HLA DR3/DR4-DQ8	
4	rs3129889	DRB1*1501	Exclusion criteria for first degree rel
5	Rs1794265	DQB1*0503	Exclusion criteria for first degree rel
	HLA class I		
6	rs1264813	HLA A 24	0.43
7	rs2395029	HLA B 5701	0.92
	Non-HLA SNPs		
8	rs2476601	PTPN22	0.76
9	rs2816316	RGS1	0.16
10	rs3024505	IL10	0.22
11	rs1990760	IFIH1	0.16
12	rs3087243	CTLA4	0.16
13	rs10517086		0.19
14	rs2069763	IL2	0.11
15	rs6897932	IL7R	0.19
16	rs3757247	BACH2	0.19
17	rs9388489	C6orf173	0.14
18	rs6920220	TNFAIP3	0.15
19	rs1738074	TAGAP	0.05
20	rs7804356	SCAP2	0.15
21	rs4948088	COBL	0.17
22	rs7020673	GLIS3	0.23
23	rs12722495	IL2RA	0.47
24	rs947474	PRKCQ	0.15
25	rs10509540	RNLS/C10orf59	0.25
26	rs689	INS	0.65

	SNP	Gene, Allele, Haplotype	Merged Score Weight
27	rs4763879	CD69	0.06
28	rs2292239	ERBB3	0.36
29	rs3184504	SH2B3	0.24
30	rs1465788	ZFP36L1	0.13
31	rs17574546		0.13
32	rs3825932	CTSH	0.15
33	rs12708716	CLEC16A	0.15
34	rs4788084	IL27	0.20
35	rs7202877		0.19
36	rs2290400	ORMDL3	0.25
37	rs7221109		0.15
38	rs45450798	PTPN2	0.09
39	rs763361	CD226	0.12
40	rs425105	PRKD2	0.21
41	rs2281808	SIRPG	0.07
42	rs3788013	UBASH3a	0.16
43	rs5753037		0.15
44	rs229541	IL2B	0.18
45	rs5979785	TLR8	0.09
46	rs2664170	GAB3	0.14

The risk score is calculated by multiplying the number of risk alleles (i. e. 0, 1 or 2 for each single SNP) with the weight assigned to each SNP (see Table 3) and then summing up the weighted contributions of all SNPs plus an additive constant for each of the two HLA class II categories, 3.15 for infants who have the HLA DR4-DQ8/DR4-DQ8 genotype or 3.98 for infants who have the HLA DR3/DR4-DQ8 genotype.

As an example, the risk score for a child with HLA DR4-DQ8/DR4-DQ8, homozygous for the risk allele of rs1264813 (weight 0.43), heterozygous for the risk allele of rs2395029 (weight 0.92), homozygous for the non-risk allele of rs2816316 (weight 0.76) and for all other SNPs in the genetic risk score is calculated as follows:

Risk score = 3.15 + (2 * 0.43) + (1 * 0.92) + (0 * 0.76) + ….

Missing genotypes at single SNPS: The following rules apply for samples that have incomplete typing at some of the SNPs:

Children without a first degree relative who has type 1 diabetes

1. Incomplete typing for SNPs 1, 2, or 3 results in an invalid test and should be either repeated or reported as invalid.
2. Incomplete typing at SNPs 4 or 5 is permissible since these are not used to calculate the genetic score.

3. Up to 5 incomplete genotyping at SNPs 6 to 46 is permissible. When incomplete, an average score weight (either positive or negative) will be added on the basis of the expected minor allele frequency in a European population (see table 4).

Children with a first degree family history of type 1 diabetes

1. SNP genotyping for SNPs 1, 3, 4 and 5 is essential.

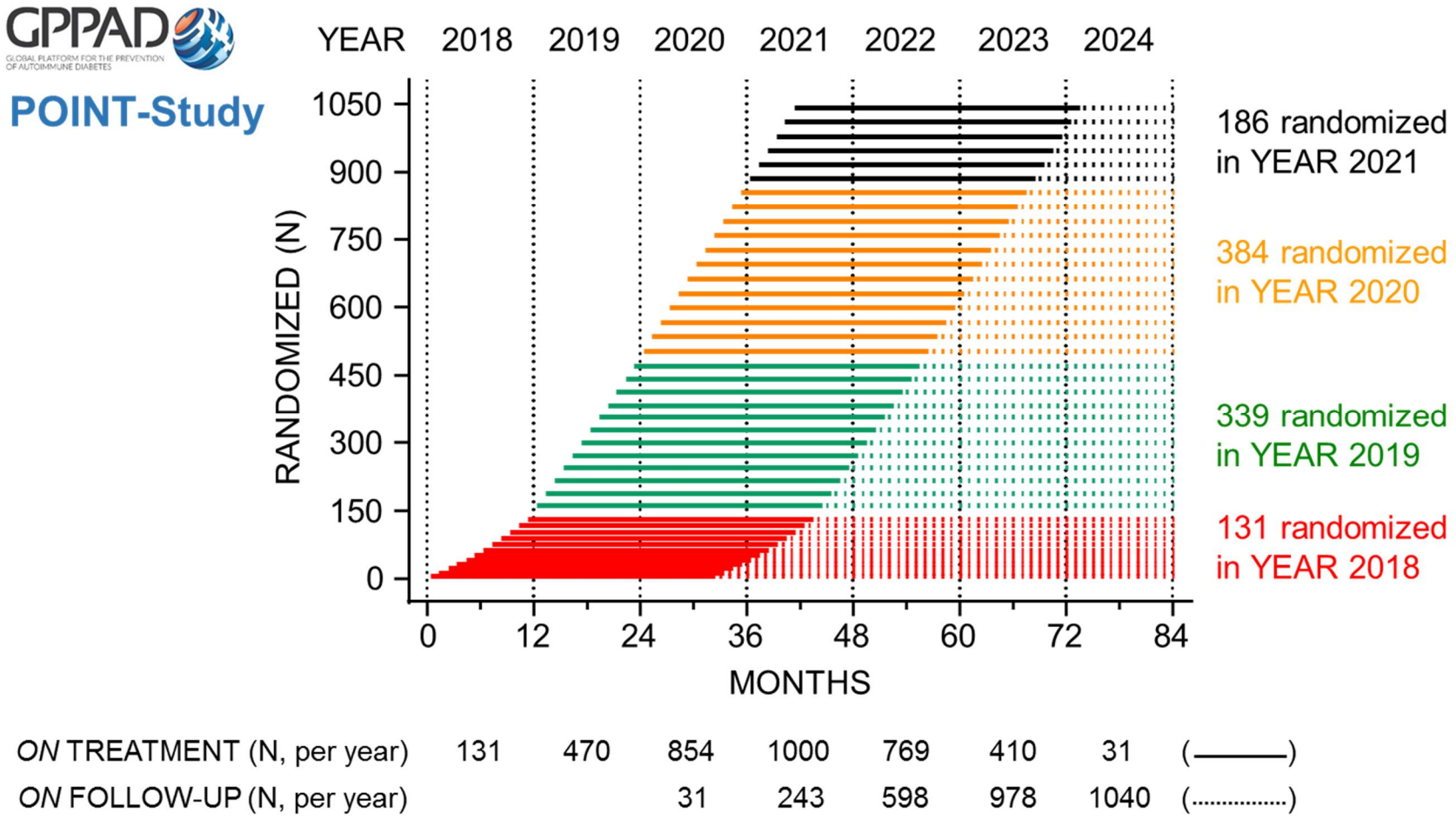
Missing genotypes for any of the other SNPs are permissible, as these are not used to determine risk.

Table 4: Average weight score for SNPs to be used if there is a missing genotype.

SNP	Gene, Allele	Minor Allele	Frequency (%)	Weight if missing
rs1264813	HLA A 24	A	9.51	0.08
rs2395029	HLA B 5701	C	0.96	1.82
rs2476601	PTPN22	A	12.11	0.18
rs2816316	RGS1	C	17.65	0.26
rs3024505	IL10	A	16.15	0.37
rs1990760	IFIH1	G	39.98	0.19
rs3087243	CTLA4	A	37.28	0.20
rs10517086	C4orf52	A	28.71	0.11
rs2069763	IL2	A	40.00	0.05
rs6897932	IL7RA	A	29.30	0.27
rs3757247	BACH2	A	41.08	0.16
rs9388489	C6orf173	G	44.65	0.13
rs6920220	TNFAIP3	A	21.38	0.06
rs1738074	TAGAP	A	40.59	0.06
rs7804356	SCAP2	G	21.64	0.24
rs4948088	COBL	A	4.51	0.32
rs7020673	GLIS3	G	49.15	0.23
rs12722495	IL2RA	G	8.29	0.86
rs947474	PRKCQ	G	18.50	0.24
rs10509540	RNLS/C10orf59	G	27.54	0.36
rs689	INS	A	37.51	0.49
rs4763879	CD69	A	38.24	0.05
rs2292239	ERBB3	A	33.19	0.24
rs3184504	SH2B3	A	45.92	0.22
rs1465788	ZFP36L1	A	29.39	0.18
rs17574546	RASGRP1	C	20.23	0.05
rs3825932	CTSH	A	36.13	0.23
rs12708716	CLEC16A	G	34.85	0.20
rs4788084	IL27	A	45.77	0.22
rs7202877	CTRB2	C	11.74	0.04
rs2290400	ORMDL3	A	47.87	0.26
rs7221109	CCR7	A	37.76	0.19
rs45450798	PTPN2	C	17.17	0.03
rs763361	CD226	A	48.45	0.12
rs425105	PRKD2	G	16.24	0.35
rs2281808	SIRPG	A	33.26	0.09

SNP	Gene, Allele	Minor Allele	Frequency (%)	Weight if missing
rs3788013	UBASH3a	A	39.81	0.13
rs5753037	RPS3AP51	A	34.93	0.10
rs229541	IL2B	A	40.47	0.15
rs5979785	TLR8	G	26.55	0.13
rs2664170	GAB3	G	30.28	0.08

18.4 APPENDIX 4: RECRUITMENT AND TREATMENT PROSPECT IN THE GPPAD-POInT STUDY



18.5 APPENDIX 5: PSYCHOLOGICAL IMPACT QUESTIONNAIRE

Psychological Questionnaire: Mother, Father or Primary Caretaker (Visit 3)

Date you completed this questionnaire

_____ / _____ / _____
(Day) (Months) (Year)

What is your relationship to the participating GPPAD-POInT Study child?

☐ Mother ☐ Father ☐ Other Primary Caretaker ☐ Other, specify _____

1. Over the last 2 weeks , how often have you been bothered by any of the following problems?

	Not at all	several days	More than half the days	Nearly every day
1.1 Little interest or pleasure in doing things	☐	☐	☐	☐
1.2 Feeling down, depressed, or hopeless	☐	☐	☐	☐
1.3 Trouble falling or staying asleep, or sleeping too much	☐	☐	☐	☐
1.4 Feeling tired or having little energy	☐	☐	☐	☐
1.5 Poor appetite or overeating	☐	☐	☐	☐
1.6 feeling bad about yourself – or that you are a failure or have let yourself or your family down	☐	☐	☐	☐
1.7 Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
1.8 Moving or speaking so slowly that other people could have noticed? Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐
1.9 Thoughts that you would be better off dead or hurting yourself in some way	☐	☐	☐	☐

2. Questions about “anxiety”:

	No	Yes
2.1 In the last 4 weeks, have you had an anxiety attack – suddenly feeling fear or panic? >> If you checked “NO”, go to question 3	☐	☐
2.2 Has this ever happened before?	☐	☐
2.3 Do some of these attacks come suddenly out of the blue – that is, in situations where you don’t expect to be nervous or uncomfortable?	☐	☐
2.4 Do these attacks bother you a lot or are you worried about having another attack?	☐	☐
2.5 During the last bad anxiety attack, did you have symptoms like shortness of breath, sweating, or your heart racing, pounding or skipping?	☐	☐

3. If you checked off any problems on this questionnaire, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

☐ not difficult at all ☐ somewhat difficult ☐ very difficult ☐ extremely difficult

4. How often do you worry that your child might get diabetes? (Mark one answer)

☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Very often

5. When you think about your child's risk for developing diabetes, you feel: (Mark one answer on each line a-f)

a.	☐ Not at all calm	☐ Somewhat calm	☐ Moderately calm	☐ Very calm
b.	☐ Not at all worried	☐ Somewhat worried	☐ Moderately worried	☐ Very worried
c.	☐ Not at all relaxed	☐ Somewhat relaxed	☐ Moderately relaxed	☐ Very relaxed
d.	☐ Not at all tense	☐ Somewhat tense	☐ Moderately tense	☐ Very tense
e.	☐ Not at all at-ease	☐ Somewhat at-ease	☐ Moderately at-ease	☐ Very at-ease
f.	☐ Not at all nervous	☐ Somewhat nervous	☐ Moderately nervous	☐ Very nervous

6. How often do you feel that each phrase applies to you in the past few weeks? (Mark one answer on each line a-f)

a. I feel that I am useful and needed:

☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

b. I have crying spells or feel like it:

☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

c. I find I can think quite clearly:

☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

d. My life is pretty full:

☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

e. I feel downhearted and blue:

☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

f. I enjoy things I do:

☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

7. Overall, how do you feel about having your child participate in the GPPAD-POInT Study?

(Mark one answer)

☐ Like it a lot ☐ Like it a little ☐ It is OK ☐ Dislike it a little ☐ Dislike it a lot

8. Do you think your child's participation in the GPPAD-POInT Study was a good decision?

(Mark one answer)

☐ A great decision ☐ A good decision ☐ An OK decision ☐ A bad decision ☐ A very bad decision

9. Would you recommend the GPPAD-POInT Study to a friend?

☐ No ☐ Yes ☐ Maybe

Thank you very much for your time!

Psychological Questionnaire: Mother, Father or Primary Caretaker (Visit 5, 8, End of Trial)

Date you completed this questionnaire

_____ / _____ / _____
(Day) (Months) (Year)

To be completed by Clinical Center personnel:
☐ V5 ☐ V8 ☐ EoT

What is your relationship to the participating GPPAD-POInT Study child?

☐ Mother ☐ Father ☐ Other Primary Caretaker ☐ Other, specify _____

1. How often do you worry that your child might get diabetes? (Mark one answer)

☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Very often

2. When you think about your child's risk for developing diabetes, you feel: (Mark one answer on each line a-f)

a.	☐ Not at all calm	☐ Somewhat calm	☐ Moderately calm	☐ Very calm
b.	☐ Not at all worried	☐ Somewhat worried	☐ Moderately worried	☐ Very worried
c.	☐ Not at all relaxed	☐ Somewhat relaxed	☐ Moderately relaxed	☐ Very relaxed
d.	☐ Not at all tense	☐ Somewhat tense	☐ Moderately tense	☐ Very tense
e.	☐ Not at all at-ease	☐ Somewhat at-ease	☐ Moderately at-ease	☐ Very at-ease
f.	☐ Not at all nervous	☐ Somewhat nervous	☐ Moderately nervous	☐ Very nervous

3. How often do you feel that each phrase applies to you in the past few weeks? (Mark one answer on each line a-f)

a. I feel that I am useful and needed:
☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

b. I have crying spells or feel like it:
☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

c. I find I can think quite clearly:
☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

d. My life is pretty full:
☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

e. I feel downhearted and blue:
☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

f. I enjoy things I do:
☐ All of the time ☐ Some of the time ☐ Occasionally ☐ Not at all

4. Overall, how do you feel about having your child participate in the GPPAD-POInT Study? (Mark one answer)

☐ Like it a lot ☐ Like it a little ☐ It is OK ☐ Dislike it a little ☐ Dislike it a lot

5. Do you think your child's participation in the GPPAD-POInT Study was a good decision? (Mark one answer)

☐ A great decision ☐ A good decision ☐ An OK decision ☐ A bad decision ☐ A very bad decision

6. Would you recommend the GPPAD-POInT Study to a friend?

☐ No ☐ Yes ☐ Maybe

Thank you very much for your time!

GPPAD-03-POInT (Global Platform for the Prevention of Autoimmune Diabetes – Primary Oral Insulin Trial)

Oral Insulin Therapy for Prevention of Autoimmune Diabetes

A study of the Global Platform for the Prevention of Autoimmune Diabetes

Protocol No.: **GPPAD-03-POInT (*path from GPPAD-02)**
EudraCT-No.: **2017-003088-36**
IMP: **Insulin**
Dose form: **7.5mg; 22.5mg; 67.5 mg insulin capsules**

Version 4.0, 09.12.2021

Protocol Chair

Prof. Dr. Anette-G. Ziegler

Protocol Authors

Prof. Dr. Anette-G. Ziegler, Prof. Dr. Ezio Bonifacio, Prof. Dr. Helena Elding Larsson

Sponsor

Technische Universität München, School of Medicine

Supported by: The Leona M and Harry B Helmsley Charitable Trust

GPPAD-Coordinating Center:
Forschergruppe Diabetes, Klinikum rechts der Isar, TUM and Institute of Diabetes Research, Helmholtz Zentrum München

Statistical advice/ data analysis/ Trial Design: IBE, LMU Munich

GPPAD

PROTOCOL APPROVAL

My signature below confirms my agreement with the design of the study as outlined within this protocol

7.2.2022

Prof. Dr. med. Bernhard Hemmer
Sponsor, Dean, Technische Universität München, School of Medicine, Munich, Germany

Date

27.1.22

Prof. Dr. med. Anette-G. Ziegler
Protocol Chair, Forschergruppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Germany

Date

28.1.22

Prof. Dr. Ezio Bonifacio
Protocol Committee Member, DFG-Center for Regenerative Therapies Dresden, Germany

Date

28.1.22

Prof. Dr. Helena Elding Larsson
Protocol Committee Member, Lund University, Department of Clinical Sciences/Malmo, Skane University Hospital SUS, Malmo, Sweden

Date

28.1.22

Andreas Weiß
Statistician, Institute of Diabetes Research, Helmholtz Zentrum München, Munich, Germany

Date

SYNOPSIS

Sponsor	Investigator Initiated Trial, Technische Universität München, represented by the School of Medicine
Title	Oral Insulin Therapy for Prevention of Autoimmune Diabetes
Short title	GPPAD-03-POInT (Global Platform of Autoimmune Diabetes – Primary Oral Insulin Trial)
Study phase	Phase IIb
Protocol Chair / Committee	Anette-G. Ziegler, MD (chair) Ezio Bonifacio, PhD Helena Elding Larsson, MD, PhD
Population/ Indication	Infants genetically at-risk for type 1 diabetes, age 4 months – 7 months
Study Design	Investigator initiated, randomized, placebo-controlled, double-blind, multi-centre primary intervention study.
Accrual Objective	1040 (1:1 randomization to oral insulin and placebo arms)
Study Objective	To determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1 diabetes reduces the cumulative incidence of beta-cell autoantibodies and diabetes in childhood.
Intervention	Eligible subjects will be randomized either into 1. oral insulin group (dose escalation: 7.5 mg for 2 months, increasing to 22.5 mg for 2 months, increasing to 67.5 mg until age 3.00 years) or 2. placebo group. Guardians of participants will self-administer the Investigational Medicinal Product (oral insulin or oral placebo). Treatment will be administered daily preferably in the morning (7-10am).
End of treatment	Children will stop treatment the day of the 3rd birthday, or when they develop diabetes.
Primary Outcome	The primary outcome is the development of persistent confirmed multiple beta-cell autoantibodies (defined as confirmed IAA, confirmed GADA, confirmed IA-2A, or confirmed ZnT8A in two consecutive samples, AND a confirmed second antibody in one sample) or diabetes.
Secondary Outcome	1. Any persistent confirmed beta-cell autoantibody, defined as at least one confirmed autoantibody in two consecutive samples, including GADA, IA-2A, IAA, ZnT8A, or TS7A, or diabetes 2. Persistent confirmed IAA. 3. Persistent confirmed GADA

	4. Abnormal glucose tolerance (AGT=dysglycemia) or diabetes.
Timeline	Recruitment: 3.17 years (projected 3.5 years) intended start (FPFV): February 2018 (projected January 2018) Intervention period: 29 to 32 months per participant Follow-up after intervention: 6-46 months Intended End (LPLV): June 2024
Inclusion criteria	1 Infant between the ages of 4 months and 7 months at the time of randomization. 2 A high genetic risk (>10%) to develop beta-cell autoantibodies by age 6 years: a. For infants without a first degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4. These represent close to 1% of all newborns. b. For infants with a first degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503. These represent around one third of infants with a first degree family history of T1D. 3 Solid foods introduced into diet of infant 4 Written informed consent signed by the custodial parent(s).
Exclusion criteria	1. Concomitant disease or treatment that may interfere with the assessments, as judged by the investigators. 2. Any condition that could be associated with poor compliance. 3. Any medical condition or medical condition coexisting, which, in the opinion of the investigator, may jeopardize the participant's safe participation in the study. 4. Diagnosis of diabetes at the time of recruitment. 5. Participation in another clinical trial.
Investigational Product	Active ingredient: insulin provided as bulk human crystals filled in capsules. Formulation of 7.5 mg, 22.5 mg and 67.5 mg insulin and microcrystalline cellulose as filling substance. The reference placebo (filling substance only: microcrystalline cellulose) is identical in appearance to the active medication. Trade name: n.a. Manufacturer: NextPharma

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LIST OF ABBREVIATIONS

AE	Adverse Event
AGT	Abnormal Glucose Tolerance
AR	Adverse Reaction
CRF	Case Report Form
CTCAE	Common Toxicity Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
FBE	Full Blood Examination
FPFV	First patient first visit
GCP	Good Clinical Practice
GPPAD	The Global Platform for the Prevention of Autoimmune Diabetes
GPPAD CC	GPPAD Coordination Center
IASP	Islet Autoantibody Standardization Program
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
ITI	Immune Tolerance Induction
ISF	Investigator Site File
IVRS/IWRS	Interactive Voice Response System / Interactive Web Response System
LPLV	Last patient last visit
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
OGTT	Oral Glucose Tolerance Test
RBQM	Risk-Based-Quality-Monitoring
RCT	Randomized controlled trial
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SDV	Source Data Verification
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1D	Type 1 diabetes mellitus
T1DGC	Type 1 Diabetes Genetic Consortia
TMF	Trial Master File
UAR	Unexpected Adverse Reaction
WTCCC	Wellcome Trust Case Control Consortium

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DECLARATION OF INVESTIGATOR

I have read the clinical study protocol and I confirm that it contains all information to accordingly conduct the clinical study. I know that the study will be done in agreement with GCP and I will cooperate with the respective Monitors and pledge the clinical study will be conducted at my study centre according to the protocol.

The first patient will be enrolled only after all ethical and regulatory requirements are fulfilled. I pledge that written informed consent for trial participation will be obtained from all patients.

I know the requirements for accurate notification of serious adverse events and I pledge to document and notify such events as described in the protocol.

I pledge to retain all trial-related documents and source data as described. All necessary documents will be provided before trial start. I agree that these documents will be submitted to the responsible Regulatory Authorities and Ethics Committees.

_____

Investigator of the site Date

GPPAD-POInT Study: Visit –schedule (Study Flow Chart)

POInT Trial	**Trial**											
	Screening Phase	**Intervention**										
Visits		baseline visit (age 4 - 7 months)	visit 2 months post baseline	visit 4 months post baseline	visit 8 months post baseline	call	visit at age 1.5 years	call	visit at age 2.0 years	call	visit at age 2.5 years	call	visit at age 3.0 years
Visit window	below 7 months of age		± 10d	± 10d	± 10d		± 10d		± 14d		± 14d		± 14d
Study visit	0	1	2	3	4		5		6		7		8
Study call						1		2		3		4	
Informed consent	X*	X											
Review Incl./Excl. Criteria	X	X											
Randomization		X											
Medical History		X											
Psychological impact Questionnaire (mother&father)				X			X						X
Antibodies measurement (IAA; GADA; IA-2A; ZnT8RA; ZnT8WA; TS7A)		X	X	X	X		X		X		X		X
Vitamin D (25OHD) [B]		X	X	X	X		X		X		X		X
Intervention													
dispense medication (+ compliance data sheet)		X	X	X	X		X		X		X		
Treatment		daily with 7,5 mg Insulin OR Placebo	daily with 22,5 mg Insulin OR Placebo	daily with 67,5 mg Insulin OR Placebo									
Investigations													
Physical examination (height, weight)		X	X	X	X		X		X		X		X
Assessment of AEs and SAEs [E]		X	X	X	X		X		X		X		X[E]
Blood glucose (0/30/60/120) [C]		X	X	X	X								
Blood glucose							X		X		X		X
Differential blood count		X											(X)[A]
OGTT (0/30/60/90/120) [D]													X[D]
Storage													
storage: serum samples		X	X	X	X		X		X		X		X
storage: plasma samples		X			X				X				X
PBMC		X			X				X				X
blood volumes for protocol parameters (mL):		5.0	3.8	3.8	3.8		3.8		3.8		3.8		5-11
blood volumes for protocol parameters (%):		0.8-1.0	0.5-0.6	0.5-0.6	0.4-0.5		0.4		0.4		0.3		0.4-0.9
additional biobank blood volumes (mL):		7.5	10.1	10.1	17.6		17.6		22.5		17.6		17.6-22.5
Total blood volumes (mL):		**12.5**	**13.9**	**13.9**	**21.4**		**21.4**		**26.3**		**21.4**		**27.5-28.6**
Total blood volume (%):**		**1.9-2.4**	**2.0-2.2**	**1.8-2.0**	**2.5-2.8**		**2.4**		**2.7**		**2.0**		**2.3-2.4**

* Start of Informed consent process

**Blood volumes are < 5% NIH/WHO allowance and in accorance to the Pre-POINT (Bonifacio et al.: Effects of high dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-1549, 2015), Pre-POINT Early (EudraCT number: 2014-005287-15, NCT02547519, BfArM no. 4040595), and Fr1da-Intervention (EudraCT number: 2015-003028-30, NCT02620072, BfArM no. 4040830) studies

[A] In autoantibody positive children who will have an OGTT, a separate blood glucose sample is **not** taken.

[B] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with vitamin D

[C] Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo)

[D] If the participant developed beta-cell-autoantibodies during trial. OGTT (0/30/60/90/120) will be performed and samples measured in laboratory
Children who seroconverted to beta-cell-autoantibodies should have a confirmation sample within 4 – 12 weeks (interim visit)

[E] AEs/SAEs/SUSARs will be noted and reported as under intervention phase for 60 days after end of treatment day

POInT Trial	Trial														
	Follow-up														
	minimum 6 months FU		variable with maximum up to 54 months FU												
Visits	call	visit at age 3.5 years	call	visit at age 4.0 years	call	visit at age 4.5 years	call	visit at age 5.0 years	call	visit at age 5.5 years	call	visit at age 6.0 years	call	visit at age 6.5 years	call
Visit window		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d	
Study visit		9		10		11		12		13		14		15	
Study call	5		6		7		8		9		10		11		12
Informed consent															
Review Incl./Excl. Criteria															
Randomization															
Medical History															
Psychological impact Questionnaire (mother&father)		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]		(X)[F]	
Antibodies measurement (IAA; GADA; IA-2A; ZnT8RA;		X		X		X		X		X		X		X	
Vitamin D (25OHD) [B]															
Intervention															
dispense medication (+ compliance data sheet)															
Treatment															
Investigations															
Physical examination (height, weight)		X		X		X		X		X		X		X	
Blood glucose (0/30/60/120) [C]															
Blood glucose		(X)[A]		(X)[A]		(X)[A]		(X)[A]		(X)[A]		(X)[A]		(X)[A]	
Differential blood count															
OGTT (0/30/60/90/120) [D]		X[D]		X[D]		X[D]		X[D]		X[D]		X[D]		X[D]	
Storage															
storage: serum samples		X		X		X		X		X		X		X	
storage: plasma samples				X				X				X			
PBMC				X				X				X			
blood volumes for protocol parameters (mL):		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8		3.8-9.8	
blood volumes for protocol parameters (%):		0.3-0.8		0.3-0.7		0.3-0.7		0.3-0.6		0.2-0.6		0.2-0.6		0.2-0.5	
additional biobank blood volumes (mL):		25.1		25.1		25.1		30		30		30		30	
Total blood volumes (mL)":		**28.9-34.9**		**28.9-34.9**		**28.9-34.9**		**33.8-39.8**		**33.8-39.8**		**33.8-39.8**		**33.8-39.8**	
Total blood volumes (%)":		**2.5-3**		**2.1-2.6**		**2.1-2.6**		**2.2-2.6**		**2.2-2.6**		**2.0-2.3**		**2.0-2.3**	

"Blood volumes are < 5% NIH/WHO allowance and in accorance to the Pre-POINT (Bonifacio et al.: Effects of high dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-1543, 2015), Pre-POINT Early (EudraCT number: 2014-005287-15, NCT02547519, BfArM no. 4040535), and Fr1da-Intervention (EudraCT number: 2015-

[A] In autoantibody positive children who will have an OGTT, a separate blood glucose sample is **not** taken.

[B] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with vitamin D

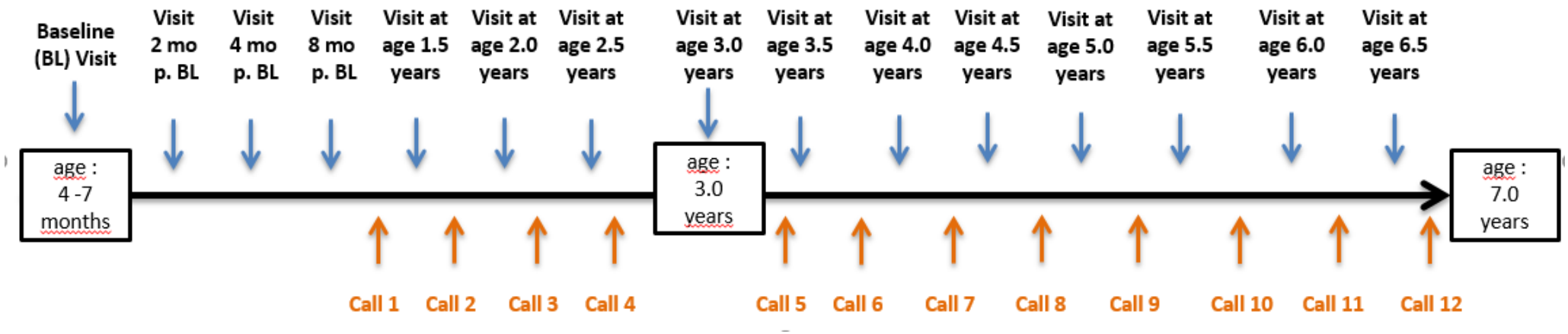
[C] Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo)

[D] If the participant developed beta-cell-autoantibodies during trial. OGTT (0/30/60/90/120) will be performed and samples measured in laboratory
Children who seroconverted to beta-cell-autoantibodies should have a confirmation sample within 4 - 12 weeks (interim visit)

[F] hand out of the Psychological Impact Questionnaire only if this visit is childs last follow-up visit (i.e. only at End of Study Visit)

GPPAD-POInT: Time schedule

Example for a participant with maximum follow-up of 46 months:



1. BACKGROUND AND SIGNIFICANCE

1.1 TYPE 1 DIABETES: DEFINITION AND METABOLIC CHARACTERISTICS

Type 1 diabetes (T1D) is an immune-mediated disease in which insulin-producing beta-cells are completely or near completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients diagnosed with T1D each year is increasing and is approaching an epidemic level in some countries that track this information (1, 2).

Compared to individuals with the more common form of diabetes, type 2 diabetes, (where individuals retain endogenous insulin production that is inadequate to maintain normal glucose and lipid metabolism), patients with T1D have a more severe metabolic impairment and a more complete loss of insulin production. At the time of diagnosis, many individuals, and children in particular, suffer significant morbidity frequently requiring ICU admission. Continuous exogenous insulin therapy is needed to prevent ketoacidosis and other catabolic effects of insulin deficiency and to promote anabolism and to maintain life. The Diabetes Control and Complications study (DCCT) showed that the long term complications could be reduced with near normal control of glucose levels but at the cost of an increased frequency of severe hypoglycemia (3). While there have been significant improvements in insulin analogs and insulin delivery systems, such as continuous subcutaneous insulin infusions with insulin pumps, normal glucose control, particularly in children, is rarely achieved. Therefore, individuals with T1D remain at risk for chronic secondary end-organ complications including visual impairment and blindness, renal failure, vascular disease and limb amputation, peripheral neuropathy, and stroke. They are also at high risk for acute complications such as severe hypoglycemia, recurrent ketoacidosis, and others.

Prevention of T1D would clearly represent a significant advancement.

1.2 NATURAL HISTORY OF TYPE 1 DIABETES

T1D results from an immune-mediated destruction of the pancreatic islet beta-cells resulting in insulin deficiency. This process is clinically silent and can be identified by circulating autoantibodies to beta-cell antigens (GADA, IA-2A, IAA and ZnT8A). Beta-cell autoantibody seroconversion has a clear peak incidence period between age 9 months and 3 years demonstrated in German (4), Finnish (5), and TEDDY studies (6) (Figure 1). In a recent combined analysis of over 13000 prospectively followed children form the BABYDIAB, DAISY, and DIPP studies, 80% of the children who developed T1D before the age of 20 years already developed beta-cell autoantibodies before the age of 5 years (median (IQR) age 2.1 (1.3-4.1) years) (7). On the basis of these findings, it is concluded that immune therapy given as a primary prevention strategy must be started early in life.

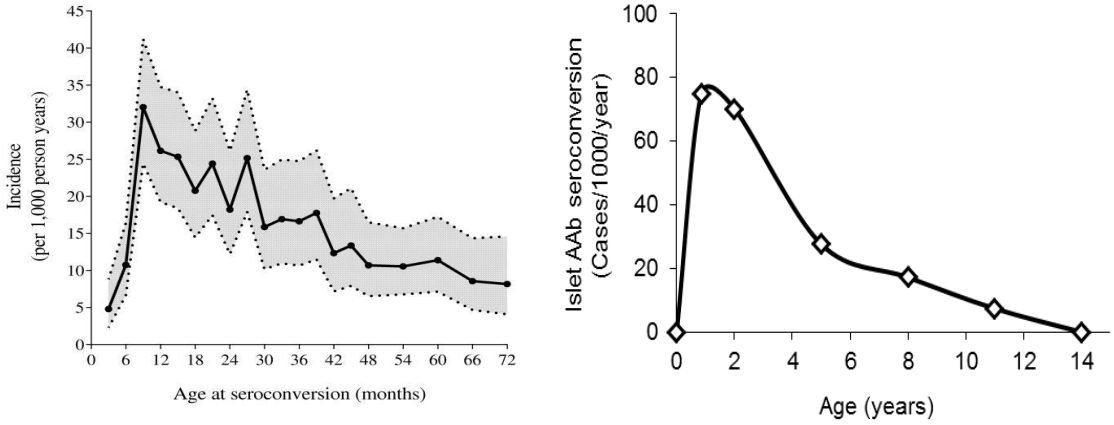


Figure 1: The incidence of beta-cell autoantibodies peaks in early childhood in children including general population at genetic risk for T1D (left) (8) and with a first degree relative with T1D (right) (9)

Almost all children who develop the stage of multiple beta-cell autoantibodies progress to clinical diabetes (Figure 2). The earlier the process of beta-cell autoimmunity is initiated, the more rapid is the progression to T1D (7).

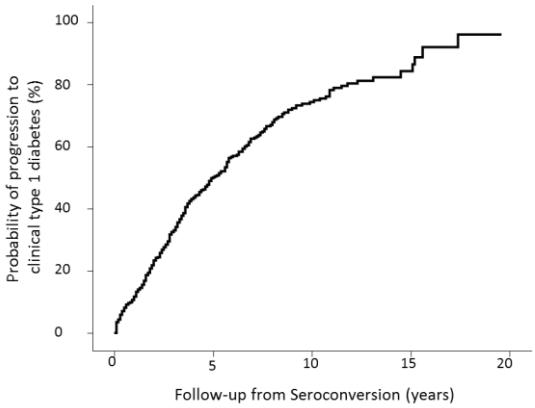


Figure 2: Children with multiple autoantibodies progress to symptomatic T1D

1.3 IDENTIFICATION OF SUBJECTS AT INCREASED RISK FOR BETA-CELL AUTOIMMUNITY AND T1D

T1D has a multifactorial etiology, which is determined by genetic and environmental factors (17). Risk in a European population is around 0.4%. A first degree family history of T1D is associated with a 5% risk for type 1 diabetes (18). There are also at least 50 known regions of the genome where genetic variation is associated with T1D risk (19). The most important of these is in the HLA DR-DQ region of chromosome 6. Certain HLA DR-DQ genotypes confer markedly elevated risk for T1D. Notably, infants who have the HLA DR3/DR4-DQ8 or the DR4-DQ8/DR4-DQ8 genotype have a risk of around 5% (20, 21). Typing at additional T1D susceptibility regions can identify infants with risks that are 10% or more (22). Thus, family history and genetic markers can be used to identify neonates or infants with 25-fold increased risk for T1D.

By analyzing data from the Type 1 Diabetes Genetic Consortia (T1DGC), it has been recently demonstrated that a genetic risk score generated from HLA class II genotypes and 40 SNPs of non-HLA genes associated with T1D predisposition can improve risk stratification for T1D over HLA alone (22). Similarly, by analyzing data from the Wellcome Trust Case Control Consortium (WTCCC), a genetic risk score of 30 SNPs was developed to estimate T1D risk (23). Both risk scores were now validated and applied to data from The Environmental Determinants of Diabetes in the Young (TEDDY) study (manuscript in preparation). In the TEDDY data, risk stratification using each genetic risk score was reproduced. Therefore, a score that merges the prior algorithms (22, 23) was calculated and used in the TEDDY children. Children with no family history of T1D who have the HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 genotype and a genetic risk score of >14.4 using the merged algorithm (corresponds to upper 75th percentile of HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 TEDDY population) had a risk of 15.9% for developing beta-cell autoantibodies by age 5 years and 11.4% for developing multiple beta-cell autoantibodies by age 6 years (figure 3 a, b). In first degree relatives of a patient with T1D, the presence of at least one HLA DR4-DQ8 haplotype and no protective HLA DR and DQB1 alleles is associated with a genetic risk of >10% for developing multiple beta-cell autoantibodies by age 6 years (18, 21).

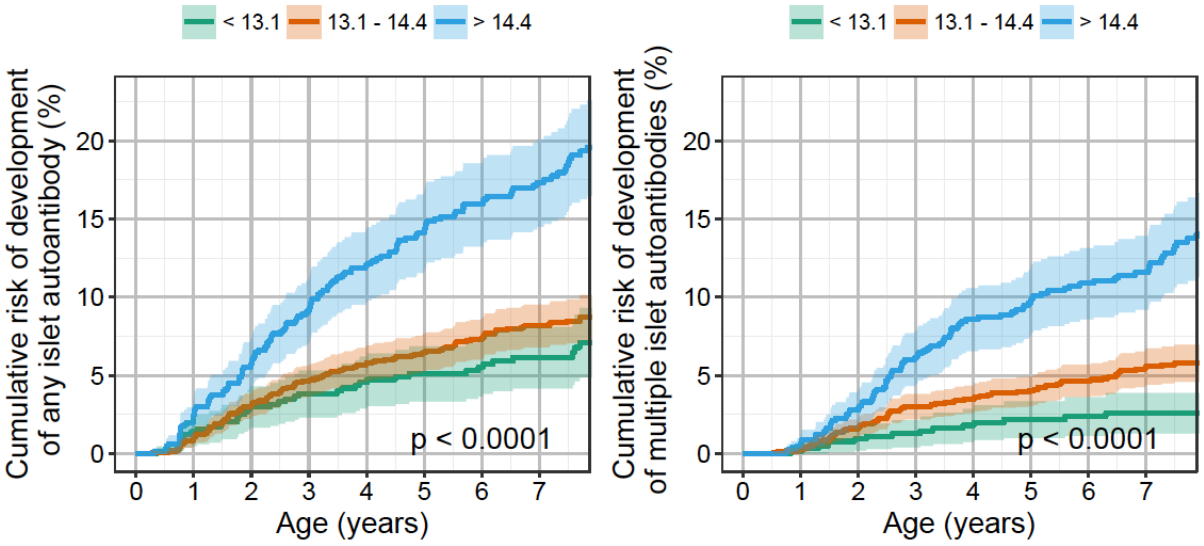


Figure 3: Risk of beta-cell autoimmunity (left, first autoantibody; right, multiple beta-cell autoantibodies) in TEDDY children with HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 genotype and a genetic risk score of > 14.4 (blue line) using the merged algorithm (corresponds to upper 75th percentile of HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 TEDDY population) compared to children with HLA DR3/DR4-DQ8 or HLA DR4/DR4-DQ8 genotype and a respective score between 13.1 and 14.4 (orange line), and below 13.1 (green line). P-values were derived from log-rank tests on differences in autoantibody risk between children in the three risk groups. Shaded areas represent the 95% confidence interval or risk estimates.

1.4 RATIONALE FOR USE OF ORAL INSULIN AS IMMUNE TOLERANCE INDUCTION THERAPY

Self-tolerance is achieved by T cell exposure to self-antigens in the thymus or in the periphery (i.e. outside the thymus or bone marrow, in secondary lymphoid tissues such as lymph nodes, gut and spleen) in a manner that deletes or anergizes autoreactive effector

T cells and induces regulatory T cells. Immunological tolerance can be achieved by administration of antigen under appropriate conditions (7, 17). Evidence is now emerging in humans that these approaches may be effective in chronic inflammatory diseases such as multiple sclerosis, allergy, and T1D (25-27).

Our goal is to introduce immune tolerance to autoantigen before the start of beta-cell autoimmunity as primary prevention for T1D (23, 28). Primary prevention of T1D has a strong rationale. There is clear evidence from man (29, 30) that insulin is the key early and primary autoantigen of childhood diabetes. There is also a strong genetic rationale for loss of tolerance against insulin as a primary cause of T1D. Allelic variation in the *insulin* gene is associated with T1D (31) and beta-cell autoimmunity (32) via an impaired mechanism of thymic T cell deletion (33). Polymorphisms in the *INSULIN (INS)* gene confer genetic risk for T1D by altering insulin expression in the thymus, thereby influencing immune tolerance to insulin and its precursors (32, 33). Children who have increased exposure to insulin in foetal and neonatal life as a result of having a mother with type 1 diabetes (34), have a reduced risk for developing beta cell autoantibodies (35). Moreover, insulin autoimmunity is closely linked to the HLA DR4-DQ8 haplotype present in the majority of children who develop T1D (36, 37).
There is a general consensus that turning back or undoing a full-fledged memory autoimmune response to multiple beta-cell autoantigens (secondary prevention) will require more aggressive therapies than preventing autoimmunity in the first place.
It is widely held that if infant tolerance to beta-cell autoantigens could be enhanced, this could prevent or delay the onset of pre- or asymptomatic T1D (defined as multiple beta-cell autoantibodies), and hence prevent or delay disease diagnosis. The key here is "infant", the time when the natural mechanisms of immune tolerance are fully active as the child becomes tolerant to commensal microorganisms and dietary components. Currently, antigen-specific tolerance approaches are attempted in individuals in whom the immune system has matured and in whom an autoimmune memory response is well established. We, however, have laid the foundation for antigen-specific tolerance induction as primary prevention (initiated prior to an autoimmune response). We have identified a dose of insulin that, when administered orally on a daily basis to genetically at-risk children who are beta-cell autoantibody negative, is safe (does not affect plasma glucose levels) and appears to engage the immune system in a manner that is consistent with immune-mediated, tolerogenic protection (38).
Hence, we believe that there are two important pillars for primary prevention to move forward – knowledge of when diabetes inducing beta-cell autoimmunity starts and demonstration that insulin-specific protective ITI is feasible.

1.5 EVIDENCE FOR ANTIGEN BASED PREVENTION IN HUMAN ALLERGY

Supporting evidence of antigen based therapies has been recently shown in a large study, which aimed to prevent peanut allergy through active exposure to peanut antigen. Unlike previous attempts based on avoidance of peanuts, the consumption trial was successful. Relevant to primary prevention, the LEAP trial enrolled 542 infants who initially had no pre-existing sensitivity to peanuts, but who had an estimated 9% risk for developing peanut allergy by age 5 years (27). Children randomized to peanut consumption were instructed to eat at least three peanut-containing meals per week - starting at age 4 to 11 months - in order to consume at least 6 g of peanut protein per week until age 5 years. The

prevalence of peanut allergy at 60 months of age was 13.7% in children who avoided peanut consumption and, remarkably, only 1.9% in children who consumed peanuts (P < 0.001). The results are even more striking in a per protocol analysis where only 0.4% of non-sensitized children developed peanut allergy in the consumption group. Another impressive aspect of the LEAP trial was that only 12 (2.2%) of the 542 enrolled children did not complete the study at age 5 years. Overall, the primary prevention part of the LEAP trial is encouraging for GPPAD attempts to introduce autoantigen-based primary prevention for T1D.

1.6 SIGNIFICANCE OF THE GPPAD-POINT STUDY

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.
Additionally, antigen-based therapy in T1D could also serve as a model for other childhood conditions and illnesses, with a major underlying goal of the promotion of better health outcomes early in life based on improved understanding of the human immune system.

2. CLINICAL AND PRE-CLINICAL DATA

2.1 PREVIOUS CLINICAL TRIALS USING ORAL INSULIN

Primary Prevention
The Pre-POINT (Primary Oral Insulin Trial)-Study (Protocol number: 80804002, BfArM number: 4034919, EudraCT number: 2005-001621-29, ISRCTN76104595).
We have conducted and completed a primary autoantigen immune tolerance dose-finding study in which children with high genetic risk for T1D were administered insulin orally daily (38). The objective of this pilot Pre-POINT study was to identify a dose of oral insulin that was safe and could engage the immune system when administered as a primary intervention to children without beta-cell autoimmunity. Pre-POINT was performed as a double-blind placebo controlled dose increasing phase I/II clinical trial. Children aged two to seven years with a family history of T1D and T1D susceptible HLA class II genotypes and without beta-cell autoantibodies (n=25) were randomized to receive placebo (n=10) or insulin (n=15) orally once a day for 3 to 12 months. The design included dose escalation so that six children were included in each of the 2.5 mg, 7.5 mg, 22.5 mg, and 67.5 mg insulin dose groups. Safety was assessed by blood glucose measurements following administration of medication, serum IgE concentrations, serum IgE against insulin, and measurement of autoantibodies to glutamic acid decarboxylase and IA-2. Activation of the immune system by the study drug was measured by insulin autoantibodies, IgG- and IgA-binding to insulin in serum and saliva, and CD4+ T cell proliferative responses /gene expression to study drug. Oral insulin at all tested doses in Pre-POINT was considered safe: None of the children who received study drug or placebo experienced hypoglycaemic episodes after administration of medication, and no allergic reactions were observed. Adverse events were similar between study drug and placebo groups. No child developed autoantibodies to glutamic acid decarboxylase or IA-2 or diabetes during the reported observation period of 6 months to maximum 3.5 years (see Appendix 1: Synopsis of Final Study Report). Important for the current protocol, five of six children exposed to a dose of

67.5 mg insulin had evidence of an antibody or T cell response to insulin (see Table 1). The response differed to the typical responses seen in children who develop diabetes in that the antibody responses were of weak affinity (not inhibitable with low concentrations of cold insulin in reference methods used to measure insulin autoantibodies) and the T cell responses had a preponderance of cells with regulatory T cell phenotypes. These results are encouraging from a safety viewpoint and indicate that oral exposure to insulin at doses that are approximately equivalent to efficacious doses in rodents may promote tolerance in children.

Table 1: Summary of immune responses to study drug (insulin) in Pre-POINT children

Response measure against insulin	Placebo	2.5 mg insulin	7.5 mg insulin	22.5 mg insulin	67.5 mg insulin
Serum IgG-IAA	1/10	0/6	1/6	1/6	3/6
Salivary IgA-IAA	0/10	0/6	0/6	1/6	0/6
$CD4^+$ T cell	1/7	1/6	0/5	1/5	2/4
Antibody or $CD4^+$ T cell response	2/10	1/6	1/6	2/6	**5/6**

The Pre-POINT-Early Study (Protocol number: 80804017, BfArM number: 4040595, EudraCT number: 2014-005287-15, NCT02547519).

Pre-POINT-Early is a study using oral insulin at early age for safety and immune efficacy. The aim of this Phase II Study was to determine whether daily administration of up to 67.5 mg insulin to young children aged 6 months to 2 years with a high genetic and familial risk for T1D is safe and induces immune responses to insulin with features of immune regulation. Autoantibody negative children at high genetic risk for T1D, age 6 months – 2 years have been randomized either into 1. Oral insulin (dose escalation: 7.5 mg for 3 months; increased to 22.5 mg for 3 months; increased to 67.5 mg for 6 months) or 2. placebo. In total, 44 subjects were enrolled and the last patient last visit of the Pre-POINT Early study was in December 2017. Safety Data from this trial confirmed that overall oral insulin at all tested doses (7.5 mg; 22.5 mg and 67.5 mg) in the Pre-POINT-Early study can be considered safe. There was no difference between treatment and placebo groups in blood glucose-, insulin- and C-peptide values, as well as insulin/C-peptide ratio, AUC glucose, AUC insulin, AUC C-peptide, or AUC insulin/C-peptide after study drug application. Two children developed the study endpoint 'persistent islet autoantibodies', one in the treatment and one in the placebo arm.

There was no significant difference in the number of adverse events, in the number of adverse events by person years, in the number of serious adverse events, and in the severity of adverse events.

When analysing single system organ classes, a higher frequency of skin and subcutaneous tissue disorder adverse events were observed in the treatment group (8 versus 1, $p = 0.01$); the overall frequency of skin and subcutaneous tissue disorder adverse events was low (6.6%); they were all classified as AE grade 1; they all resolved during the study. The safety data for Pre-POINT-Early are provided in appendix D of the Investigator Brochure.

Secondary Prevention

The TN07 study (Oral Insulin for Prevention of Diabetes in Relatives at Risk for Type 1 Diabetes Mellitus) (Protocol number: TN07, 80804005, EudraCT number: 2006-006550-96, NCT00419562).

A secondary prevention multicentre study using 7.5 mg oral insulin administered daily is conducted by the TrialNet Study Group. This study included multiple sites in Europe such as the Forschergruppe Diabetes, Klinikum rechts der Isar der Technischen Universität München, and the Lund University, Skåne University Hospital SUS. Autoantibody, normoglycemic subjects aged 3 to 45 years are treated with oral insulin. There are over 500 subjects enrolled (479 children <18 years and 102 subjects <5 years respectively). No safety issues have been observed thus far (a regularly updated AE list is available on the internal TrialNet TN07 website and available upon request at trialnetinfo@epi.usf.edu). The TN07 results were presented orally at the American Diabetes Association Conference (San Diego) in June 2017. There was no evidence that treatment with 7.5 mg oral insulin accelerated disease in any of the pre-specific participant Strata. In the secondary stratum with first phase insulin response to glucose below threshold (n=55), time to diabetes was significantly longer with oral insulin: HR=0.45 (95% CI 0.22, 0.91), p=0.01, indicating protection. In the other secondary stratum (n=116), and the entire cohort (n=560), there was no significant difference between groups: HR=1.03 (95% CI 0.44, 2.42), p=0.95 and HR= 0.79 (95% CI: 0.58, 1.06) p=0.10. The most common adverse event was infection (n=255) but there were no significant study related adverse events.

The Fr1da-Insulin-Intervention Study (Mechanistic study using oral insulin for immune efficacy in secondary prevention of type 1 diabetes) (Protocol number: 808040019, EudraCT number: 2015-003028-30, NCT02620072).

The objective of this phase II study is to determine the bioavailability and immune efficacy of high dose oral insulin in children with multiple beta-cell autoantibodies in a secondary intervention study. Immune efficacy is defined as a change in the immune response to the treatment that is associated with a reduction in the progression to dysglycemia. Children in the oral insulin group receive increasing dose of daily oral insulin: 7.5 mg for a duration of 3 months and increasing to 67.5 mg for 9 months of intervention. Children in the placebo group will receive 12 months of daily oral placebo. The study aims to recruit 220 participants. No safety concerns have been observed thus far.

2.2 PRECLINICAL DATA AND OTHER HUMAN STUDIES

Previous studies in rodents had indicated that mucosal administration of insulin is effective in inducing regulatory immune responses that can prevent autoimmune diabetes (39-42). Mouse studies indicated that the dose of oral insulin is important (41). For Pre-POINT we had reasoned that doses above 50 mg per day were required in children in order to match efficacious doses in mouse models of autoimmune diabetes (41), and the results shown in table 1 support this reasoning.

Other previous human studies had given oral insulin at doses between 2.5 mg and 15 mg to diabetic or non-diabetic subjects without side effects (43-45). The studies demonstrated no obvious benefit in diabetic subjects with respect to preservation of residual beta-cell function. The administration of oral insulin (7.5 mg per day) to prediabetic ICA and IAA positive first degree relatives of T1D patients in the DPT-1 study showed no significant beneficial effect in the intention to treat analysis. A sub-analysis of the data, however, showed significant benefit in those relatives with higher titer IAA (43), that was persistent (46).

3. STUDY DESIGN

3.1 OVERVIEW

The GPPAD-POInT Study is designed as a randomized, placebo-controlled, double blind, multicentre, multinational primary prevention phase IIb study aiming to induce immune tolerance to beta-cell autoantigens through regular exposure to oral insulin for a period of 29 to 32 months.

3.2 HYPOTHESIS

We hypothesize that regular exposure to oral insulin throughout the period in life where beta-cell autoimmunity usually initiates will tolerize against insulin and train the body's immune system to recognize the treatment product without reacting adversely to it in a manner seen in children who develop T1D. This immune tolerance induction therapy would reduce the likelihood of beta-cell autoimmunity.

3.3 OBJECTIVES

The study objective is to determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1 diabetes reduces the cumulative incidence of beta-cell autoantibodies and diabetes in childhood.

3.4 SUMMARY OF INCLUSION / EXCLUSION CRITERIA

3.4.1 Inclusion criteria

Participants must meet all entry criteria for the protocol as outlined below.

1. Infant between the ages of 4 months and 7 months at the time of randomization.
2. A high genetic risk (>10%) to develop multiple beta-cell autoantibodies by age 6 years:
   a. For infants without a first degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4[1]. These represent close to 1% of all newborns.
   b. For infants with a first degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503. These represent around one third of infants with a first degree family history of T1D.
3. Solid foods introduced into diet of infant

[1] The genetic risk score is calculated by multiplying the number of risk alleles (i. e. 0, 1 or 2 for each single SNP) with a specific weight assigned to each SNP (see Table 2) and then summing up the weighted contributions of all SNPs plus an additive constant for each HLA category. All 47 SNPs as listed in Table 2 will be determined in each child.

4. Written informed consent signed by the custodial parent(s)

3.4.2 Exclusion criteria

Participants meeting any of the following criteria will NOT be eligible for inclusion into the study:

1. Concomitant disease or treatment that may interfere with the assessments, as judged by the investigators.
2. Any condition that could be associated with poor compliance.
3. Any medical condition or medical condition coexisting, which, in the opinion of the investigator, may jeopardize the participant's safe participation in the study.
4. Diagnosis of diabetes at the time of recruitment.
5. Participation in another clinical trial.

3.5 ENROLLMENT

Potential study subjects will be identified through the GPPAD-02 study or through similar studies testing for type 1 diabetes risk in infancy. In the GPPAD-02 study, testing for genetic risk of T1D is offered either at delivery (cord blood), together with the regular newborn screening, or at a pediatric baby-visit with collection of blood using GPPAD-02 filter paper cards. Infants are tested for genetic risk of T1D based on risk scores derived from SNPs that define HLA DR3, HLA DR4, and HLA DQ8 alleles as well as SNPs from HLA class I, and non-HLA T1D susceptibility genes, and from HLA class II protective alleles. Infants with a predicted risk of >10% to develop multiple beta-cell autoimmunity by age 6 years and who fulfill the inclusion criteria as stated above will be asked to participate in the GPPAD-POInT Study.

3.6 DESCRIPTION OF TREATMENT GROUPS

The intervention will be conducted only in children for whom consent to participate has been provided. Eligibility will be verified by the study physician shortly before randomization and at the baseline visit. Infants will be randomized to receive either oral insulin or placebo. Treatment will be provided at approved GPPAD clinical sites with appropriate facilities. Blood and serum samples for the primary and secondary outcome determinations will be sent to central laboratories for analysis. Clinical safety parameters may be done at the local sites.

Participants will be randomly assigned in a 1:1 ratio to the following two groups:

- to receive daily oral insulin 7.5 mg for 2 months, followed by 22.5 mg for 2 months, followed by 67.5 mg until age 3.0 years. Close monitoring for beta-cell autoantibodies, dysglycemia, and diabetes will occur through the duration of study.
- to receive daily oral placebo until age 3.0 years. Close monitoring for beta-cell autoantibodies, dysglycemia, and diabetes will occur through the duration of study.

3.7 DOSAGE FORM

The IMP used in the GPPAD-POInT Study will be essentially identical to the IMP used in the Pre-POINT [2] (36), Pre-POINT-Early[3], and Fr1da-Insulin-Intervention[4] studies. Human insulin for oral administration is provided by Lilly Pharmaceuticals, Indianapolis, Indiana USA. It is provided as bulk human insulin crystals. This insulin is sold by Lilly as an injectable formulation known as Humulin-R. In the GPPAD-POInT Study three doses are given: Dose 1 is 7.5 mg rH-insulin crystals; dose 2 is 22.5 mg rH-insulin crystals; dose 3 is 67.5 mg rH-insulin crystals. The insulin crystals are formulated together with filling substance (microcrystalline cellulose to a total weight of 200 mg) and contained in hard gelatin capsules. The dose was chosen based on demonstrated immune efficacy in children in the Pre-POINT study, and demonstrated safety in children participating in the Pre-POINT, Pre-POINT-Early, and Fr1da-Insulin-Intervention study (see also part 2.1).
The conversion of the mg unit into IU for the 7.5 mg of the oral insulin results in 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.
For further information about the IMPs please also refer to the Investigator's brochure.

3.8 APPLICATION, DOSE AND DOSAGE REGIMEN

The study treatment will be given orally as a powder spread on a small quantity meal serving e.g. with infant formula, tea spoon of water, commercial baby food or yogurt. The insulin will be provided in a capsule box à 32 hard gelatin capsules containing rH-insulin crystals or placebo.
The investigational product (oral insulin or placebo) will be self-administered by the child's parents as content of one capsule per day. Treatment will be administered preferably in the morning (7-10am). Parent(s) will be instructed in the administration and storage of study drug at their baseline visit.
Participants will be observed for 2 hours after administration of the study drug at visits 1, 2, 3, and 4. They are advised to immediately report any adverse events experienced following treatment.

3.9 DOSE WITHHOLDING, WITHDRAWAL OR DROP OUT OF PARTICIPANTS

Withdrawal Criteria
Participants will be withdrawn from study treatment if they:

- develop diabetes (study endpoint)
- report moderate to severe intolerance of study treatments
- develop an intercurrent illness deemed incompatible with the study, as judged by the investigators
- have consent withdrawn by custodial parent(s)

[2] Protocol ID: 80804002; EudraCT-No.: 2005-001621-29; CurrentControlledtrials ID: ISRCTN76104595
[3] Protocol ID: 808040017; EudraCT-no.: 2014-005287-15; Clinicaltrials.gov ID: NCT02547519
[4] Protocol ID: 808040019; EudraCT-no.: 2015-003028-30; Clinicaltrials.gov ID: NCT02620072

The participant and/or his or her parent(s) will be informed that being in the trial is voluntary and that he or she may withdraw consent from the study at any time, for any reason. 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". The reason for discontinuation of study treatment and/or withdrawal from the study will be captured on the Case Report Form. In case custodial parent(s) withdraw consent re treatment but agree to continue the observation of the infant as specified in the trial protocol, this will be done. Every effort will be made to follow all participants enrolled in the study (including those that do not complete the treatment period).

3.10 TREATMENT ASSIGNMENT

Trial inclusion and registration will take place at the baseline visit. Infants will be included and registered if they meet the inclusion criteria and none of the exclusion criteria and after written consent has been obtained by the custodial parent(s). As this trial is double-blind, the study drug manufacturer will provide the medication packages using package numbers. The study drug packages and thus the participants too will be randomized in a 1:1 ratio to each arm. A separate randomization list is used for multiples to ensure that these are assigned to the same treatment arm.

3.11 PROCEDURES FOR UNMASKING

Emergency unmasking will be available through the IVRS/IWRS system. In case of temporary unintended unavailability of the IVRS/IWRS system a 24h hotline is available likewise.
Regular (non-emergency) unblinding of study drug assignment is planned to be conducted upon completion and verification, closure of database and completion of all parameter analyses. It will occur upon notification of the GPPAD CC and approval of the POInT Protocol Chair. There are special provisions for the DSMB.

3.12 STUDY ASSESSMENT

During the course of the study, participants will be tested for beta-cell autoantibodies, glucose levels, and will be assessed for their overall health and well-being. In children with beta-cell autoantibodies, OGTTs will be performed at six month intervals starting from age 3.0 years. Individuals in both of the study arms will have examinations performed as detailed in the Flow Table (see page 13).

As an **ancillary** component of the trial, Biobank repository samples will be drawn for storage in local biobanks at each study site and for storage of aliquots at a central biobank for future research related to T1D. Samples will be pseudonymised before transferred to the biobank. Drawing and storage of samples that are assigned for the biobank is not considered a trial violation.

3.13 QUALITY ASSURANCE

Investigational sites have a clinical study centre with adequate medical space and equipment. Study physicians and study nurses have experience in the conduct of clinical

trials and are trained according to GCP Guidelines. Study medication will be stored in a closed, limited access area at the clinical study centre. Approval from the competent ethics committees will be obtained. Medical and research records are maintained at the clinical study centre in the strictest confidence.
Beta-cell autoantibodies as the primary outcome marker are assessed in two central GPPAD autoantibody laboratories. The performance of both laboratories in the Islet Autoantibody Standardization Program (IASP) will be documented and monitored. Additionally, quality assurance of local routine laboratories is guaranteed by choosing accredited laboratories which must present valid ring test certificates or equivalent certificates throughout the study. Study centres must keep documentation of all laboratory certificates.

3.14 STUDY TIMELINE

Study Duration
It is estimated that the enrollment period will last approximately 3.5 years and a total study duration of 7.0 years will provide a sufficient number of events to detect the assumed treatment difference.
Enrolled participants will start treatment from earliest age of 4 months to latest 7 months for a period of 29 up to 32 months (until 3.00 years of age). Participants will be followed until completion of the trial for a period of 6 to 54 months at last follow-up visit. Note that the accrual period and the study sample are only projections since the actual accrual rate and the lost to follow-up rate are unknown. Participants who discontinue treatment or withdraw from the study will not be replaced.
Participants who develop beta-cell autoantibodies will remain on study and will be followed according to protocol to assess the secondary outcomes until completion of the trial or until diabetes development.
Individuals who develop diabetes may be eligible for interventional studies available through other consortia such as INNODIA.

Update after completion of enrollment:
The enrollment period lasted 3.17 years instead of 3.5 years, and has been completed in March 2021 (1050 subjects enrolled). The end of study (LPLV) is now expected in June 2024, which is when the last child completed 6 months of follow-up after the end of treatment Additional trial follow-up after end of enrollment will be 3.25 years.
The last visit (final close-out visit) including collection of blood samples for final assessments should be performed for all children within the last 6 months before the last child completed 6 months of follow-up.

4. PARTICIPANT MANAGEMENT

4.1 IDENTIFICATION OF ELIGIBLE SUBJECTS FOR POINT

This study will mainly draw participants from the GPPAD-02 that tests the genetic risk for beta-cell autoantibodies in newborns and infants.
Potential participants in the oral trial will have a high genetic risk (>10%) to develop multiple beta-cell autoantibodies by age 6 years.
Inclusion- and exclusion criteria are listed in chapter 3.4.

4.2 SCREENING AND INFORMED CONSENT PROCESS FOR POINT

Prior to randomization, in appropriate time before the baseline visit a screening visit will be conducted. During the screening visit, inclusion and exclusion criteria will be assessed and medical history information will be obtained. Furthermore, the GPPAD-POInT Study will be described to the custodial parent(s) of potential participants by a GPPAD-POInT Study physician. After reading the informed consent form and clarifying all questions to satisfaction with the study physician/investigator(s), all eligible participants' custodial parent(s) will be invited to provide written consent. They will have the opportunity to read the consent document and to discuss any questions concerning the consent or trial participation. If participation in the clinical trial is considered by the family, they may take the consent document home to discuss with family, friend or advocate. The families will be given enough time to consider whether or not to participate. The custodial parent(s) will then be asked to sign and date an informed consent document describing the purpose, risks, and benefits of the trial prior to or at the baseline visit. The signature of the custodial parent(s) indicates that he/she understands the potential risks and benefits of study participation.

The assessment at screening therefore include the following procedures:

1) Assessment of **inclusion and exclusion criteria**
2) Assessment of **medical history** (only cases that lead to medical consultation will be recorded)
3) **Informed consent** process

4.3 BASELINE VISIT (BETWEEN 4 AND 7 MONTHS OF AGE)

After written informed consent has been properly obtained, the participant will be randomized to a treatment group (see part 3.10 Treatment Assignment). Participant eligibility must be reviewed and confirmed once more by the study physician immediately prior to randomization.

The clinical assessment at baseline includes the following procedures:
Before first study drug intake:

1) Collection of **venous blood** for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) Blood glucose (before study drug administration)
   c) 25-OH-Vitamin D3
      A single missing vitamin D value will not be considered as protocol violation.
   d) FBE (differential blood count)
      Note: If it's not possible to collect enough blood for all assessments at baseline differential blood count can be postponed to another visit during the first year instead. Single missing blood count parameters (except leucocytes and hemoglobine) will not be considered as protocol violation.

e) As ancillary storage (subject to ethical approval and separate informed consent): Serum, plasma, PBMC

2) General **physical examination** (weight, height)
3) Administration **and dispensation of blinded study drug** (7.5 mg insulin OR placebo) will be performed after collecting baseline biological samples (at time point 0 minutes)
4) Monitoring of blood glucose over 2 hours from when blinded study drug is administered:

   Collection of venous blood or capillary blood after study drug intake (30min, 60min, 120min) for blood glucose. Single missing glucose values will not be considered as protocol violation as long as at least 2 values after administration of study drug and the value before administration of study drug are available.

After first study drug intake

1) Documentation and assessment of **AEs and SAEs**

Single missing lab values or any other single missing test results are not considered as protocol deviation as long as adequate attempts have been taken to get the result as required per protocol. Any missing data due to non-compliance of study participants are also not considered as protocol deviation.

4.4 INTERVENTION - VISITS 2, 3 AND 4

The study visits 2 (2 months post baseline visit ± 10 days), visit 3 (4 months post baseline visit ± 10 days), visit 4 (8 months post baseline visit ± 10 days), follow the same schedule. At visit 2, a dose escalation to 22.5 mg Insulin and at visit 3, a dose escalation to the final dose of 67.5 mg Insulin are designated.

The following procedures are scheduled:

1) Collection of **venous blood** for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) Blood glucose (before study drug administration)
   c) 25-OH-Vitamin D3
      A single missing vitamin D value will not be considered as protocol violation.
   d) As ancillary storage (subject to ethical approval and separate informed consent): serum, plasma, PBMC (at visit 4 only)
2) **Physical examination** (weight, height)
3) Documentation and assessment of **AEs and SAEs**
4) **<u>At visit 3 only:</u> Psychological Impact Questionnaire** completed by mother and father (Appendix 5)
5) Collection of information on **medication compliance**
6) **Administration and dispensation of blinded study drug** will be performed after collecting baseline biological samples (at time point 0 minutes). Return of opened/ broken study medication.

7) Monitoring of blood glucose over 2 hours from when blinded study drug is administered:

   Collection of venous blood or capillary blood after study drug intake (30min, 60min, 120min) for blood glucose. Single missing glucose values will not be considered as protocol violation as long as at least 2 values after administration of study drug and the value before administration of study drug are available.
   Single missing lab values or any other single missing test results are not considered as protocol deviation as long as adequate attempts have been taken to get the result as required per protocol. Any missing data due to non-compliance of study participants are also not considered as protocol deviation.

4.5 INTERVENTION - VISITS 5, 6 AND 7

The study visit 5 (visit at age 18 months ± 10 days), visit 6 (visit at age 24 months ± 14 days) and visit 7 (visit at age 30 months ± 14 days) follow the same schedule.

The following procedures are scheduled:

1) Collection of **venous blood** for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) blood glucose by glucose meter and laboratory assessment
   c) 25-OH-Vitamin D3
      A single missing vitamin D value will not be considered as protocol violation.
   d) As ancillary storage (subject to ethical approval and separate informed consent): Serum (at visit 5, 6 and 7), plasma (at visit 6 only), PBMC (at visit 6 only)
2) **Physical examination** (weight, height)
3) Documentation and assessment of **AEs and SAEs**
4) Collection of information on **medication compliance**
5) **Administration and dispensation of blinded study drug** will be performed after collecting baseline biological samples (at time point 0 minutes). Return of opened/ broken study medication.
6) **Psychological Impact Questionnaire** completed by mother and father (Appendix 5) (only at visit 5)

Single missing lab values or any other single missing test results are not considered as protocol deviation as long as adequate attempts have been taken to get the result as required per protocol. Any missing data due to non-compliance of study participants are also not considered as protocol deviation.

4.6 TELEPHONE CALLS BETWEEN VISITS

Regular telephone calls between visits will be made to keep closely in touch with the participants and their families. The first call will be between visit 4 and visit 5 at age 12-

18 months ± 14 days. Further calls will be at age 21 months ± 14 days, 27 months ± 14 days, and 33 months ± 14 days.
During the telephone call, the following will be made:

1) Assessment of **general compliance**
2) Documentation and assessment of **AEs and SAEs**

4.7 INTERVENTION VISIT 8 (END OF TREATMENT)

Study visit 8 will be conducted at age 3.00 years (± 14 days), intake of study medication ends the day before this visit.
The following procedures are scheduled for this visit:

1) Collection of **venous blood** for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A
   b) blood glucose
   c) 25-OH-Vitamin D3
      A single missing vitamin D value will not be considered as protocol violation.
   d) FBE (differential blood count)
   e) As ancillary storage (subject to ethical approval and separate informed consent): Serum, plasma, PBMC
2) **Physical examination** (weight, height)
3) Documentation and assessment of **AEs and SAEs (record and reporting until 60 days after the last treatment with the study drug)**
4) Collection of information on **medication compliance**
5) **Psychological Impact Questionnaire (Appendix 5)** completed by mother and father
6) **Return of study medication** and return of opened/broken study medication.

Single missing lab values or any other single missing test results are not considered as protocol deviation as long as adequate attempts have been taken to get the result as required per protocol. Any missing data due to non-compliance of study participants are also not considered as protocol deviation.

4.8 FOLLOW-UP VISITS 9, 10, 11, 12, 13, 14 AND 15

The minimum number of follow-up visits is 1 visit and 1 telephone call; the maximum number of follow-up visits is 7 visits and 7 telephone calls.
The study visit 9 will be conducted at age 42 months (± 30 days), the study visit 10 will be conducted at age 48 months (± 30 days), visit 11 at age 54 months (± 30 days), visit 12 at age 60 months (± 30 days), visit 13 at age 66 months (± 30 days), visit 14 at age 72 months (± 30 days) and visit 15 at age 78 months (± 30 days). Visit 15 will be the last visit (maximum).
The following procedures are scheduled:

1) Collection of **venous blood** for
   a) Beta-cell autoantibodies, including IAA, GADA, IA-2A, ZnT8RA, ZnT8WA and TS7A

b) blood glucose

c) As ancillary storage (subject to ethical approval and separate informed consent): Serum; at visits 10, 12 and 14additionally storage of plasma and PBMC

2) **Physical examination** (weight, height)

3) If this visit is the End of Study Visit for the child, **Psychological Impact Questionnaire** (Appendix 5) completed by mother and father

4.9 TELEPHONE CALL BETWEEN VISITS

Regular telephone call visits will be made to keep closely in touch with the participants and their families. These will be at age 39 months ± 14 days, 45 months ± 14 days, 51 months ± 14 days, 57 months ± 14 days, 63 months ± 14 days, 69 months ± 14 days, 75 months ± 14 days and 81 months ± 14 days.
During the telephone visit, the following will be made:

- inquiry on well-being of the participant

4.10 FOR PARTICIPANTS WHO DEVELOPED POSITIVE BETA-CELL AUTOANTIBODIES

If the participant develops one or more beta-cell autoantibodies during the study, the beta-cell autoantibody positive status will be confirmed in the same blood sample by a second central autoantibody laboratory. Study sites will be informed about a positive result.
If the sample is confirmed autoantibody positive by both trial central autoantibody laboratories, the participant should have **a confirmation sample drawn** within 4-12 weeks. The collection of venous blood for the confirmation sample can be obtained by a local physician and shipped to the study centre.
Additionally, the participant will have a fasting glucose and OGTT evaluation at the regular scheduled study visits from age 3.00 years or from when the child has a confirmed persistent beta-cell autoantibody status, whichever is last. **OGTT** (0/30/60/90/120)
For the OGTT, the participant needs to be fasting for at least 8 hours. Venous blood samples will be taken immediately prior to the start of intake of 1.75 g/kg glucose solution (time point 0 minutes) at the study centre. The child must drink the entire glucose solution consistently and within 5 minutes.[5] Additional venous blood samples for OGTT are taken 30, 60, 90 and 120 minutes after start of drinking the glucose solution. Blood glucose is measured at the central laboratory on each sample. Capillary blood glucose will be tested immediately at these time points with a glucose meter.

Custodial parent(s) will be informed when a child has developed persistent confirmed beta-cell autoantibodies (early pre-symptomatic stage of T1D). The child will remain in the study and continues to be treated or followed as planned until the child has developed T1D. The

[5] Time point `0 minutes is the time the child starts drinking the glucose solution. If a child needs more than 5 minutes, this must be documented in the participant chart and eCRF accordingly.

parents will be asked to participate in an educational program informing about the diagnosis of beta-cell autoantibody positivity. Contents of the education will be:

- Information what the diagnosis "beta-cell-autoantibodies" means
- How to recognize clinical symptoms of T1D
- How to self-monitor blood glucose in case of diagnosed early pre-symptomatic stage of T1D

4.11 VITAMIN D SUPPLEMENTATION

Vitamin D levels are often low in children who develop T1D. Vitamin D is also considered important for a healthy immune system. Therefore, 25-OH-Vitamin D3 concentrations will be monitored at every visit of the GPPAD-POInT Study. If concentrations are below 75 nmol/l, the family pediatrician will be notified and the family and pediatrician advised to introduce a daily vitamin D supplement or increase the dose of supplementation.

4.12 LABORATORY ASSESSMENT

Safety clinical laboratory assessments will be determined locally at the sites qualified laboratory (as described in chapter 3.13) and include the following parameters:
WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH/HbE, MCHC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils as well as blood glucose.

Beta-cell autoantibodies will be determined centrally at the Institute of Diabetes Research in Munich, Germany. In case of a positive test result, a sample will be shipped from the first GPPAD Central autoantibody laboratory to the second GPPAD Central autoantibody laboratory for confirmation. The second central autoantibody laboratory is Southmead Hospital, Department of Diabetes and Metabolism in Bristol, UK.
25-OH-vitamin D3 concentrations will be determined locally.
Fasting blood glucose and blood glucose after OGTT will be determined centrally in participants with beta-cell autoantibodies.

5. SAFETY AND EFFICACY ASSESSMENT

5.1 MEASUREMENT OF SAFETY PARAMETERS

Local and systemic adverse effects will be elicited by direct questioning of the participant (if already feasible) and/or parent. Systemic effects will be sought by questioning about any untoward symptoms or signs, and graded as mild, moderate, or severe according to level of intervention required.

5.1.1 Hypoglycemia

Blood glucose measured before and 30, 60, and 120 minutes after drug has been administered during study visits at baseline (placebo or 7.5 mg oral insulin), and 2 months (dose increase to placebo or 22.5 mg oral insulin) and 4 months (placebo or dose increase to 67.5 mg oral insulin) and 8 months (placebo or 67.5 mg oral insulin) post baseline. Venous blood will be collected and measured at the local certified laboratory. Additionally, a capillary measurement with a glucose meter will be used for immediate test result only.

Families will also be instructed to report suspected hypoglycaemic events. Families will be instructed to monitor their participating child for symptoms or hypoglycemia after study drug intake. In case of a suspected hypoglycaemic event, with symptoms such as trembling, sweating and impaired consciousness, parents are instructed to immediately give their child something to drink or eat that contains fast absorbable carbohydrates e.g. juice or dextrose. Additionally, parents will be provided with an emergency number and can contact a study physician at any time.

5.1.2 Clinical examination and laboratory tests

A physical examination including measurement of height, weight and oral inspection will be performed. Blood samples will be taken for differential blood count at visit 1 and visit 8.

5.1.3 Signs of allergy to study drug

No allergy to orally administered insulin has been reported. IgE anti insulin levels have been measured in Pre-POINT and in Pre-POINT-Early and no child has developed an IgE response to insulin. Nevertheless, families are asked to report any adverse reaction such as wheezing seen within 2 hours of taking study medication that may be considered as indicative of a hypersensitive response to study drug. Families will report these to the study physician. The study physician will discuss these with the protocol chairs in order to determine whether administration of study drug should be continued or temporarily ceased in such cases.

5.2 MEASUREMENT OF EFFICACY MARKERS

Beta-cell autoantibodies

Venous blood samples obtained at all visits will be tested for antibodies to insulin (IAA), GAD (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8RA; ZnT8WA) and tetraspanin 7 (TS7A). IAA will be measured by competitive immuno-precipitation of ^{125}I-insulin (47), antibodies to IA-2 and ZnT8 by RBA (48), antibodies to GAD by RBA, LIPS, or ELISA, and TS7A by LIPS (49). The clinical study site will be notified if a participant is beta-cell autoantibody positive (see chapter 4.10). The clinical study site will communicate persistent confirmed positive autoantibody results to families by telephone and in written form.

5.3 STORED SAMPLES

As an ancillary component to the study and with the participant's custodial parent(s) consent, serum, plasma, and peripheral blood mononuclear cell (PBMC) samples will be stored at a biobank for further analyses and assays valuable to test drug efficacy for prevention of T1D or investigate T1D pathogenesis. Analyses performed on the samples may include (but will not necessarily be limited to) expression of RNA and its protein products, T cell functional assessments including cytokine expression, and beta-cell autoantibody isotypes.

5.4 FOLLOW-UP OF PARTICIPANTS AFTER END OF STUDY

Participants will be contacted regularly for up to 10 years to determine whether the child has developed diabetes. Children will also be invited to participate in an available follow-up study for the detection of beta-cell autoantibodies. Follow-up will be organized by each clinical study centre by separate consents outside of the GPPAD-POInT Study.

Participants who reach the study end point and develop T1D during the trial will pass over to the regular diabetes care centre and their local physician for management of their diabetes.

6. ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1 DEFINITIONS

Adverse Event Reporting
The investigators are responsible for the conduct of the study in accordance with GCP regulations, which includes the recording and reporting of adverse events observed during and 60 days after the last treatment with the study drug.

Adverse Event
An adverse event is any untoward medical occurrence in a clinical investigational subject, which may or may not be related to the administered pharmaceutical product regardless of the causal relationship with treatment. An event can therefore be an unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the investigational product. Events such as misuse of study medication and medication errors are deemed adverse events and will be recorded as such.

Serious Adverse Event
Each of the following events is defined as a 'serious adverse event' for the purposes of this protocol, to meet or exceed the requirements of the ICH Guideline for Good Clinical Practice:

- Results in death.
- Life-threatening event. A 'life-threatening event' is present when the participant was, in the view of the investigator, at immediate risk of death from the event as it occurred. Note that this definition does not include an event that, had it occurred in a more serious form, might have caused death.
- Events, which develop during the study and require inpatient hospitalization or prolongation of existing hospitalization.
- Events, which are permanently disabling or incapacitating or cause a severe or permanent disruption of one's ability to carry out normal life functions or daily activities.
- Any congenital anomaly.

Note: Hospitalization for elective treatment of a pre-existing condition that did not worsen beyond the natural course of the pre-existing condition during the study is NOT considered a serious adverse event unless a complication occurs during the hospitalization.
Furthermore, hospitalization due to dysglycemia or the onset of T1D is **not considered** as an adverse event or serious adverse event, but the frequency of these events will be monitored by the Medical Monitor and the DSMB.

Assessment of Causality
The relationship, or attribution, of an adverse event to an investigational medicinal product will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate eCRF and if applicable also on the 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).

Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:
☐ _A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
☐ _One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the populations exposed to the drug
☐ _An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Assessment of Severity
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 4.03 (published June 14, 2010).

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.

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

6.2 HYPOGLYCEMIA DEFINITION

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

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

- 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, or
- symptoms commonly associated with severe hypoglycaemia (e.g. loss of consciousness, convulsion, stupor) that are reversed by treatment with intravenous glucose or subcutaneous glucagon.

6.3 EXPEDITED REPORTING

This requirement applies if the adverse event is considered serious, unexpected, and drug related (SUSAR). This type of SAE must be reported by the Sponsor to the appropriate health authorities and Ethics committees within 15 days; fatal or life-threatening events must be reported within 7 days.

6.4 POST-STUDY ADVERSE EVENTS

The investigator should notify the Medical Monitor, Ethics Committee and regulatory authorities of any death or adverse event occurring at any time after a participant has been signed out of a clinical study but no longer than 3 months, when such death or adverse event may reasonably be related to the investigational product. However, the investigator is not obligated to seek adverse events in former study participants.
Investigators should notify the Medical Monitor, Ethics Committee and regulatory authorities if they become aware of a former study participant who has developed cancer.

6.5 REPORTING OF UNDESIRABLE EVENTS AND ADVERSE REACTIONS

6.5.1 Reporting obligation of the investigator (§ 13 (1) – (6) GCP Guideline)

The investigator shall report any serious adverse event (SAE), which occurs in a subject immediately to the sponsor.
When an investigator identifies a SAE, he or she must notify the Sponsor/ Pharmacovigilance and the POInT Protocol Committee within 24 hours of discovering the event.
This will be done via **Fax: +49 700 DRUGSAFETY / +49 700 3784723389 or email: GPPAD@DRUGSAFETY.DE**, to *Dr. Nibler & Partner.*

For documentation purposes it is recommended to the study centre to note date and time of learning of the event. In addition to telephone reporting, the investigator must ensure that these events are entered on the adverse event eCRF and documented on the paper SAE report form (see Appendix 2). The SAE report form must be faxed to the Sponsor/Pharmacovigilance within 24 hours.

Adverse Events (AEs)
Throughout the study the investigator will record all adverse events on the appropriate adverse event Case Report Form (eCRF) 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.
Where the event reported consists of, or results in, the death of a subject, the investigator shall supply the sponsor with any additional information requested by the sponsor. Where the death has been reported to the relevant ethics committee, the investigator shall supply any additional information requested by that committee.

6.5.2 Reporting obligation of the Sponsor (§ 13 (1) – (6) GCP Guideline)

The sponsor shall keep detailed records of all adverse events relating to a clinical trial, which are reported to him by the investigators for that trial. The Competent Authority may require the sponsor to provide those records.
The sponsor shall ensure that all relevant information about a SUSAR, which occurs during the course of a clinical trial and **is fatal or life-threatening** is reported as soon as possible to the Competent Authority in which the trial is being conducted, and the relevant ethics committee.
This needs to be done **not later than seven days** after the sponsor was first aware of the reaction. Any additional relevant information should be sent within eight days of the report.
A sponsor shall ensure that a **SUSAR** which is **not fatal or life-threatening** is reported as soon as possible, and in any event **not later than 15 days** after the sponsor is first aware of the reaction to the competent authorities of any EEA State, in which the trial is being conducted and the relevant ethics committee. SUSARs will be reported to the national competent authority of the Member State concerned directly or indirectly through the EudraVigilance Clinical Trial Module for electronic reporting of SUSARs as required by Directive 2001/20/EC.

Updating source documentation
Documents describing the safety profile of a drug, such as the investigator's brochure, will be amended as needed by the sponsor or delegated responsible person or principal investigator 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.

Annual Safety Report (DSUR)
In addition to the expedited reporting required for SUSAR, the sponsor will submit a safety report to the Competent Authority and Ethics Committee, once a year throughout the clinical trial or upon request. The annual safety report should take into account all new available safety information received during the reporting period. Serious Adverse Events and Adverse Events will be reported with the DSUR on a yearly basis or upon request.

Final Study Report
According to point 5.1.4 of the ICH-GCP, a final study report will be prepared and provided by the sponsor. The report will be prepared within 12 months after completion of the clinical study.

7. PARTICIPANT SAFETY

7.1 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS

7.1.1 Benefits

The potential benefit for a participating child would be the prevention of emerging beta-cell autoantibodies. A benefit would also be a marked delay in the development of beta-cell autoantibodies, dysglycemia, or diabetes. Because all participating children, including children who receive placebo, have a high risk (>10%) of developing beta-cell autoantibodies and diabetes, testing blood samples in the study will allow early recognition of an immune response against the beta-cells, close monitoring and regular blood glucose testing. Children identified as beta-cell autoantibody positive, will be invited to receive education and teaching to learn about the risk of hyperglycemia and means to prevent diabetic ketoacidosis. Participation in ongoing prevention trials aiming to prevent disease progression may be possible (TrialNet studies, or others, separate consents, https://www.diabetestrialnet.org/). If a participating child develops T1D during the study, the disease can be diagnosed very early, i.e. before the child shows the typical symptoms of severe metabolic dysfunction, and an appropriate therapy could be started immediately. Early diagnosis and therapy of T1D reduces complications at onset of diabetes (50, 51) and potentially later in life. Furthermore, information about available treatments and intervention studies that include children with new-onset T1D in order to preserve the remaining beta-cells can be given to families.

7.1.2 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 anaesthetic cream at the puncture site. The volume of blood drawn at each visit that is strictly for the study protocol is $\leq$1% of the total blood volume and within the suggested limits from the European guidelines for a paediatric population. Additional blood volumes are requested for **ancillary** purposes and storage. These require separate informed consent and are subject to local ethical approval. The total blood volume for study protocol and ancillary purposes is less than 3%, which is within the limits of NIH guidelines for a paediatric population. A volume of up to 3% of total blood volume has been collected at 3 month intervals from children in three studies conducted in Munich with no clinically relevant reductions in blood counts or haemoglobin concentrations and no reported adverse events that suggested anaemia. Thus, we consider the proposed blood draw volumes in the study to pose no added risk to the participant safety.
Oral insulin does not lower blood sugar. Unexpected hypoglycemia is theoretically possible, but hasn't been shown in preceding studies (Pre-POINT Study, TrialNet Oral Insulin Study, Pre-POINT-Early, Fr1da-Insulin-Intervention).

There were also no reported allergic reactions or alterations in routine chemistry laboratory values in individuals receiving oral insulin. IgE anti insulin levels have been measured in Pre-POINT and in Pre-POINT-Early and no child has developed an IgE response to insulin. Detailed safety data from the Pre-POINT-Study and Pre-POINT-Early Study and Fr1da-Insulin-Intervention Study are discussed in chapter 1 and are shown in the Investigator's Brochure.
Oral insulin has not been shown to increase the risk of beta-cell autoimmunity or diabetes. The DSMB will monitor the development of beta-cell autoantibodies and diabetes in study participants and can request unblinding of data if there is reasonable concern that the frequency of beta cell autoantibodies or diabetes development in participants exceeds expectations. Parents of participating children will be soundly informed about the likelihood of their child to develop beta-cell autoantibodies, dysglycemia and T1D.

8. PSYCHOLOGICAL IMPACT OF STUDY PARTICIPATION ON FAMILIES

The psychological effect of study participation will be monitored by a questionnaire at visit 3 (appendix 5; 52) and visit 5, visit 8 and at the last visit at the end of participation (appendix 5; 53). The questionnaire is preferably completed by each of both parents or custodial parent(s). A similar questionnaire has been used in the Pre-POINT Study (38) as well as the TEDDY Study (54, 55).
When a parent is identified with high levels of anxiety and/or distress (PHQ-D/A; diabetes-specific items), a structured concept of psychological care will be provided (see also Figure 4). 1) direct (phone) contact and structured assessment of burden and need of support; 2) if the psychological burden is a consequence of study participation psychological measures that were proven in the care of families with newly diagnosed children with type 1 diabetes are provided by psychologists (experienced in the care of children with diabetes and their parents), e. g. personalized information focusing on diabetes specific fears and feelings of guilt, elements of cognitive behavioral therapy (CBT) focusing on negative thoughts, support to diabetes-specific parenting; family discussion; if necessary referral to psychotherapy.

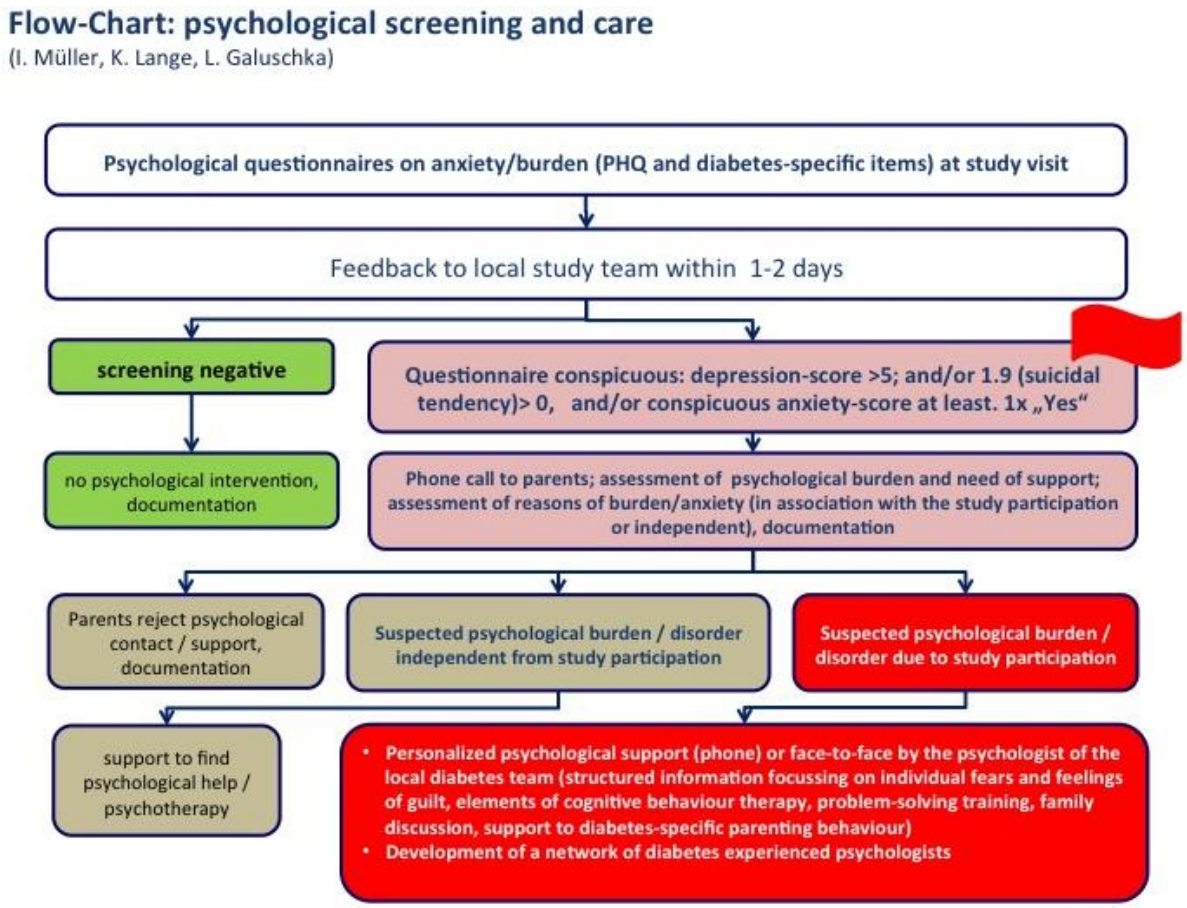


Figure 4: Psychological screening and care

9. PARTICIPANT, STUDY AND SITE DISCONTINUATION

9.1 PARTICIPANT DISCONTINUATION

A participant has the right to voluntarily withdraw from the study at any time. In addition, the investigator has the right to withdraw a participant from the study at any time. Reasons for withdrawal from the study may include but are not limited to the following:

- Withdrawal of consent for participant at any time
- Any medical condition that the investigator or Sponsor determines may jeopardize the participant's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the participant

Every effort should be made to obtain information on participants who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF.

9.2 STUDY AND SITE DISCONTINUATION

The **Sponsor** has the right to terminate this study due to different reasons. Reasons for terminating the study may include but are not limited to the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to participants
- Unsatisfactory participant enrolment
- There is convincing evidence regarding the superiority of the investigational treatment
- The continuation of study is unethical or it has been proven that the therapy has a clearly negative influence;
- Unforeseen complications arise that no longer justify a continuation of the study;
- The results of the over morbidity assessment fulfil the criteria to stop the trial prematurely

The **Sponsor** and POInT Protocol Committee will be advised by the DSMB with respect to study termination for any of these reasons. The DSMB will receive all and complete data for safety parameters, including the islet autoantibodies and diabetes in all participants, along with the expected frequencies for each of these outcomes in the placebo group in each 6 monthly DSMB report. If the DSMB has concerns of over-morbidity, the DSMB will be able to ask for unblinding at any stage of the trial and will advise the POInT Protocol Committee and the **Sponsor** as to whether the study should be continued, modified, suspended, or terminated.

Guidelines for considering suspending and/or stopping the study include:

- A >1.7-fold and significant increase in the frequency multiple beta cell autoantibody (10% expected cumulative frequency at the end of the study) in the participant group that receives the study drug as compared to the participant group receiving placebo.
- A >2-fold and significant increase in the frequency of diabetes (<5% expected cumulative frequency at the end of the study) in the participant group that receives the study drug as compared to the participant group receiving placebo.
- A statistically significant effect is observed.
- The results of the over morbidity assessment fulfil the criteria to stop the trial prematurely.

The **Sponsor** will notify the investigator of a decision to discontinue the study.

The **Sponsor** has the right to **close a site** at any time. Reasons for closing a site may include, but are not limited to the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice
- No further study activity (i.e., all participants have completed and all obligations have been fulfilled)

The **investigator** may discontinue the clinical study at his site if he no longer considers the continuation of the study, for example because of ethical and/or medical concerns.

10. DOCUMENTATION

It is the investigator's responsibility to ensure the conduct of the study in accordance with the GCP Guidelines as well as with the national regulatory requirements and the study protocol and that all data is correctly documented. All collected data must be noted and filed in the corresponding eCRF's by authorized personnel. This also applies to data that refers to subjects that have been excluded from the study.
The investigator keeps a record of all participating subjects on a specific subject identification list. This list contains subject ID, full name, date of birth, date of enrolment into the trial (i.e. randomization date) and serves as a possibility for later identification of the participants. This list will be kept at a secured place in the clinical centre throughout the study and after the study according to local laws and regulations.
Additionally it has to be ensured that each personnel that is responsible for the documentation of eCRF's can be identified. Therefore a staff signature log with signature and acronym of authorized personnel will be filed and kept updated. The log will be filed in the investigator site file (ISF) and trial master file (TMF).

10.1 CASE REPORT FORMS

All study relevant subject data and laboratory results must be documented in corresponding eCRFs.
Corrections on the source documents must be made so that the initial record is still readable (i.e. whiteout is not permitted!). Corrections on eCRFs will be recorded and tracked through an approved audit trail database system. Furthermore, corrections have to be dated and signed. Missing data or data that was not collected has to be indicated as such (i.e. n.a. or n.d.), where appropriate reasons for missing data should be documented.
The investigator must ensure that study source data of participating subjects are documented instantly, readable, complete, and transferred correctly from patient's records on the eCRFs.

Source documents and the respective data entries in the eCRF as specified in the monitoring manual will be reviewed by a monitor for correctness, plausibility and completeness and will be archived for 10 years after completion of the study. CRF data will be entered into the study database.

10.2 INVESTIGATOR SITE FILE

An Investigator Site File (ISF) will be kept at the clinical centre. All essential and required information according to GCP documents will be filed in the ISF.
Upon completion or termination of the study, the ISF must be archived for 10 years. The investigator is responsible for the completeness and archiving of the ISF.

10.3 ARCHIVING OF DATA

10.3.1 Archiving obligations of the sponsor

Following closure of the study, the sponsor must maintain all study related records in a safe and secure location for at least 10 years.

10.3.2 Archiving obligations of the investigator

Following closure of the study, the investigator must maintain all study related records and source documents in a safe and secure location for at least 10 years.

11. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address the primary, secondary and exploratory objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

All efficacy analyses will be conducted under the intention-to-treat principle whereby all outcome data in all randomized subjects who have received at least one dose of study drug or placebo will be included in all analyses as appropriate. Subjects who drop-out of the study will not be replaced. All data acquired prior to termination will be included in the primary analysis unless a participant withdraws consent. Every effort will be made to conduct a final study visit with the participant who drops out of the study and participants will be followed clinically until, if applicable, all adverse events resolve.

11.1 PRIMARY OUTCOME

The primary outcome is the elapsed time from random treatment assignment to the development of persistent confirmed multiple beta-cell autoantibodies or diabetes among those enrolled in the primary analysis cohort consisting of subjects with an elevated genetic risk. For subjects who developed persistent confirmed multiple beta-cell autoantibodies, the elapsed time will be from the random treatment assignment to the confirmed autoantibody positive sample used in defining the persistent confirmed multiple beta-cell autoantibody positive status. It is expected that beta-cell autoantibodies will be detected prior to diabetes onset; however, the presence of diabetes in the absence of beta-cell autoantibodies is also considered as a primary outcome endpoint, and in this case situation, the date of diagnosis is the time of the end point.
The study end point is realized with either persistent confirmed multiple beta-cell autoantibodies or OGTT criteria for diabetes or clinical criteria for diabetes.
Although children who develop persistent confirmed multiple beta-cell autoantibodies will have reached the primary study endpoint, these children will continue to receive assigned treatment and will be followed in the study for continued monitoring of glucose tolerance and diabetes development and safety assessments.

Criteria for persistent confirmed beta-cell autoantibodies:

Criteria are based on the measurement of beta-cell autoantibodies against insulin (IAA), GAD65 (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8A) tested in the GPPAD central autoantibody laboratory and, if positive, confirmed in the GPPAD confirmatory laboratory. Children who are IAA positive in both laboratories will be further tested by methods that provide an assessment of affinity of insulin autoantibodies. Autoantibodies to tetraspanin 7 (TS7A) will be measured, but will not be considered in the primary outcome.

- Confirmed IAA is defined as sample positive for IAA in both the GPPAD central and confirmatory laboratories. Positivity for IAA in two laboratories is a feature of high affinity insulin autoantibodies. High affinity insulin autoantibodies are not expected to be induced by the study drug.
- Confirmed GADA is defined as sample positive for GADA in both the GPPAD central and confirmatory laboratories.
- Confirmed IA-2A is defined as sample positive for IA-2A in both the GPPAD central and confirmatory laboratories.
- Confirmed ZnT8A is defined as sample positive for ZnT8RA or ZnT8WA in both the GPPAD central and confirmatory laboratories.

Persistent confirmed multiple beta-cell autoantibodies (primary outcome) is defined as confirmed IAA, confirmed GADA, confirmed IA-2A, or confirmed ZnT8A in two consecutive samples, AND a confirmed second antibody from these four antibodies in one sample. Persistent confirmed beta-cell autoantibodies that are considered maternally derived GADA or IA-2A are NOT included as positive for the primary outcome. Maternally derived autoantibodies are defined as follows:
In children who are positive for any of the four beta-cell autoantibodies in the first sample taken and where there is no negative sample prior to this sample, the likelihood they have maternally derived autoantibodies will be considered. The status of the autoantibodies will be classified as maternally derived beta cell autoantibodies if they become negative in a subsequent sample taken before age 3 years. Maternally derived beta cell autoantibodies are not a primary outcome endpoint and are not considered as a positive outcome in the statistical analysis.
If children are positive for beta-cell autoantibodies at their first sample and therefore potentially have maternally derived beta cell autoantibodies, they are still eligible for randomization and treatment. The elapsed time from randomization to primary outcome in children with maternally derived beta-cell autoantibodies will be determined as:
For children who become beta-cell autoantibody negative before age 3 years, the primary outcome is defined as the first confirmed beta-cell autoantibody positive sample after the negative sample.
Children who are positive for a beta-cell autoantibody from the start of sample collection and remain positive for the same autoantibody until age 3 years will be classified as beta-cell autoantibody positive and outcome positive from baseline.
Children who are positive for two or more beta-cell autoantibodies from the start of sample collection and remain positive for only one of these autoantibodies until age 3 years will be classified as multiple beta-cell autoantibody positive from when they subsequently develop multiple beta cell autoantibodies.
Children who are positive for multiple beta-cell autoantibodies from the start of sample collection and remain positive for the same autoantibodies until age 3 years will be classified as multiple beta-cell autoantibody positive from baseline. These are expected to

be rare cases*.
*Maternal autoantibodies are expected in a minority of infants at baseline and expected to be randomly distributed between treatment groups. The presence of masking maternal autoantibodies decreases rapidly over the first year of life and is only present at 12 months in a minority of those who have maternally transferred autoantibodies at birth. Thus, the majority of these antibodies will be lost by the time the child starts the highest dose of study drug (between age 8 and 11 months). We assume that at most, 10% of randomized infants will have a mother with type 1 diabetes, we expect that around 50% of these will have antibodies at baseline and that less than 1% of the other recruited infants will have maternally derived antibodies at baseline (age 4 to 7 months). The majority (>50%) of these have only one islet autoantibody and not confound the definition of the primary outcome of the study. Thus, 3% of children in each treatment group may have maternally transferred multiple beta-cell autoantibodies that may mask a primary outcome. By age 3 years, the expected frequency of the primary outcome is around 5% in the placebo group. Thus, at most we may expect a maximum of 5% of 3% (ie 0.15%) with maternally transferred multiple beta-cell autoantibodies at baseline who could reach 3 years without losing their maternally derived multiple islet autoantibody state. Even this is highly inflated expectation, however, since we also expect that the majority of the children with maternally derived multiple islet autoantibody positive children to have lost at least one of their antibodies prior to the development of multiple islet autoantibody development. Hence, we expect maternally transferred islet autoantibodies to have no significant effect on the primary outcome of the study. A larger potential bias may affect some of the secondary analysis such as single islet autoantibodies and the potential effect of the bias will be considered. Evidence for bias will be assessed by considering the frequency of islet autoantibody positivity assigned to baseline samples in insulin and placebo-treated groups. If evidence of bias is found, then an analysis will also be performed in children who are negative for islet autoantibodies (including maternally acquired autoantibodies) at baseline.

Criteria for T1D onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis).
One of the following criteria must be met **on two occasions** as soon as possible but no less than 1 day apart for diabetes to be defined:

1. Symptoms of diabetes and a casual plasma glucose ≥200 mg/dL (11.1mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
   OR
2. Fasting plasma glucose (FPG) ≥126 mg/dL (7 mmol/L), fasting is defined as no caloric intake for at least 8 hours
   OR
3. Two-hour plasma glucose (PG) ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75g anhydrous glucose dissolved in water.

It is preferred that at least one of the two testing occasions involve an OGTT. Cases identified will be confirmed as having diabetes if the glucose values to make these

determinations were obtained in a GPPAD central autoantibody laboratory as part of an OGTT. Cases diagnosed with diabetes by symptoms and casual glucose ≥ 200mg/dL or by other criteria than the above will be adjudicated by the GPPAD Endpoint Adjudication Committee. Trial treatment will be terminated if T1D is reached.

Primary Analysis
The study objective is to determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1 diabetes reduces the cumulative incidence of multiple beta-cell autoantibodies and diabetes in childhood.

The cumulative incidence of beta-cell autoantibodies over time since randomization within each treatment group will be estimated from a Kaplan-Meier estimate of the "beta-cell autoantibody-free" survival function. The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.05 level, two-sided, using the Cox regression including site as covariate. The estimates of cumulative incidence and the test will adjust for periodic outcome assessment visits to assess beta-cell autoantibody status. The critical value for the test statistic, and confidence intervals in this primary analysis will be determined by the group-sequential procedure.

In case the assumptions of the sample size estimation in section 11.3 hold, it will be possible to reject the null hypothesis of equal hazard rates with the power of 80%, if 832 children will be uniformly randomized over 3.5 years and afterwards, all 832 patients will be followed up for another 3.5 years. We have assumed a drop-out rate of 20%, and therefore we need to randomize 1040 children to support an 80% power by a complete follow-up of 832 children ranging from 3.5 to 7 years. All randomized children will be included in the analysis. Those children meeting the inclusion criteria and randomized who drop out of the study before their last study visit will also be considered for the analyses of the primary endpoint. Their observation time until the point of drop-out will add to the Wald statistic of the Cox model and increase the power of the study.

Update after accrual completion:
Enrolment lasted 3.17 years, the follow-up period will be 3.25 years. The total trial duration will be ~6.5 years. As outlined in section 11.3, the study will have >80% power to reject the null hypothesis of equal hazard rates despite the shorter median observation time.

11.2 SECONDARY OUTCOMES AND ANALYSES

In addition to the primary outcome of multiple islet autoantibodies, four secondary outcomes will be included for analysis.

1. Any persistent confirmed beta-cell autoantibody, defined as at least one confirmed autoantibody, in two consecutive samples, including GADA, IA-2A, IAA, ZnT8A, or TS7A, or diabetes.
2. Persistent confirmed IAA.
3. Persistent confirmed GADA
4. Abnormal glucose tolerance (AGT=dysglycemia) or diabetes.

Dysglycemia is defined as:

• impaired fasting plasma glucose of ≥110 mg/dL (6.1 mmol/L), or
• impaired 2-hour glucose of ≥140 mg/dL (7.8 mmol/L), or
• high glucose levels at intermediate time points on OGTT (30, 60, 90 min levels of ≥200mg/dL (11.1 mmol/L))

Diabetes is defined as described in chapter 11.1.

The treatment arms will be compared on the corresponding incidence rates of each secondary outcome using the log rank statistic.

A variety of secondary analyses are planned after completion of the trial. These include the following.

Subgroup analyses will be conducted comparing the effects of oral insulin versus placebo on the risk of multiple beta-cell autoantibodies with a test of the group by subgroup factor interaction in a Cox proportional hazard (PH) Model. Subgroups of the population classified by sex, race/ethnicity (if appropriate), first degree relative status, beta-cell autoantibody status at baseline, maternally transferred beta cell autoantibody status at baseline, genetic risk score tertiles, and INS genotype. Differences in the treatment effect between subgroups will be tested using a covariate by treatment group effect in a Cox PH model.

Similar analyses will be conducted using the values of quantitative baseline factors including weight (z score), height (z score), BMI (z score), and genetic risk score. The dependence of the treatment effect on the quantitative levels of a covariate will also be assessed by a covariate of treatment group interaction in a PH model.

Analysis will also be performed considering outcomes that occur during the treatment period vs the non-treatment period. Incidence rates will be determined for each of these periods within the oral insulin and placebo groups and compared in a PH model.

Additional factors may be defined before unmasking of the study data to the investigators. The analyses will distinguish between factors specified prior to unmasking, and those identified post-hoc during analysis. If the assumption of proportional hazard is not appropriate, the data will be examined to determine the cause of non-proportional hazard, such as the presence of a decaying, diverging, or crossing effect of hazard ratios over time. Based on the cause of the non-proportional hazard, post-hoc analyses such as frailty models, parametric models, or models with interactions and time-dependent covariates may be employed.

Longitudinal analyses will assess the effects of oral insulin versus placebo treatment on immunologic and metabolic markers over time up to the onset of beta-cell autoantibodies. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors if the assumption of multivariate normal random errors is violated.

Immunologic and metabolic markers will be modelled to determine the effects of oral insulin versus placebo treatment while adjusting for subject characteristics for each follow-up time point of interest. For continuous endpoints that lend themselves to normal error linear models, ANOVA and ANCOVA models will be employed. Generalized linear modelling will be employed for dichotomous and categorical endpoints by using the most appropriate link functions. Longitudinal analyses maybe employed in order to characterize the relationship among the repeated measures during the treatment period and possibly beyond. Due to the exploratory nature of the longitudinal modelling, treatment effect hypothesis testing will not conducted.

The association of demographic, genetic, immunologic, metabolic, and other factors, both at baseline and over time, with the risk of beta-cell autoantibodies onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on beta-cell autoantibodies risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the oral insulin and placebo groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Models will then be assessed within the two groups combined, taking account of any group by covariate interactions.

Subgroup analyses analogous to those described for the beta-cell autoantibodies endpoint will be conducted on the secondary outcome endpoints.

11.3 STUDY POWER AND ACCRUAL TARGET

The study has been designed to provide 80% power to detect a 50% risk reduction in the rate of beta-cell autoantibodies using a two-sided test at the 0.05 level after 7.0 years of study duration. A total of approximately 1040 infants will be allocated in a 1:1 ratio to the two groups.

For the sample size estimation, the following scenario was chosen:
Overall alpha level = 0.05 (two-sided).
Overall beta level = 0.2, i.e. power = 0.8.
In the placebo group, at 3.5 years (approximate age of participants, 4 years), an event probability of 7.5% was assumed. Based on the exponential distribution, this leads to a hazard of 0.02227.
It is expected that the hazard is halved by the treatment.
Accrual time is 3.5 years.
Follow-up time is 3.5 years.
A dropout rate of 20% was expected.

Adjustment of power-calculation and trial duration (October 2021) according to observed drop-out rate, the accrual period, and event rate:

1) Drop-out rate:

The median observation time in 10/21 was 19.9 months. Drop-out rate at this time was 4.05%. Extrapolating this to the expected final median observation time of 4 years and 10 months results in an expected drop-out rate of 12% (95% confidence interval, 8 – 15%). **With 12%, the currently extrapolated drop-out rate at the final median observation time 4 years and 10 months is smaller than the assumed total drop-**

out rate of 20%.

2) Hazard assumptions:
In our original sample size estimation, a hazard rate of 0.02227 for the placebo group, and 0.011135 for the verum group were expected, providing an overall hazard rate of 0.0167. Estimating the hazard rate for the total group of 1050 patients, the hazard rate is 0.02456 95% confidence interval, 0.01822 - 0.03312).**This means a potentially higher power to detect a significant difference between placebo and verum.**

3) Enrollment:
Recruitment was completed in 3.17 years instead of 3.5 years. With follow-up planned for 6 months after the third birthday of the three youngest children randomized most recently, a follow-up until around June 2024 would be needed. Thus, duration of the trial after end of recruitment in March 2021 would be 3.25 years. This would result in a median observation time of 4.835 years (instead of 5.25) or 4 years and 10 months instead of 5 years and 3 months.
As a consequence, the median observation time would be 5 months less than originally planned.
The implication of the 5 month reduction of the median observation time on study power to reject the null hypothesis if the original 20% drop-out rate and hazard rates of 0.011135 and 0.02227 were true would be a reduction to 77.6% according to PROC POWER of SAS9.4 based on Lakatos formula (Lakatos, Biometrics , 1988, Vol. 44, No. 1, pp. 229-24). However, using the current total hazard of 0.02456 for the placebo group and assuming half this hazard (i.e. 0.01228) for the verum group while keeping all other parameters fixed, the power would be 81.3%.

Therefore, based on the currently available data, there is no power issue preventing the end of the trial 6 months after the last date of treatment as originally planned in the study protocol and is now expected to be in June 2024.

12. DATAMANAGEMENT, MONITORING AND AUDIT

12.1 QUALITY ASSURANCE

The sponsor will implement study specific quality risk management processes in order to manage quality during the planning and implementation phase.

For quality assurance purposes monitoring will be conducted throughout the clinical study (RBQM).
The Investigator(s)/institution(s) will permit trial related monitoring, audits, Ethics Committee review and regulatory inspection, providing direct access to source data/documents.
A representative of the sponsor will visit the investigator periodically to monitor the progress of the study in accordance with national and ICH-GCP guidelines. A monitoring manual describing the scope of the monitoring activities in detail will be prepared. All data pertaining to a subject's participation in this study must be made available to the monitor during these visits. The investigator agrees to cooperate with the monitor to ensure that

any problems detected in the course of these monitoring visits are resolved.
A monitoring visit report is prepared for each visit describing the progress of the clinical trial and all identified problems.

Designated personnel may perform an audit at any time during or after completion of the study. All study-related documentation must be made available to the designated auditor. A regulatory authority may also audit the study. Access to case report forms, source documents and study files must be made available for monitoring and audit purposes, at reasonable times, during the course of the study and after completion.

12.2 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 eCRFs.
Safety data will be recorded on eCRFs specifically designed for this purpose. All the SAEs will be reported on a paper SAE report form. The data forms will be checked by the clinical study centre and families called if necessary (incomplete or incorrectly completed forms). The clinical study centre will report the data from the forms into the GPPAD-POInT Study database system. The clinical study centre will store all data forms electronically or as paper versions, depending on national requirements.

12.3 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 in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the clinical site must permit authorized representatives of the sponsor and health authorities to examine (and where required by applicable law) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. 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.

13. REPORTS

13.1 STUDY REPORT

Confidentiality and assurance of security of confidential documents such as the protocol, eCRF, Investigator's Brochure, final study reports, manuscript drafts, unpublished data, correspondence, etc. will be maintained throughout the study.
The statistical report and compilation of the final study report will be provided by the PI in cooperation with the data management and will be evaluated and signed by the PI, co-Investigator(s) and other responsible persons. All information and data of the report will be kept confidential.

13.2 PUBLICATIONS

Any publications resulting from the GPPAD-POINT Study (including meeting abstracts) will be agreed between the principal investigators and co-authors prior to submission. Patient names or other identifiers will not be disclosed. The major publication will provide registration details to www.clinicaltrials.gov.

14. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GCP

14.1 RESPONSIBILITIES OF SPONSOR AND INVESTIGATORS

The sponsor of the clinical study (*Technische Universtät München represented by the Fakultät für Medizin*) has the responsibility for commissioning, organisation and financing of the clinical study being undertaken (ICH-GCP 1.5.3). Sponsor and investigator must ensure that the conduct of the clinical study is in accordance with current laws and regulations, corresponding to ICH-GCP Guidelines, Declaration of Helsinki and the national Pharmaceutical Act and GCP regulation (2004).
The investigator accepts the requirements of the signed study protocol.

The investigators responsibilities are inter alia:

- Comprehension of the IMP's characteristics as outlined in the Investigator's Brochure,
- Comprehension for the implementation of the intervention plan,
- Securing that there is sufficient amount of time and capacity for the conduct of the study,
- Correct collection and documentation of the data, reporting,
- Supply all data for the sponsor, monitor and relevant regulatory authorities for audits and/or inspections,
- Securing that information of participants as well as all information from the sponsor of all persons involved in the trial are treated strictly confidentially
- Where applicable, statement for the involvement of persons dependent on sponsor or investigator
- Statement of conflicts of interest of investigator(s) in relation to the IMP

According to ICH-GCP 4.1.1 the investigator has the responsibility for the correct conduct of the clinical study at the clinical centre.

14.2 ETHICAL APPROVAL AND REPORTING TO REGULATORY AUTHORITIES

Prior to study initiation, the protocol will be reviewed and approved by an appropriate Ethics Committee and regulatory authority. Also, the informed consent documents will be approved by the leading Ethics Committee. Any amendments to the protocol or consent materials must also be approved by the study DSMB and where appropriate, the relevant competent authorities and ethics committees before they are implemented. The participating GPPAD clinical sites will obtain approval from their corresponding ethics committee in accordance with their local procedures and institutional requirements.
Application for regulatory approval will be submitted by the sponsor or sponsor delegate in accordance with § 7 GCP-Guideline.

14.3 PARTICIPATING CENTRES

Participating GPPAD clinical sites must have sufficiently qualified staff and time, as well as visit room- and laboratory space (sample processing) and equipment in order to conduct the trial.
The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

14.4 INFORMED CONSENT

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. The custodial parent(s) must read, sign, and date a consent form before entering the study, taking the study drug, or undergoing any study-specific procedures. As statement of the information process and assurance that they have understood all obligations and trial procedures, date and signature of the study investigator will also be obtained on the consent form.

Consenting requirements currently vary between countries that participate in the POInT study. It is necessary to also adhere to country specific requirements. In particular, for participants who are consented in Sweden or in Germany, signed consent must be obtained from both parents or custodial parent(s) of the child prior to randomization.

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 handed out. The custodial parent(s) of the prospective participant will be told that being in the trial is voluntary and that the participant may withdraw from the study at any time, for any reason.
Once the participant infant is able to understand that he/she is participating in a clinical trial it has to be informed in an appropriate way and assent should be asked for.

14.5 CLINICAL TRIAL INSURANCE

On behalf of the sponsor, obligatory clinical trial insurance according to ICH-GCP point 5.8. is being set up for all study participants (before the first participant is recruited) with the following insurance company:

HDI Global SE

Germany
HDI-Gerling Industrie Versicherung AG
Ganghoferstrasse 37-39
80339 München, Germany
Phone: 089 92431 - 87000

UK and Sweden

HDI Global SE
10 Fenchurch Street
London EC3M 3BE, United Kingdom
Phone: +44 20 7696 8099

Poland

TUIR WARTA S.A.
ul. Chmielna 85/87
00-805 Warszawa, Poland
Phone: +48 22 581 01 00

Belgium

HDI Global SE
Avenue de Tervuren 273 bte 1
1150 Bruxelles, Belgium
Phone: +32 2 773 08 11

Study participants will be insured for any adverse event occurring as a result of study participation covering max. 500.000 Euro per participant. The insurance covers all direct or indirect damages that participants have experienced in the course of intervention with the study drug or by any study related test and examination procedures.

14.6 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 and not names will be used to collect, store, and report participant information.

The nature of the study requires retaining identifying data; however, confidentiality of study subjects and subject materials will be provided by filing and storage at the clinical centres. Information stored on computers will be accessible through passwords available only to authorized study personnel and -investigators. Hard copy data will be stored in locked filing cabinets kept in locked rooms. All data processing will comply with the European regulations in their current version.

The trial may be audited by designated qualified auditors who are independent of the GPPAD-POINT Study clinical trial/data collection systems.

15. STUDY ADMINISTRATION

15.1 ORGANIZATIONAL STRUCTURE

GPPAD is a network of collaborating investigators from European countries. The network was created to allow for a coordinated, multi-disciplinary approach to prevent T1D by early intervention.
The following organizational structures will be relevant for POInT:

15.1.1 GPPAD Coordination Centre

The GPPAD Coordination Centre (GPPAD CC) is part of the GPPAD platform and provides support for any trial conducted under GPPAD. The GPPAD CC will provide communication and coordination among the POInT clinical study centres, and manage the collection, analysis and storage of clinical data. The GPPAD CC will work together with external partners in order to establish and maintain the data acquisition, transfer, and management system; provide procedures for ensuring subject confidentiality and safety; provide procedures for quality control, and supervise the orderly collection and transmission of data. The GPPAD CC will overview monitoring activities, CRO activities, the pharmacy, and the central laboratories.

The GPPAD CC puts in place a work system for a well-functioning trial conduct and logistic. It will meet on a regular basis to discuss current status of the trial, protocol compliance issues, and current topics in the trial conduct. The GPPAD CC will work closely with the protocol committee and provide status updates to the Sponsor, POInT Protocol Committee, DSMB, POInT Medical Monitor, and the competent authorities.
The GPPAD CC will organize telephone conferences and meetings between the POInT Clinical site PIs, the POInT Protocol Committee, the POInT Medical Monitor, The POInT DSMB and between the local POInT clinical study centres as required.

15.1.2 GPPAD-POInT Protocol Committee

The GPPAD POInT Protocol Committee (GPPAD PC) consists of the protocol authors Prof. Dr. Anette-G. Ziegler (chair), Prof. Dr. Ezio Bonifacio and Associate Prof. Dr. Helena Elding Larsson.
Significant changes that occur to this protocol during the course of the trial require the formal approval of the Protocol Committee.
The GPPAD PC committee members will receive periodic reports from the GPPAD CC; these will include accrual rates and baseline demographic characteristics, and AE and SAE reports and protocol compliance.

If required, further functions of the committee may include e.g.:

- the review of protocol deviations on a regular basis
- address and work out protocol amendments as they become necessary
- review of inclusion and exclusion criteria upon request by clinical site investigator or study physician
- assessment of new clinical study centres on cooperation with GPPAD CC

The committee will make decision on endpoint on the basis of protocol definitions. Consultation of the committee's opinion will also be available on demand in case of ambiguous cases.

15.1.3 POInT Medical Monitor

The medical monitor will be selected by the GPPAD CC in agreement with the protocol committee. The functions of the medical monitor are

- periodic review all adverse event reports, masked to treatment assignment
- periodic review and second assessment of SAEs

- provide updates on the assessment of the overall safety of the trial where they become necessary
- periodic review of multiple beta cell autoantibody and diabetes outcome reports, masked to treatment assignment
- periodic review of laboratory parameters during intervention and follow-up of participants throughout the study
- periodic review of protocol and GCP compliance

The GPPAD CC will provide periodic reports to the Medical Monitor.

15.1.4 POInT DSMB

The Data and Safety Monitoring Board (DSMB) is an independent group of experts that advises the sponsor. The major responsibility of the DSMB is to safeguard the well-being and safety of the trial participants and to provide pertinent advice to the sponsor and the POInT Protocol Committee. The DSMB will review each substantial protocol amendment for any major concern prior to implementation.

Functions and responsibilities:

a. Monitors safety parameters during the study, e.g. the DSMB will receive each case of a SUSAR after receipt within 96 hours for assessment
b. Monitors the proper conduct of the trial, e.g. recruitment rate, rate of drop outs and lost to follow ups, compliance with the trial protocol;
c. Reviews the 6-monthly DSMB reports and advises pertinent recommendations.
d. Safeguards confidentiality of and interests of individuals included in the study

Data as defined in the DSMB Charta will be presented to and reviewed by the DSMB. The DSMB will meet six-monthly during the conduct of the study. Conference calls are permitted.

At six-monthly intervals, the DSMB will receive a report with all relevant data for safety and efficacy, including the islet autoantibodies and diabetes data for each participant, along with the expected frequencies for each of these outcomes in the placebo group. The DSMB will be asked to examine this data and comment on this data with respect to safety, and at later stages also for efficacy. If the DSMB has concerns of over-morbidity, the DSMB will be able to ask for unblinding at any stage of the trial.

The DSMB will conclude each review with their recommendations to the sponsor and the POInT Protocol Committee as to whether the study should continue without change, be modified, suspended, or terminated.

15.2 THE PARTICIPATING POINT CLINICAL STUDY CENTRES

The participating clinical study centres are responsible to obtain ethical approval and regulatory approval with support of the GPPAD CC and protocol committee. Any amendments to the protocol or consent materials must also be approved before they are implemented. The participating clinical study centres are responsible for coordinating the collection and validation of clinical data. They will adhere to SOPs/MOO to support the study protocol.

15.3 THE GPPAD-POINT STUDY GROUP

This includes all site PIs and sub-investigators, as well as study nurses and further involved study personnel that contribute to the conduct of POInT.

15.4 ROLE OF INDUSTRY

The Insulin Crystals used for manufacture of the IMP will be provided by Eli Lilly and Company.

15.5 FINANCING OF RESEARCH STUDY

The GPPAD-POInT Study is financed by research grants from the Leona M. and Harry B. Helmsley Charitable Trust. There will be no cost to the participating families.

16. AMENDMENTS TO THE PROTOCOL

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. Any substantial amendments to the protocol or to the consent materials must also be approved before they are implemented.

In exceptional cases where an amendment becomes necessary the reason must be outlined in writing and must be signed by all responsible parties. All substantial amendments will be reviewed by the DSMB prior to getting into force. Amendments will only be implemented in agreement with the Protocol Committee. If an amendment is considered substantial (according to GCP-Guidance §10) the amended protocol and other concerned study documents have to be approved by the respective ethics committee and regulatory authority.

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18. APPENDIX

18.1 APPENDIX 1: SYNOPSIS OF FINAL STUDY REPORT (PRE-POINT)

Name of Sponsor/Company: Technische Universität Dresden	**Individual Study Table Referring to Part of the Dossier** Not applicable	*(For National Authority Use only)*
Name of Finished Product: Oral Insulin capsules at doses 2.5mg, 7.5mg, 22.5 mg and 67.5mg	**Volume:** Not applicable	
Name of Active Ingredient **Human recombinant insulin**	**Page:** Not applicable	

Title of Study:

Pre-POINT (*P*rimary *O*ral *IN*sulin *T*rial) - 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

Investigators:

Bonifacio, Ezio, PhD, Ziegler, Anette-G., MD, Klingensmith, Georgeanna, MD, Bingley, Polly, MD, Schober, Edith, MD, Hasford, Joerg, MD, Achenbach, Peter, MD, Roth, Roswith, PhD,

Study centre(s):

Germany/Munich:	Forschergruppe Diabetes, Technische Universität München
USA/Denver:	Barbara Davis Center for Childhood Diabetes, University of Colorado
Austria/Vienna:	Universitätsklinik für Kinder und Jugendheilkunde
UK/ Bristol:	Diabetes and Metabolism, University of Bristol, Southmead Hospital

Publication (reference):

Achenbach P, Barker J, Bonifacio E. Modulating the natural history of type 1 diabetes in children at high genetic risk by mucosal insulin immunization. Curr Diab Rep 2008; 8:87–93.

Studied period (years):	4	**Phase of development:**
date of first enrolment:	23.10.2009	Phase II Pilot-Study
date of last completed:	15.09.2013	

Objectives:

To determine the feasibility, safety and bioavailability of oral insulin in children with high genetic risk for type 1 diabetes (T1D) in a dose escalation primary intervention pilot study.

Methodology:

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 daily

Children were treated for the duration of 2.5 months to 20 months. Study visits were scheduled for each dose at baseline, day 15, at 3 months, and 6 months and semi-annually thereafter. Follow-up until the 3 month visit was sufficient for study completion.

Safety assessment included monitoring of hypoglycemia through capillary blood glucose measurement at 30, 60 and 120 minutes after drug had been administered on the first day of treatment at each dose; and home capillary blood glucose measurements 60 minutes after drug administration.

Laboratory tests included blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start and end of treatment for each dose. Allergy to the study drug was monitored by total IgE and IgE antibodies to insulin, and family self-reporting. Diabetes, GAD and IA-2 autoantibodies were measured to assess possible increase in diabetes risk.

Bioavailability was measured by antibody and T cell response to study drug.

Psychosocial effects of study participation were assessed through questionnaires completed by families prior to and after 3 and 9 months participation in the study.

Number of subjects (planned and analysed):

Twenty-five children were enrolled into the study. Subjects were randomized in a 3:2 ratio to either insulin or placebo. Children were allowed to increase the dose of the study drug once during the dose-finding Pre-POINT study. In this way, each dose was given to 6 children, and 10 children received placebo. All 25 children were analysed.

Diagnosis and main criteria for inclusion:

1. Children aged 2 years to 7 years who:

A. have a multiplex first degree family history of T1D (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 T1D;

and are identical by descent for the HLA DR3/DR4-DQ8 genotype with their diabetic sibling;

2. Islet autoantibody negative at time of recruitment.

Test product, dose and mode of administration, batch number:

Daily intake of insulin capsules administered orally:

2.5 mg: 80804002/032010-25; 80804002/252010-25; 80804002/042011-25

7.5 mg: 80804002/402010-75; 80804002/072011-75; 80804002/412011-75; 100715-1; 100815-1

22.5 mg: 80804002/042011-225; 80804002/272011-225; 80804002/062012-225; 80804002/422012-225; 80804002/032013-225; 101905-8

67.5 mg: 80804002/062012-675; 80804002/282012-675; 80804002/422012-675; 80804002/052013-675; 100815-2; 100815-3

Duration of treatment:

The treatment duration per subject per dose varied between 2.5 months and 12 months.

Cumulative treatment duration per dose is 36 months for 2.5 mg, 54 months for 7.5 mg, 50 months for 22.5 mg and 48 for 67.5 mg insulin capsules.

Reference therapy, dose and mode of administration, batch number:

Daily intake of placebo capsules administered orally:

Placebo: 80804002/032010-25; 80804002/252010-25; 80804002/042011-25; 80804002/402010-75; 80804002/072011-75; 80804002/382012-75; 80804002/422012-75; 80804002/272011-225; 80804002/062012-225; 80804002/052013-675; 090819-37; 090724-14; 100613-1; 100615-2; 090819-90; 100615-3; 101908-3; 101908-4; 102013-2; 090819-37

Criteria for evaluation:

Safety: 1. Potential risk of hypoglycaemia was assessed by blood glucose after administration. Bood glucose values below 50 mg/dl were considered an adverse event and reportable. 2. General effects were assessed by blood count; differential blood count; blood glucose, electrolytes, liver enzymes, protein, albumin, urea, and creatinine at start and end of treatment for each dose. 3. Allergy was assessed by family reports, total IgE concentrations and IgE against insulin. 4. A potential increase in diabetes risk caused by study drug was assessed by measuring autoantibodies to GAD65 and to IA-2.

Efficacy: Immune bioavailability of the study drug was assessed by islet autoantibody titer, IgG- and IgA-binding to insulin in serum and saliva, and by CD4+ T cell responses to insulin.

Statistical methods:

The number of hypoglycaemic events at each dose were compared by Kruskall-Wallis test, and the blood glucose concentrations after taking study drug at each dose were compared to placebo using ANOVA.

IgE concentrations were compared to baseline at each study dose using students t test.

The number of adverse events (total and separately for event type) in each dose and for all doses were compared to the placebo group using Fischer's exact test.

The number of children with antibody and/or T cells responses to study drug at each dose were compared to placebo using Fischer's exact test and Chi squared test for trend.

SUMMARY – CONCLUSIONS

EFFICACY RESULTS:

Increases in IgG-binding to insulin, salivary IgA-binding to insulin, and/or $CD4^+$ T cell proliferative responses to insulin were observed in two of ten placebo-treated children, one treated with 2.5 mg insulin, one 7.5 mg-treated, two 22.5 mg-treated, and five of six 67.5 mg-treated children (p=0.017). FOXP3/IFNg signature ratios of insulin-responsive cells increased (p=0.02) in children who received oral insulin treatment.

SAFETY RESULTS:

None of the children who received oral insulin or placebo experienced hypoglycaemia after administration of medication. There were no increases in IgE concentration or IgE responses to insulin. Adverse events were similar between insulin and placebo groups. No child developed autoantibodies to glutamic acid decarboxylase or IA-2 or diabetes.

CONCLUSION:

This pilot study indicates that daily oral administration of insulin to islet autoantibody negative children who are genetically at risk for T1D appears safe, and at a daily 67.5 mg dose can actively engage the immune system with features of immune regulation.

Date of the report: 15. September 2014

18.2 APPENDIX 2: SAE REPORT FORM

Do not send source documents unless requested by Dr. Nibler & Partner
Summarise all pertinent information from the records on this form in BLOCK CAPITALS

Serious Adverse Event Report Form

DR. NIBLER & PARTNER

1. Study Information

Short Title:	GPPAD-POInT
Clinical Trial Code:	GPPAD-03-POInT
EudraCT:	2017-003088-36
Indication:	Type 1 diabetes

2. Subject Information

Patient ID: ___ - ___ - ___ ___
Country Site Subj Case No.

Age: ___ months

Sex: M ☐ F ☐ Weight (kg): ___ , ___ Height (cm): ___

3. Report Information

Date of Awareness: ___

Date of Report: ___ (dd/mm/yyyy) Patient was unblinded: Yes ☐ No ☐

Type of Report: Initial ☐ Follow-Up ☐ Follow-Up number ☐

4. Investigator Information

Name of Investigator:	
Name of Institution:	
Country:	
E-Mail-Address:	
Phone-No.:	
Fax-No.:	

PLEASE FAX FORM WITHIN 24 HOURS TO : +49 700 DRUGSAFETY / +49 700 3784723389

OR E-MAIL: GPPAD@DRUGSAFETY.DE

Dr. Nibler & Partner SAE Form Version 1.0, March 2019

DR. NIBLER & PARTNER **Serious Adverse Event Report Form**
Study: GPPAD-POInT

Patient ID: Country ID | Site ID | Subj ID | Case No.

5. Information on Serious Adverse Event(s)

Serious Adverse Event	Mark most serious diagnosis (x)	Seriousness Criteria	Severity	Onset Date			Outcome	Date of resolution			Relationship between SAE and IMP
Please record each event in one form; if possible, record the diagnosis instead of symptoms		1. Death 2. Life threatening 3. Inpatient hospitalization or prolongation of existing hospitalization 4. Results in persistant or significant disability / incapacity or permanent disruption of one's ability to carry out normal life functions or daily activities 5. Any congenital anomaly (not applicable) 6. Important medical event					1. Recovered/ Resolved 2. Recovering/Resolving 3. Not recovered/ not resolved 4. Recovered/ resolved with sequelae 5. Fatal** 6. Lost to follow-up				**Related** 1 = Related 2 = Probable 3 = Possible **Not related** 4 = unlikely 5 = unrelated
		Please fill in **1 - 6**	Insert Grade **1–5***	**dd**	**mm**	**yyyy**	**1 - 6**	**dd**	**mm**	**yyyy**	Please fill in **1- 5**

* 1=mild; 2=moderate; 3=severe and undisireable;
* 4=life-threatening or disabling; 5=death

**If subject died, was an autopsy performed? yes ☐ no ☐ unknown ☐ if yes, date of autopsy (dd/mm/yyyy):_____

Cause(s) of death as of autopsy:_____

Is there a reasonable possibility that any other medication caused the event? yes ☐ no ☐ If yes, please fill in name of medication: _____

Are there any other possible contributory factors? yes ☐ no ☐ unknown ☐ If yes, please fill in:

Contributory Factor	
Progression of concomitant disease	☐
Study conduct	☐
Others	☐

Name and date of last visit before onset of SAE:

DR. NIBLER & PARTNER **Serious Adverse Event Report Form** **Study: GPPAD-POInT**

Patient ID: Country ID | Site ID | Subj ID | Case No.

6. Study Treatment Information

Study treatment	Kit No.	Treatment Dates		Dosage		Study Treatment Action because of SAE			Restart of Study Treatment	
	Kit Number	Date dose first received (dd/mm/yyyy)	Date of last dose prior to SAE (dd/mm/yyyy)	Frequency	Route	1. No change 2. Drug interrupted 3. Stopped permanently 4. Not applicable 5. Unknown — 1-5	Date of Action (dd/mm/yyyy)	Mark with (x) if event improved	Restart of Study treatment date (dd/mm/yyyy)	Mark with (x) if event reoccurred
Insulin 7,5 mg / Placebo				1 x per day	p.o.					
Insulin 22,5 mg / Placebo				1 x per day	p.o.					
Insulin 67,5 mg / Placebo				1 x per day	p.o.					

7. Relevant concomitant medication excluding those used to treat the SAE

Name of medication	Treatment Dates		Dosage				Medication Action		Reason for use
	Date dose first received (dd/mm/yyyy)	Date of last dose prior to SAE (dd/mm/yyyy)	Amount	Unit	Frequency	Route	1. Medication unchanged 2. Medication withdrawn 3. Dose reduced 4. Dose increased 5. Medication interrupted 6. Unknown — 1-6	Date of Action (dd/mm/yyyy)	

☐ none

DR. NIBLER & PARTNER **Serious Adverse Event Report Form** **Study: GPPAD-POInT** Page 4/5

Patient ID: Country | Site | Subj | Case No.

8. Treatment of the reported events (Countermeasures)

No countermeasures ☐ **Drug treatment or others** ☐ **→ Please fill in subsequent list**

Treatment	Treatment dates			Dosage			
Details of drug and non-drug treatment	Start date (dd/mm/yyyy)	Stop date (dd/mm/yyyy)	ongoing	Amount (e.g. 200	Unit mg	Frequency 3 times per day	Route oral)

9. Relevant medical history

Medical history relevant to the SAE	Dates		
(including concurrent and pre-existing conditions)	Start date (dd/mm/yyyy)	Ongoing at time of SAE? (Y / N)	If no, End date (dd/mm/yyyy)

☐ **None**

10. Relevant laboratory findings or investigations

Laboratory, test or scan relevant to the SAE	Date (dd/mm/yyyy)	Result Value with Unit	Normal value Range of Values	CTCAE Grade 1-5*

☐ **None**

*if CTCAE grade 4 or above, or considered to be serious for any other reason, please also list in section 5

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DR. NIBLER & PARTNER **Serious Adverse Event Report Form** **Study: GPPAD-POInT** Page 5/5

Patient ID: Country | Site | Subj | Case No.

11. Investigator's Comment

Please provide additional information relevant to the SAE here
Please do not attach discharge summaries, copies of medical records or examination results unless specifically requested

12. Investigator's Signature

Date (dd/mm/yyyy)	Signature

PLEASE FAX FORM WITHIN 24 HOURS TO : +49 700 DRUGSAFETY / +49 700 3784723389

OR E-MAIL: GPPAD@DRUGSAFETY.DE

Dr. Nibler & Partner SAE Form Version 1.0, March 2019

18.3 APPENDIX 3: RISK SCORE

Table 2: List of SNPs determined in the GPPAD-02 Study

	SNP	Gene, Allele, Haplotype	Merged Score Weight
	HLA class II		
1	rs17426593	HLA DR4-DQ8/DR4-DQ8	3.15
2	rs2187668		3.98
3	rs7454108	HLA DR3/DR4-DQ8	
4	rs3129889	DRB1*1501	Exclusion criteria for first degree rel
5	Rs1794265	DQB1*0503	Exclusion criteria for first degree rel
	HLA class I		
6	rs1264813	HLA A 24	0.43
7	rs2395029	HLA B 5701	0.92
	Non-HLA SNPs		
8	rs2476601	PTPN22	0.76
9	rs2816316	RGS1	0.16
10	rs3024505	IL10	0.22
11	rs1990760	IFIH1	0.16
12	rs3087243	CTLA4	0.16
13	rs10517086		0.19
14	rs2069763	IL2	0.11
15	rs6897932	IL7R	0.19
16	rs3757247	BACH2	0.19
17	rs9388489	C6orf173	0.14
18	rs6920220	TNFAIP3	0.15
19	rs1738074	TAGAP	0.05
20	rs7804356	SCAP2	0.15
21	rs4948088	COBL	0.17
22	rs7020673	GLIS3	0.23
23	rs12722495	IL2RA	0.47
24	rs947474	PRKCQ	0.15
25	rs10509540	RNLS/C10orf59	0.25
26	rs689	INS	0.65
27	rs4763879	CD69	0.06
28	rs2292239	ERBB3	0.36

	SNP	Gene, Allele, Haplotype	Merged Score Weight
29	rs3184504	SH2B3	0.24
30	rs1465788	ZFP36L1	0.13
31	rs17574546		0.13
32	rs3825932	CTSH	0.15
33	rs12708716	CLEC16A	0.15
34	rs4788084	IL27	0.20
35	rs7202877		0.19
36	rs2290400	ORMDL3	0.25
37	rs7221109		0.15
38	rs45450798	PTPN2	0.09
39	rs763361	CD226	0.12
40	rs425105	PRKD2	0.21
41	rs2281808	SIRPG	0.07
42	rs3788013	UBASH3a	0.16
43	rs5753037		0.15
44	rs229541	IL2B	0.18
45	rs5979785	TLR8	0.09
46	rs2664170	GAB3	0.14

The risk score is calculated by multiplying the number of risk alleles (i. e. 0, 1 or 2 for each single SNP) with the weight assigned to each SNP (see Table 2) and then summing up the weighted contributions of all SNPs plus an additive constant for each of the two HLA class II categories, 3.15 for infants who have the HLA DR4-DQ8/DR4-DQ8 genotype or 3.98 for infants who have the HLA DR3/DR4-DQ8 genotype.

As an example, the risk score for a child with HLA DR4-DQ8/DR4-DQ8, homozygous for the risk allele of rs1264813 (weight 0.43), heterozygous for the risk allele of rs2395029 (weight 0.92), homozygous for the non-risk allele of rs2476601 (weight 0.76) and for all other SNPs in the genetic risk score is calculated as follows:

Risk score = 3.15 + (2 * 0.43) + (1 * 0.92) + (0 * 0.76) + ….

Missing genotypes at single SNPS: The following rules apply for samples that have incomplete typing at some of the SNPs:

Children without a first degree relative who has type 1 diabetes

1. Incomplete typing for SNPs 1, 2, or 3 results in an invalid test and should be either repeated or reported as invalid.
2. Incomplete typing at SNPs 4 or 5 is permissible since these are not used to calculate the genetic score.

3. Up to 5 incomplete genotyping at SNPs 6 to 46 is permissible. When incomplete, an average score weight (either positive or negative) will be added on the basis of the expected minor allele frequency in a European population (see table 4).

Children with a first degree family history of type 1 diabetes

1. SNP genotyping for SNPs 1, 3, 4 and 5 is essential.

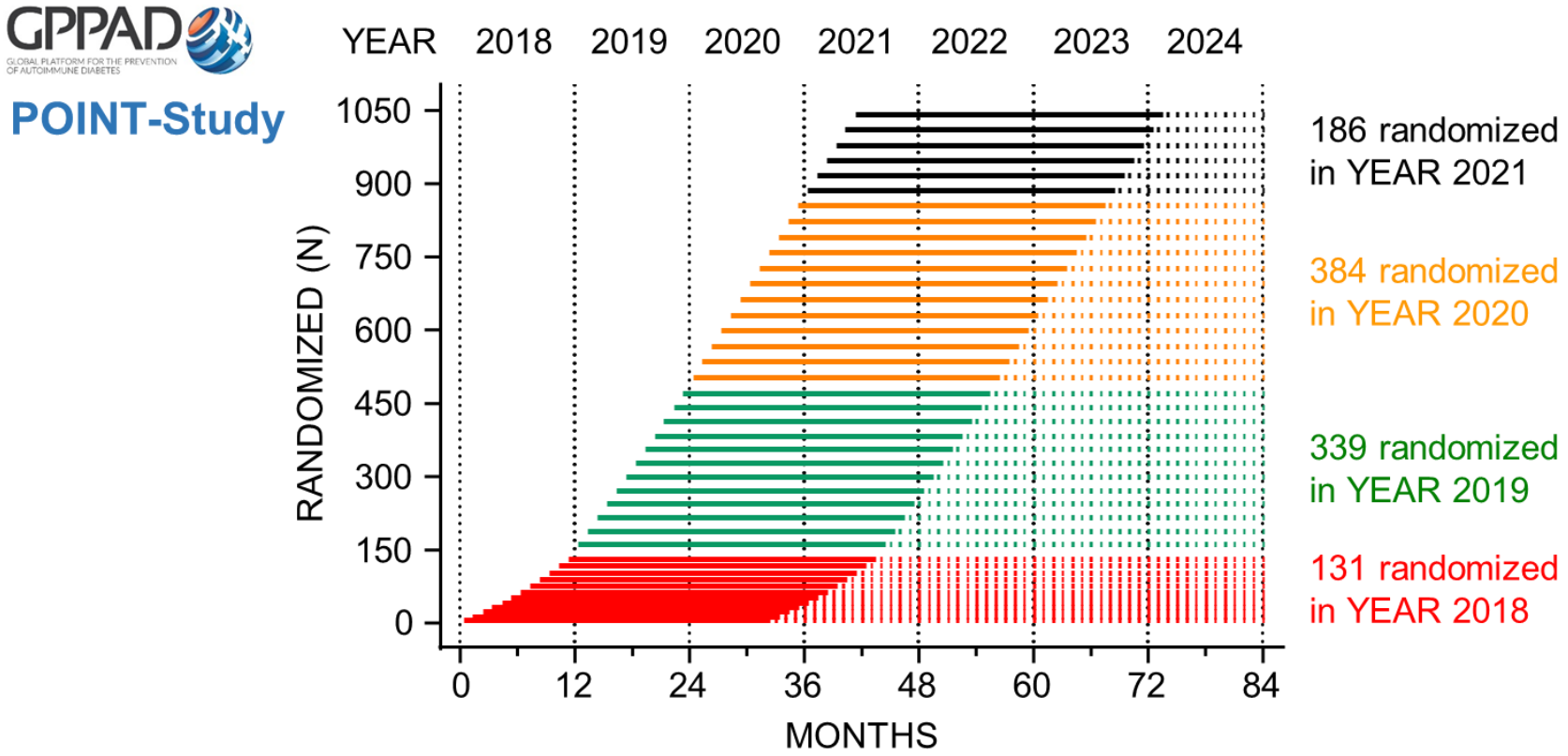
Missing genotypes for any of the other SNPs are permissible, as these are not used to determine risk.

Table 3: Average weight score for SNPs to be used if there is a missing genotype.

SNP	Gene, Allele	Minor Allele	Frequency (%)	Weight if missing
rs1264813	HLA A 24	A	9.51	0.08
rs2395029	HLA B 5701	C	0.96	1.82
rs2476601	PTPN22	A	12.11	0.18
rs2816316	RGS1	C	17.65	0.26
rs3024505	IL10	A	16.15	0.37
rs1990760	IFIH1	G	39.98	0.19
rs3087243	CTLA4	A	37.28	0.20
rs10517086	C4orf52	A	28.71	0.11
rs2069763	IL2	A	40.00	0.05
rs6897932	IL7RA	A	29.30	0.27
rs3757247	BACH2	A	41.08	0.16
rs9388489	C6orf173	G	44.65	0.13
rs6920220	TNFAIP3	A	21.38	0.06
rs1738074	TAGAP	A	40.59	0.06
rs7804356	SCAP2	G	21.64	0.24
rs4948088	COBL	A	4.51	0.32
rs7020673	GLIS3	G	49.15	0.23
rs12722495	IL2RA	G	8.29	0.86
rs947474	PRKCQ	G	18.50	0.24
rs10509540	RNLS/C10orf59	G	27.54	0.36
rs689	INS	A	37.51	0.49
rs4763879	CD69	A	38.24	0.05
rs2292239	ERBB3	A	33.19	0.24
rs3184504	SH2B3	A	45.92	0.22
rs1465788	ZFP36L1	A	29.39	0.18
rs17574546	RASGRP1	C	20.23	0.05
rs3825932	CTSH	A	36.13	0.23
rs12708716	CLEC16A	G	34.85	0.20
rs4788084	IL27	A	45.77	0.22
rs7202877	CTRB2	C	11.74	0.04
rs2290400	ORMDL3	A	47.87	0.26
rs7221109	CCR7	A	37.76	0.19
rs45450798	PTPN2	C	17.17	0.03
rs763361	CD226	A	48.45	0.12
rs425105	PRKD2	G	16.24	0.35
rs2281808	SIRPG	A	33.26	0.09

SNP	Gene, Allele	Minor Allele	Frequency (%)	Weight if missing
rs3788013	UBASH3a	A	39.81	0.13
rs5753037	RPS3AP51	A	34.93	0.10
rs229541	IL2B	A	40.47	0.15
rs5979785	TLR8	G	26.55	0.13
rs2664170	GAB3	G	30.28	0.08

18.4 APPENDIX 4: RECRUITMENT AND TREATMENT PROSPECT IN THE GPPAD-POInT STUDY



ON TREATMENT (N, per year)	131	470	854	1000	769	410	31	(———)
ON FOLLOW-UP (N, per year)			31	243	598	978	1040	(.....)

18.5 APPENDIX 5: PSYCHOLOGICAL IMPACT QUESTIONNAIRE

Well-being Questionnaire: mother, father, other guardian (Study Visit 3, 5, 8, End of Trial)

Today's date:

_____ / _____ / _____
(day) (month) (year)

Filled out by the study team:
☐ V3 ☐ V5 ☐ V8 ☐ EoT

Who has filled out the questionnaire?

☐ Mother ☐ Father ☐ Other guardian:_____

1. Over the last 2 weeks, how often have you been bothered by any of the following problems?

	not at all	several days	more than half the days	nearly every day
1.1 Little interest or pleasure in doing things	☐	☐	☐	☐
1.2 Feeling down, depressed, or hopeless	☐	☐	☐	☐
1.3 Trouble falling or staying a sleep, or sleeping too much	☐	☐	☐	☐
1.4 Feeling tired or having little energy	☐	☐	☐	☐
1.5 Poor appetite or overeating	☐	☐	☐	☐
1.6 Feeling bad about yourself — or that you are a failure or have let yourself or your family down	☐	☐	☐	☐
1.7 Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
1.8 Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐
1.9 Thoughts that you would be better off dead or of hurting yourself in some way	☐	☐	☐	☐

2. Questions about "anxiety":

	No	Yes
2.1 In the last 4 weeks, have you had an anxiety attack — suddenly feeling fear or panic? >> If you checked „NO", go to question #3.	☐	☐
2.2 Has this ever happened before?	☐	☐
2.3 Do some of these attacks come suddenly out of the blue — that is, in situations where you don't expect to be nervous or uncomfortable?	☐	☐
2.4 Do these attacks bother you a lot or are you worried about having another attack?	☐	☐
2.5 Think about your last bad anxiety attack? Have you been bothered by short of breath, sweat, heart race or pound, feeling dizzy or faint, tingling or numbness, nausea or an upset stomach?	☐	☐

3. If you checked off any problems on the questions 1 and 2, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

☐ not difficult at all ☐ somewhat difficult ☐ very difficult ☐ extremly difficult

4. How often do you worry that your child will get diabetes? (mark one answer)

☐ never ☐ rarely ☐ sometimes ☐ often ☐ very often

5. When you think about your child's risk for developing diabetes do you feel: (mark one answer on each line a – f)

a.	☐ not at all calm	☐ somewhat calm	☐ moderately calm	☐ very calm
b.	☐ not at all worried	☐ somewhat worried	☐ moderately worried	☐ very worried
c.	☐ not at all relaxed	☐ somewhat relaxed	☐ moderately relaxed	☐ very relaxed
d.	☐ not at all tense	☐ somewhat tense	☐ moderately tense	☐ very tense
e.	☐ not at all at-ease	☐ somewhat at-ease	☐ moderately at-ease	☐ very at-ease
f.	☐ not at all nervous	☐ somewhat nervous	☐ moderately nervous	☐ very nervous

6. Overall, how do you feel about having your child participate in the GPPAD-POInT-Study? (mark one answer)

☐ Like it a lot ☐ Like it a little ☐ It is ok ☐ Dislike it a little ☐ Dislike it a lot

7. Do you think your child's participation in the GPPAD-POInT-Studie study was a good decision? (mark one answer)

☐ a great decision
☐ a good decision
☐ an ok decision
☐ a bad decision
☐ a very bad decision

8. Would you recommend the GPPAD-POInT-Study to other paretns?

☐ No, not at all ☐ rather not ☐ it depends ☐ rather yes ☐ Yes, at any case

Thanks for taking time to complete this questionnaire!

Section / Substantial OR non-substantial amendment	**Previous wording**	**New wording**	**Rationale**
Title Page Footer Non-substantial	Version 2, 05.09.2017	Version 2.1, 23.11.2017	Administrative Change
Protocol approval Page Non-substantial	PD Dr. Markus Pfirrmann Prof. Dr. med. Joerg Hasford Statisticians, IBE, Ludwig-Maximilians-Universität München, Munich, Germany	Dr. Verena Hoffmann; Prof. Dr. med. Joerg Hasford Statisticians, Institut of Diabetes Research, Helmholtz Zentrum München	Change of Trial statistician
Protocol approval Page Non-substantial	Prof. Dr. med. Joerg Hasford Statisticians~~, IBE, Ludwig-Maximilians-Universität München~~	Prof. Dr. med. Joerg Hasford Statisticians, Institut of Diabetes Research, Helmholtz Zentrum München	Change of affiliation of second trial statistician
Synopsis Non-substantial	Short title: GPPAD-POInT (Global Platform of Autoimmune Diabetes – Primary Oral Insulin Trial)	Short title: GPPAD-03-POInT (Global Platform of Autoimmune Diabetes – Primary Oral Insulin Trial)	Correction of short title
Synopsis Non-substantial	4. Abnormal glucose tolerance (AGT) ~~defined by dysglycemia~~ or diabetes	4. Abnormal glucose tolerance (AGT=dysglycemia) or diabetes.	Clarification and consistent wording throughout the protocol
Synopsis Non-substantial	Inclusion criteria: 1. For infants without a first-degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4.	Inclusion Criteria: 1. For infants without a first-degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4. These represent close to 1% of all newborns. 2. For infants with a first degree family history of type 1 diabetes, high genetic	Correction: Added last sentence for consistency throughout the protocol and to be in line with chapter 3.4.1 inclusion criteria

	2. For infants with a first degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503.	risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503. These represent around one third of infants with a first degree family history of T1D.	
List of abbreviations Non-substantial	None	IVRS/IWRS - Interactive Voice Response System / Interactive Web Response System	Updated abbreviation list
General Information/ Study organisation Non-substantial	**Protocol-Chair/ Principal Investigator:** Prof. Dr. med. Anette-G. Ziegler	**Protocol-Chair/Coordinating and Principal Investigator:** Prof. Dr. med. Anette-G. Ziegler	Corrected title
General Information/ Study organisation Non-substantial	~~Co-Investigator:~~ Prof. Dr. Ezio Bonifacio, PhD ~~Co-Investigator:~~ Prof. Dr. Helena Elding Larsson, MD, PhD	**Protocol-Committee Members:** Prof. Dr. Ezio Bonifacio, PhD Prof. Dr. Helena Elding Larsson, MD, PhD	Corrected title
General Information/ Study organisation Non-substantial	Manufacturer of IMP: NextPharma,	Manufacturer of IMP: NextPharma, allphamed PHARBIL Arzneimittel GmbH	Corrected to the complete name
General Information/ Study organisation Non-substantial	Statistics: ~~PD Dr. Markus Pfirrmann, Institut f. Medizin. Informationsverarbeitung Biometrie und Epidemiologie (IBE)Ludwig-Maximilians-Universität München Marchioninistr. 15~~	Statistics: Dr. Verena Hoffmann, Institute of Diabetes Research, Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany	Change of Trial statistician

	~~81377 Munich, Germany~~		
General Information/ Study organisation Non-substantial	Statistics: Prof. Dr. med. Joerg Hasford ~~Institut f. Medizin. Informationsverarbeitung Biometrie und Epidemiologie (IBE), Ludwig-Maximilians-Universität München Marchioninistr. 15 81377 Munich, Germany~~	Statistics: Prof. Dr. med. Joerg Hasford Institute of Diabetes Research Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany	Change of affiliation of second trial statistician
GPPAD-POInT Study: Visit – schedule (Study Flow Chart) Non-substantial			Clarifications on blood glucose collection, footnote reference for OGTT, TS7A antibody measurement not restricted to autoantibody positive as is its not restricted in the protocol
3.4 Non-substantial	2. A high genetic risk (>10%) to develop beta-cell autoantibodies by age 6 years:	2. A high genetic risk (>10%) to develop multiple beta-cell autoantibodies by age 6 years:	Corrected for precise clarification regarding the status of high genetic risk of over 10% does refer to multiple autoantibody status
3.5 4.1 Non-substantial	GPPAD-02 ~~(Freder1k)~~	GPPAD-02	Deleted Study name as there are different names for GPPAD-02 in each country
3.5 Non-substantial	In the ~~Freder1k~~ study, testing for genetic risk of T1D is offered either at delivery (cord blood), together with the regular newborn screening, or at a pediatric baby-visit ~~before the age of 3 months,~~ with collection of blood using ~~Freder1k~~ filter paper cards.	In the GPPAD-02 study, testing for genetic risk of T1D is offered either at delivery (cord blood), together with the regular newborn screening, or at a pediatric baby-visit with collection of blood using GPPAD-02 filter paper cards.	Clarified exact screening age Deleted Study name as there are different names for GPPAD-02 in each country And deleted specific information of the GPPAD-02 trial from this protocol and deemed not necessary in this protocol
3.5 5 Non-substantial	Infants with a predicted risk ~~for T1D~~ of >10% to develop beta-cell autoimmunity by age 6 years and	Infants with a predicted risk of >10% to develop beta-cell autoimmunity by age 6 years and who fulfill the inclusion criteria	Corrected error

	who fulfill the inclusion criteria as stated above will be asked to participate in the GPPAD-POInT Study.	as stated above will be asked to participate in the GPPAD-POInT Study.	
3.10 Non-substantial	Trial pharmacy […] provide packages sequentially numbered	Study drug manufacturer […] using package numbers	Correction and Clarification
Non-substantial	The Münchner Studienzentrum will generate a unique randomization list ~~for each trial centre~~ centrally and provide these lists to the ~~trial pharmacy~~ only.	The Münchner Studienzentrum will generate a unique randomization list and medication list centrally and provide these lists to the study drug manufacturer only.	adapted procedure to final process
3.11 Non-substantial	Emergency unmasking will ~~occur upon notification of the POInT Medical Monitor and the GPPAD CC via the 24 hour emergency number and approval by the POInT Protocol chair.~~	Emergency unmasking will be available through the IVRS system. In case of temporary unintended unavailability of the IVRS/IWRS system a 24h hotline is available likewise.	An IVRS/IWRS system is used in the trial. Emergency unblinding will occur through the IVRS system.
4.1 Non-substantial	Potential participants in the oral trial will have a high ge-netic risk (>10%) to develop beta-cell autoantibodies by age 6 years.	Potential participants in the oral trial will have a high genetic risk (>10%) to develop multiple beta-cell autoantibodies by age 6 years.	Corrected for precise clarification regarding the status of high genetic risk of over 10% does refer to multiple autoantibody status
4.2 Non-substantial	2) Assessment of **medical history**	2) Assessment of **medical history** (only cases that lead to medical consultation will be recorded)	clarification
4.3 4.4 Non-substantial	none	b) Blood glucose (before study drug administration)	Clarification that a first blood glucose sample needs to be taken before study drug administration together with first blood sample
4.3 Non-substantial	~~3) Documentation and assessment of **AEs and SAEs**~~	*After first study drug intake* 1) Documentation and assessment of **AEs and SAEs**	Moved documentation and assessment of EAs and SAE to an outlined visit section "after first study drug intake" for clarification, as AEs

			and SAE will not be collected before the first baseline application of study drug
4.5 Non-substantial	d) As ancillary storage (subject to ethical approval and separate informed consent): Serum, plasma, PBMC	d) As ancillary storage (subject to ethical approval and separate informed consent): Serum (at visit 5, 6 and 7), plasma (at visit 6 only), PBMC (at visit 6 only)	Clarification at which visit each ancillary storage sample will be collected
4.6 Non-substantial	Regular telephone calls between visits will be made to keep closely in touch with the participants and their families. These will be at age ~~15~~ months ± 14 days	Regular telephone calls between visits will be made to keep closely in touch with the participants and their families. These will be at age 12-18 months ± 14 days	Corrected timeline
4.7 4.7 6.1 Non-substantial	3) Documentation and assessment of **AEs and SAEs (record and reporting until 60 days after ~~End of Treatment~~)**	3) Documentation and assessment of **AEs and SAEs (record and reporting until 60 days after the last treatment with the study drug)**	Clarified timepoint
4.12 Non-substantial	WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH/HbE, MCHC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils.	WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH/HbE, MCHC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils as well as blood glucose.	Glucose has been added to the local laboratory assessments
5.1.1 Non-substantial	Blood glucose measured before and 30, 60, and 120 minutes after drug has been administered during study visits at baseline (placebo or 7.5 mg oral insulin), 2 months (dose increase to placebo or 22.5 mg oral insulin) and 4 months (dose increase to 67.5 mg oral insulin). ~~Capillary blood will be used for measurements~~ […]	Blood glucose measured before and 30, 60, and 120 minutes after drug has been administered during study visits at baseline (placebo or 7.5 mg oral insulin), and 2 months (dose increase to placebo or 22.5 mg oral insulin) and 4 months (placebo or dose increase to 67.5 mg oral insulin) and 8 months (placebo or 67.5 mg oral insulin) post baseline. Venous blood will be collected and measured at the local certified laboratory. Additionally	Clarification purpose: Hypoglycemia must be confirmed by a certified local laboratory result […] Deleted repetitive sentence

	Families will also be instructed to report suspected hypoglycaemic events.	a capillary measurement with a glucose meter will be used for immediate test result only […] none	
6.1 6.5.1 12.2 Non-substantial	The site investigator will also record the determination of attribution on the appropriate eCRF and~~/ or a~~ SAE report form.	The site investigator will also record the determination of attribution on the appropriate eCRF and if applicable also on the SAE report form.	Clarification purpose: causality assessment is applicable to AEs that are documented in eCRF and also for SAE's that are documented on paper SAE form.
8. Non-substantial	None	The questionnaire is preferably completed by each of both parents or custodial parent(s).	Added clarification sentence
11.1 Non-substantial	[…], the elapsed time will be from the random treatment assignment to the ~~first~~ confirmed autoantibody positive sample used in defining the persistent confirmed multiple beta-cell autoantibod~~ies~~ positive status.	[…], the elapsed time will be from the random treatment assignment to the confirmed autoantibody positive sample used in defining the persistent confirmed multiple beta-cell autoantibody positive status.	Corrected sentence for more precise clarification
Synopsis 3.6 6.1 7.1.1 7.2 11.2 Non-substantial	4. Abnormal glucose tolerance (AGT) ~~defined by dysglycemia~~ or diabetes.	4. Abnormal glucose tolerance (AGT=dysglycemia) or diabetes.	For clarification: wording AGT=Dysglycemia is used equally in this protocol
14.4 Non-substantial	None	Consenting requirements currently vary between countries that participate in the POInT study. It is necessary to also adhere to country specific requirements. In particular, for participants who are consented in Sweden or in Germany,	Added according to Regulatory VHP request from VHP approval letter

		signed consent must be obtained from both parents or custodial parent(s) of the child prior to randomization.	
15.1.3 Non-substantial	Medical Monitor:[…] - ~~Source data verification~~	Medical Monitor:[…]	Source data verification will not be done by medical monitor
15.1.4 5. Non-substantial	Monitors safety parameters during the study, e.g. the DSMB will receive each case ~~of serious AE~~ a SUSAR after receipt within 96 hours for assessment	Monitors safety parameters during the study, e.g. the DSMB will receive each case of a SUSAR after receipt within 96 hours for assessment	Timeline does not apply to SAE reports
18.2 Non-substantial			Replaced SAE form by a finalised new SAE form provided by pharmacovigilance service provider
18.5 Non-substantial			Replaced psychological questionnaire with a Correction version provided by the psychologist advisor
General formatting corrections and spelling mistake corrections Non-substantial			

Section	Previous wording	New wording	Rationale	Substantial OR non-substantial amendment
Title Page Footer Non-substantial	Version 2.1, 23.11.2017	Version 2.2, 16.06.2018	Administrative Change	Non-substantial
List of abbreviations Non-substantial	None	OGTT – Oral Glucose Tolerance Test	Updated abbreviation list	Non-substantial
General Information/ Study organisation Non-substantial	**Pharmacovigilance:** Münchner Studienzentrum, Klinikum rechts der Isar der Technischen Universität 81675 München, Germany	**Pharmacovigilance:** CenTrial GmbH, Paul-Ehrlich-Straße 5, 72076 Tübingen, Germany	Updated Contact Details for Pharmacovigilance due to transition of Pharmacovigilance responsibility to CenTrial	Non-substantial
General Information/ Study organisation Non-substantial	**Monitoring supervision:** Münchner Studienzentrum Klinikum rechts der Isar der Technischen Universität München Ismaninger Straße 22 81675 Munich, Germany AND European Monitoring acquisition and coordination: CenTrial GmbH Paul-Ehrlich-Straße 5 72076 Tübingen, Germany	**Monitoring supervision:** ~~Münchner Studienzentrum~~ ~~Klinikum rechts der Isar der Technischen Universität München~~ ~~Ismaninger Straße 22~~ ~~81675 Munich, Germany~~ ~~AND~~ ~~European Monitoring acquisition and coordination:~~ CenTrial GmbH Paul-Ehrlich-Straße 572076 Tübingen, Germany	Updated Contact Details for Monitoring supervision due to transition of Monitoring supervision to CenTrial for all sites	Non-substantial

GPPAD-POInT Study: Visit – schedule (Study Flow Chart) Non-substantial	--	Visit 8 reference Footnote A: Correction as footnote A is related to blood glucose - not differential blood count	Correction	Non-substantial
GPPAD-POInT Study: Visit – schedule (Study Flow Chart) Non-substantial	Footnote B: [B]If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with 1000 IU vitamin D daily	Footnote B: [B]Measurement of Vitamin D level is recommended at each visit however a single missing vitamin D value will not be considered as protocol violation. If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with 1000 IU vitamin D daily	Clarification that single missing lab values will not be considered as protocol violation	Non-substantial
GPPAD-POInT Study: Visit – schedule (Study Flow Chart) Non-substantial	Footnote C: [C]Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo).	Footnote C: [C]Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo). Single missing glucose values will not be considered as protocol violation as long as at least 2 values after administration of study drug and the value before administration of study drug are available.	Clarification that single missing lab values will not be considered as protocol violation	Non-substantial

Section	Previous wording	New wording	Rationale	Substantial OR non-substantial amendment
Title Page Footer Non-substantial	Version 2.2, 16.06.2018	Version 2.3, 15.03.2019	Administrative Change	Non-substantial
Title Page Non-substantial	Site Principal Investigator: Site Co Investigator:	~~Site Principal Investigator:~~ ~~Site Co Investigator:~~	Formal correction	Non-substantial
General Information/ Study organisation Non-substantial	**Protocol Chair / Coordinating and Principal Investigator:** Prof. Dr. med. Anette-G. Ziegler Forschergruppe Diabetes Klinikum rechts der Isar Technische Universität München Kölner Platz 1 80939 Munich, Germany	**Protocol Chair / Coordinating and Principal Investigator:** Prof. Dr. med. Anette-G. Ziegler Forschergruppe Diabetes Klinikum rechts der Isar Technische Universität München Heidemannstr. 1 80939 Munich, Germany	Administrative Change	Non-substantial
General Information/ Study organisation Non-substantial	**Project Management:** GPPAD Coordinating Center (CC) Forschergruppe Diabetes / Klinikum rechts der Isar / Technische Universität München Kölner Platz 1, 80939 Munich, Germany	**Project Management & Monitoring Supervision:** GPPAD Coordinating Center (CC) Forschergruppe Diabetes Klinikum rechts der Isar Technische Universität München Heidemannstr. 1 80939 Munich, Germany	Administrative Change	Non-substantial

General Information/ Study organisation Non-substantial	**Data Management:** Dr. Florian Haupt Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany and CenTrial GmbH Paul Ehrlich-Straße 5 72076 Tübingen, Germany	**Data Management:** PHARMALOG Institut für klinische Forschung GmbHOskar-Messter-Str. 29 85737 Ismaning, Germany	Updated Contact Details for Data Management due to transition of responsibility from CRO CenTrial to Pharmalog	Non-substantial
General Information/ Study organisation Non-substantial	**Pharmacovigilance:** CenTrial GmbH Paul Ehrlich-Straße 5 72076 Tübingen, Germany	**Pharmacovigilance:** PHARMALOG Institut für klinische Forschung GmbHOskar-Messter-Str. 29 85737 Ismaning, Germany Subcontractor: Dr. Nibler & Partner Fürstenrieder Str. 105 80686 München, Germany	Updated Contact Details for Pharmacovigilance due to transition of Pharmacovigilance responsibility from CenTrial to Pharmalog / Dr. Nibler & Partner	Non-substantial
General Information/ Study organisation Non-substantial	**Randomisation:** Münchner Studienzentrum Klinikum rechts der Isar der Technischen Universität München Ismaninger Straße 22 81675 Munich, Germany	~~**Randomisation:**~~ ~~Münchner Studienzentrum Klinikum rechts der Isar der Technischen Universität München~~ ~~Ismaninger Straße 22~~ ~~81675 Munich, Germany~~	Administrative Change	Non-substantial
General Information/ Study organisation Non-substantial	**Monitoring supervision:** CenTrial GmbH Paul-Ehrlich-Straße 572076 Tübingen, Germany	~~**Monitoring supervision:**~~ ~~CenTrial GmbH~~ ~~Paul-Ehrlich-Straße 572076 Tübingen,~~ ~~Germany~~	Deleted due to transition of Monitoring supervision from CenTrial to GPPAD CC	Non-substantial

3.10 Non-substantial	… The study drug packages and thus the participants too will be randomized in a 1:1 ratio to each arm. The Münchner Studienzentrum will generate a unique randomization list and medication list centrally and provide these lists to the study drug manufacturer only.	… The study drug packages and thus the participants too will be randomized in a 1:1 ratio to each arm. A separate randomization list is used for multiples to ensure that these are assigned to the same treatment arm.	Updated as Münchner Studienzentrum is no longer involved and to clarify special situation for randomization of multiples.	Non-substantial
4.5 /4.6 Non-substantial	SINGLE MISSING LAB VALUES OR ANY OTHER SINGLE MISSING TEST RESULTS ARE NOT CONSIDERED AS PROTOCOL DEVIATION AS LONG AS ADEQUATE ATTEMPTS HAVE BEEN TAKEN TO GET THE RESULT AS REQUIRED PER PROTOCOL. ANY MISSING DATA DUE TO NON-COMPLIANCE OF STUDY PARTICIPANTS ARE ALSO NOT CONSIDERED AS PROTOCOL DEVIATION	Single missing lab values or any other single missing test results are not considered as protocol deviation as long as adequate attempts have been taken to get the result as required per protocol. Any missing data due to non-compliance of study participants are also not considered as protocol deviation	Correction of format	Non-substantial
6.5.1 Non-substantial	When an investigator identifies a SAE, he or she must notify the Sponsor/ Pharmacovigilance and the POInT Protocol Committee within 24 hours of discovering the event.This will be done via Fax: +49	When an investigator identifies a SAE, he or she must notify the Sponsor/ Pharmacovigilance and the POInT Protocol Committee within 24 hours of discovering the event. This will be done via Fax: +49 700 DRUGSAFETY / +49 700 3784723389 or	Updated contact details for SAE-Reporting	Non-substantial

	7071 9992 297 or email: pv.centrial@centrial.de, addressed to CenTrial GmbH, Paul-Ehrlich-Straße 5, 72076 Tübingen, Germany.	email: GPPAD@DRUGSAFETY.DE addressed to Dr. Nibler & Partner.		
18.2 Appendix 2 SAE Report Form Non-substantial			Updated Form with new Pharmacovigilance contact information	Non-substantial

Section	Previous wording	New wording	Rationale	Substantial OR non-substantial amendment
Title Page Footer Non-substantial	Version 2.3, 15.03.2019	Version 3.0, 23.09.2019	Administrative Change	Non-substantial
General Information/ Study organisation Non-substantial	**Clinical Study Centers:** United Kingdom Dr. Matthew Snape Department of Paediatrics Clinical Vaccine Research and Immunisation Education, Oxford, UK	**Clinical Study Centers:** United Kingdom Dr. Matthew Snape Department of Paediatrics Clinical Vaccine Research and Immunisation Education, Oxford, UK Dr. Loredana Marcovecchio University Department of Paediatrics, Cambridge Biomedical Campus, Cambridge, UK Dr. Catherine Owen Royal Victoria Infirmary, Newcastle upon Tyne, UK	Administrative Change	Non-substantial
General Information/ Study organisation Non-substantial	**Statistics:** Dr. Verena Hoffmann Institute of Diabetes Research Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany Prof. Dr. med. Joerg Hasford	**Statistics:** ~~Dr. Verena Hoffmann~~ ~~Institute of Diabetes Research~~ ~~Helmholtz Zentrum München~~ ~~Ingolstädter Landstr. 1~~ ~~85764 Neuherberg, Germany~~ Prof. Dr. med. Joerg Hasford Institute of Diabetes Research Helmholtz	Administrative Change	Non-substantial

	Institute of Diabetes Research Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany	Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany		
VISIT – SCHEDULE (STUDY FLOW CHART) Non-substantial	[B] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with 1000 IU vitamin D daily	[B] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with vitamin D.	Change of formatting and modification of footnote B	Non-substantial
2.1 Previous Clinical Trials Using Oral Insulin Non-substantial	… The Pre-POINT-Early Study (Protocol number: 80804017, BfArM number: 4040595, EudraCT number: 2014-005287-15, NCT02547519). Pre-POINT-Early is a study using oral insulin at early age for safety and immune efficacy. The aim of this Phase II Study is to determine whether daily administration of up to 67.5 mg insulin to young children aged 6 months to 2 years with a high genetic and familial risk for T1D is safe and induces immune	… The Pre-POINT-Early Study (Protocol number: 80804017, BfArM number: 4040595, EudraCT number: 2014-005287-15, NCT02547519). Pre-POINT-Early is a study using oral insulin at early age for safety and immune efficacy. The aim of this Phase II Study was to determine whether daily administration of up to 67.5 mg insulin to young children aged 6 months to 2 years with a high genetic and familial risk for T1D is safe and induces immune responses to insulin with features of immune regulation. Autoantibody negative children at high genetic risk for T1D, age 6 months – 2 years have been randomized either into 1. Oral insulin (dose escalation: 7.5 mg for 3 months; increased to 22.5 mg for 3 months; increased to 67.5 mg for 6	Section updated; (study completed and final study report now available)	Non-substantial

	responses to insulin with features of immune regulation. Autoantibody negative children at high genetic risk for T1D, age 6 months – 2 years are randomized either into 1. Oral insulin (dose escalation: 7.5 mg for 3 months; increased to 22.5 mg for 3 months; increased to 67.5 mg for 6 months) or 2. placebo. Currently, all 44 subjects are enrolled. There have been no safety issues observed thus far (no hypoglycemia, 215 AEs, 5 SAE). All blood glucose measurements and all SAE and AE that have occurred in participants of the Pre-POINT-Early study are provided in appendix D.2 and D.6 of the Investigator Brochure. A regularly updated AE list is available on demand from GPPADregulatory@helmholtz-muenchen.de. The safety data for Pre-POINT-Early without unblinding is provided in appendix D4.7 of	months) or 2. placebo. In total, 44 subjects were enrolled and the last patient last visit of the Pre-POINT Early study was in December 2017. Safety Data from this trial confirmed that overall oral insulin at all tested doses (7.5 mg; 22.5 mg and 67.5 mg) in the Pre-POINT-Early study can be considered safe. Safety Data from this trial confirmed that overall oral insulin at all tested doses (7.5 mg; 22.5 mg and 67.5 mg) in the Pre-POINT-Early study can be considered safe. There was no difference between treatment and placebo groups in blood glucose-, insulin- and C-peptide values, as well as insulin/C-peptide ratio, AUC glucose, AUC insulin, AUC C-peptide, or AUC insulin/C-peptide after study drug application. Two children developed the study endpoint 'persistent islet autoantibodies', one in the treatment and one in the placebo arm. There was no significant difference in the number of adverse events, in the number of adverse events by person years, in the number of serious adverse events, and in the severity of adverse events. When analysing single system organ classes, a higher frequency of skin and subcutaneous tissue disorder adverse events were observed in the treatment group (8 versus 1, p = 0.01); the overall frequency of skin and subcutaneous tissue disorder adverse events was low (6.6%); they were all classified as		

	the Investigator Brochure. Thus, far 2 (4.5%, 95%CI, 1.3% to 15%) of the 44 participating children have developed autoantibodies to GAD, IA-2 or ZnT8, which is not more than the expected frequency in at risk children of similar age and follow-up time. Also consistent with Pre-POINT, insulin autoantibody data has demonstrated the development of high affinity insulin autoantibodies in only one of the participants so far; some participants have developed low or moderate affinity insulin autoantibodies (without developing autoantibodies to GAD, IA.2 or ZnT8) as a potential sign of treatment response – ie, responses which are not unintended).	AE grade 1; they all resolved during the study. The safety data for Pre-POINT-Early are provided in appendix D of the Investigator Brochure.		
2.1 Previous Clinical Trials Using Oral Insulin Non-substantial	The Fr1da-Insulin-Intervention Study (Mechanistic study using oral insulin for immune efficacy in secondary prevention of type 1 diabetes) (Protocol number: 808040019,	The Fr1da-Insulin-Intervention Study (Mechanistic study using oral insulin for immune efficacy in secondary prevention of type 1 diabetes) (Protocol number: 808040019, EudraCT number: 2015-003028-30, NCT02620072). The objective of this phase II study is to	Section updated (study still ongoing)	Non-substantial

	EudraCT number: 2015-003028-30, NCT02620072). The objective of this phase II study is to determine the bioavailability and immune efficacy of high dose oral insulin in children with multiple beta-cell autoantibodies in a secondary intervention study. Immune efficacy is defined as a change in the immune response to the treatment that is associated with a reduction in the progression to dysglycemia. Children in the oral insulin group receive increasing dose of daily oral insulin: 7.5 mg for a duration of 3 months and increasing to 67.5 mg for 9 months of intervention. Children in the placebo group will receive 12 months of daily oral placebo. The study aims to recruit 220 participants. As of February 2017, there are 68 children enrolled in the trial. No safety concerns have been observed thus far (129 AEs, 3 SAEs). A regularly updated	determine the bioavailability and immune efficacy of high dose oral insulin in children with multiple beta-cell autoantibodies in a secondary intervention study. Immune efficacy is defined as a change in the immune response to the treatment that is associated with a reduction in the progression to dysglycemia. Children in the oral insulin group receive increasing dose of daily oral insulin: 7.5 mg for a duration of 3 months and increasing to 67.5 mg for 9 months of intervention. Children in the placebo group will receive 12 months of daily oral placebo. The study aims to recruit 220 participants. No safety concerns have been observed thus far.		

	AE list is available on demand from GPPADregulatory@helmholtz-muenchen.de.			
2.1 Previous Clinical Trials Using Oral Insulin Non-substantial	Table 1: Cumulative exposure to study drug in Pre-POINT-Early and Fr1da-Insulin-Intervention (as of 17.02.2017)	*Table 2 deleted*	Table deleted as data are no longer up to date	Non-substantial
4.6 Non-substantial	… Regular telephone calls between visits will be made to keep closely in touch with the participants and their families. These will be at age 12-18 months ± 14 days, 21 months ± 14 days, 27 months ± 14 days, and 33 months ± 14 days.	… Regular telephone calls between visits will be made to keep closely in touch with the participants and their families. The first call will be between visit 4 and visit 5 at age 12-18 months ± 14 days. Further calls will be at age 21 months ± 14 days, 27 months ± 14 days, and 33 months ± 14 days.	Wording modified for clarification	Non-substantial
15.1.4 Non-substantial	…The DSMB will review each protocol amendment for any major concern prior to implementation.	…The DSMB will review each substantial protocol amendment for any major concern prior to implementation.	Wording modified for clarification	Non-substantial
16. Non-substantial	… Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Ethics Committee. Any amendments to the protocol or to the consent materials must also be approved	… Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Ethics Committee. Any substantial amendments to the protocol or to the consent materials must also be approved before they are implemented. In exceptional cases where an amendment	Wording modified for clarification	Non-substantial

	before they are implemented. In exceptional cases where an amendment becomes necessary the reason must be outlined in writing and must be signed by all responsible parties. All amendments will be reviewed by the DSMB prior to getting into force.	becomes necessary the reason must be outlined in writing and must be signed by all responsible parties. All substantial amendments will be reviewed by the DSMB prior to getting into force. .		

Section	Previous wording	New wording	Rationale	Substantial OR non-substantial amendment
Title Page Footer Non-substantial	Version 3.0, 23.09.2019	Version 4.0, 09.12.2021	Administrative Change	Non-substantial
Synopsis Timeline Substantial	Recruitment: 3.5 years intended start (FPFV): January 2018 Intervention period: 29 to 32 months per participant Follow-up after intervention: 6-54 months Intended End (LPLV): January 2025 Interim Analysis: ~4.5 years after first randomization	Recruitment: 3.17 years (projected 3.5 years) intended start (FPFV): February 2018 (projected January 2018) Intervention period: 29 to 32 months per participant Follow-up after intervention: 6-46 months Intended End (LPLV): June 2024	Timelines updated because recruitment completed earlier than expected. Maximum follow-up time reduced and interim analysis cancelled	Substantial
General Information/ Study organisation Non-substantial	**Clinical Study Centers:** United Kingdom Dr. Matthew Snape Department of Paediatrics Clinical Vaccine Research and Immunisation Education, Oxford, UK Dr. Loredana Marcovecchio University Department of Paediatrics, Cambridge Biomedical Campus, Cambridge, UK Dr. Catherine Owen	**Clinical Study Centers:** United Kingdom Dr. Matthew Snape Department of Paediatrics Clinical Vaccine Research and Immunisation Education, Oxford, UK	Administrative Change	Non-substantial

	Royal Victoria Infirmary, Newcastle upon Tyne, UK			
General Information/ Study organisation Non-substantial	**Statistics:** Prof. Dr. med. Joerg Hasford Institute of Diabetes Research Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany	**Statistics:** Andreas Weiß Institute of Diabetes Research Helmholtz Zentrum München Ingolstädter Landstr. 1 85764 Neuherberg, Germany	Administrative Change	Non-substantial
VISIT – SCHEDULE (STUDY FLOW CHART) Non-substantial			Follow-up visits at age 7 years and 7.5 years and call 13 deleted	Non-substantial
TIME Schedule Non-substantial			Follow-up visits at age 7 years and 7.5 years and call 13 deleted	Non-substantial
3.14 Study Timeline Non-substantial		Update after completion of enrollment: The enrollment period lasted 3.17 years instead of 3.5 years, and has been completed in March 2021 (1050 subjects enrolled). The end of study (LPLV) is now expected in June 2024, which is when the last child completed 6 months of follow-up after the end of treatment Additional trial follow-up after end of enrollment will be 3.25 years. The last visit (final close-out visit) including collection of blood samples for final assessments should be performed for all children within the last 6 months before the last child completed 6 months of follow-up	Update after completion of enrollment added	Non-substantial

4.8 Follow-Up visits Non-substantial	4.8 FOLLOW-UP VISITS 9, 10, 11, 12, 13, 14, 15, 16 AND 17 The minimum number of follow-up visits is 1 visit and 1 telephone call; the maximum number of follow-up visits is 9 visits and 9 telephone calls. The study visit 9 will be conducted at age 42 months (± 30 days), the study visit 10 will be conducted at age 48 months (± 30 days), visit 11 at age 54 months (± 30 days), visit 12 at age 60 months (± 30 days), visit 13 at age 66 months (± 30 days), visit 14 at age 72 months (± 30 days), visit 15 at age 78 months (± 30 days), visit 16 at age 84 months (± 30 days), and visit 17 at age 90 months (± 30 days). Visit 17 will be the last visit (maximum). … A missed visit during the follow-up period is not a protocol violation.	4.8 FOLLOW-UP VISITS 9, 10, 11, 12, 13, 14 AND 15 The minimum number of follow-up visits is 1 visit and 1 telephone call; the maximum number of follow-up visits is 7 visits and 7 telephone calls. The study visit 9 will be conducted at age 42 months (± 30 days), the study visit 10 will be conducted at age 48 months (± 30 days), visit 11 at age 54 months (± 30 days), visit 12 at age 60 months (± 30 days), visit 13 at age 66 months (± 30 days), visit 14 at age 72 months (± 30 days) and visit 15 at age 78 months (± 30 days). Visit 15 will be the last visit (maximum). … ~~A missed visit during the follow-up period is not a protocol violation.~~	Follow-up visits 16 and 17 deleted Correction: Missed visits are considered as PD.	Non-substantial
4.9 Telephone calls between visits Non-substantial	Regular telephone call visits will be made to keep closely in touch with the participants and their families. These will be at age 39 months ± 14 days, 45 months ± 14 days, 51 months ± 14 days, 57 months ± 14 days, 63 months ± 14 days, 69 months ± 14 days, 75 months ± 14 days, 81 months ± 14 days, and 87	Regular telephone call visits will be made to keep closely in touch with the participants and their families. These will be at age 39 months ± 14 days, 45 months ± 14 days, 51 months ± 14 days, 57 months ± 14 days, 63 months ± 14 days, 69 months ± 14 days 75 months ± 14 days and 81 months ± 14 days.		

	months ± 14 days.			
9.2 Study and site discontinuation Substantial	The Sponsor has the right to terminate this study due to different reasons. Reasons for terminating the study may include but are not limited to the following: …. • The results of the pre-planned interim analysis (see 11.4) fulfil the criteria to stop the trial prematurely	The Sponsor has the right to terminate this study due to different reasons. Reasons for terminating the study may include but are not limited to the following: …. • The results of the over morbidity assessment fulfil the criteria to stop the trial prematurely	Updated because interim analysis is cancelled	Substantial
11.1 Primary Outcome Non-substantial	… We assume that at most, 10% of randomized infants with have a mother with type 1 diabetes…	…We assume that at most, 10% of randomized infants will have a mother with type 1 diabetes…	Typo correction	Non-substantial
Primary Analysis Non-substantial		Update after accrual completion: Enrolment lasted 3.17 years, the follow-up period will be 3.25 years. The total trial duration will be ~6.5 years. As outlined in section 11.3, the study will have >80% power to reject the null hypothesis of equal hazard rates despite the shorter median observation time.	Update added	Non-substantial
11.3 Study Power and accrual target Substantial	… An interim analysis will be performed when 53% of the total information is obtained.	… ~~An interim analysis will be performed when 53% of the total information is obtained~~ Adjustment of power-calculation and trial duration (October 2021) according to observed drop-out rate, the accrual period, and event rate:	Interim analysis cancelled and power calculation adjusted	Substantial

		1) Drop-out rate: The median observation time in 10/21 was 19.9 months. Drop-out rate at this time was 4.05%. Extrapolating this to the expected final median observation time of 4 years and 10 months results in an expected drop-out rate of 12% (95% confidence interval, 8 – 15%). With 12%, the currently extrapolated drop-out rate at the final median observation time 4 years and 10 months is smaller than the assumed total drop-out rate of 20%. 2) Hazard assumptions: In our original sample size estimation, a hazard rate of 0.02227 for the placebo group, and 0.011135 for the verum group were expected, providing an overall hazard rate of 0.0167. Estimating the hazard rate for the total group of 1050 patients, the hazard rate is 0.02456 95% confidence interval, 0.01822 - 0.03312).This means a potentially higher power to detect a significant difference between placebo and verum. 3) Enrollment: Recruitment was completed in 3.17 years instead of 3.5 years. With follow-up planned for 6 months after the third birthday of the three youngest children randomized most recently, a follow-up until around June 2024 would be needed. Thus, duration of the trial after end of recruitment in March 2021 would be 3.25 years. This would result in a median observation time of 4.835 years (instead of 5.25) or 4 years and 10 months instead of 5		

		years and 3 months. As a consequence, the median observation time would be 5 months less than originally planned. The implication of the 5 month reduction of the median observation time on study power to reject the null hypothesis if the original 20% drop-out rate and hazard rates of 0.011135 and 0.02227 were true would be a reduction to 77.6% according to PROC POWER of SAS9.4 based on Lakatos formula (Lakatos, Biometrics , 1988, Vol. 44, No. 1, pp. 229-24). However, using the current total hazard of 0.02456 for the placebo group and assuming half this hazard (i.e. 0.01228) for the verum group while keeping all other parameters fixed, the power would be 81.3%. Therefore, based on the currently available data, there is no power issue preventing the end of the trial 6 months after the last date of treatment as originally planned in the study protocol and is now expected to be in June 2024.		
11.4 Interim Analysis Plan Substantial			Section deleted because interim analysis is cancelled	Substantial
15.1.4 POInT DSMB Non-substantial	…Functions and responsibilities: … c. Reviews the 6-monthly DSMB reports and the report of the interim analysis and advises pertinent recommendations..	… Functions and responsibilities: … c. Reviews the 6-monthly DSMB reports and advises pertinent recommendations.	Updated because interim analysis is cancelled	Non-substantial

PRIMARY ORAL INSULIN TRIAL

Statistical Analysis Plan

Version 1.0 / 22.01.2024
EudraCT-No.: 2017-003088-36

Prevention of Diabetes Autoimmunity with Oral Insulin Therapy

A study of the Global Platform for the Prevention of Autoimmune Diabetes

Andreas Weiss, Anette-G. Ziegler, Ezio Bonifacio

GPPAD
GLOBAL PLATFORM FOR THE PREVENTION OF AUTOIMMUNE DIABETES

Statistical Analysis Plan
GPPAD-03

Signatures

28. März 2024

Prof. Dr. med. Anette-G. Ziegler — Date
Protocol Chair, Forschergruppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Germany, Institute of Diabetes Research, Helmholtz Munch, Germany

28/03/24

Andreas Weiss — Date
Trial Statistician, Institute of Diabetes Research, Helmholtz Munch, German, Helmholtz Munich, Germany

28/03/24

PD Dr. Markus Pfirrmann — Date
Independent Trial Statistician, IBE, LMU Munich

This SAP was prepared in accordance with the Guidelines for the Content of Statistical Analysis Plans in Clinical Trials published in JAMA. 2017;318(23):2337-2343.

Statistical Analysis Plan
GPPAD-03

Introduction

The study objective is to determine whether daily administration of oral insulin from age 4 months - 7 months until age 3.00 years to children with elevated genetic risk for type 1 diabetes reduces the cumulative incidence of beta-cell autoantibodies and diabetes in childhood.

We hypothesize that regular exposure to oral insulin throughout the period in life where beta-cell autoimmunity usually initiates will tolerize against insulin and train the body's immune system to recognize the treatment product without reacting adversely to it in a manner seen in children who develop T1D. This immune tolerance induction therapy would reduce the likelihood of beta-cell autoimmunity.

Eligible children will be randomized either into oral insulin (dose escalation: 7.5 mg for 2 months, increasing to 22.5 mg for 2 months, increasing to 67.5 mg until age 3.00 years) or placebo. Guardians of participants will self-administer the Investigational Medicinal Product once every day (oral insulin or oral placebo). Treatment will be administered preferably in the morning (7-10am).

Children will stop treatment the day of the 3rd birthday, or when they develop clinical diabetes. During the course of the study, participants will be tested for beta-cell autoantibodies, glucose levels, and will be assessed for their overall health and well-being. In children with beta-cell autoantibodies, Oral Glucose Tolerance Tests (OGTTs) will be performed at six-month intervals starting from age 3.0 years. After the intervention period, participants will be followed until completion of the trial for a period of 6 to 46 months. The trial started in February 2018, and the Last Patient Last Visit is intended to be June 2024.
The primary outcome is the development of persistent confirmed multiple beta-cell autoantibodies (see definition below) or diabetes. Secondary outcomes are 1) the development of at least one persistent confirmed beta-cell autoantibody, 2) the development of persistent confirmed IAA, 3) the development of persistent confirmed GADA, and 4) the development of dysglycemia or type 1 diabetes (see definitions below).

Primary Outcome	The primary outcome is the development of persistent confirmed multiple beta-cell autoantibodies (definition see below) or diabetes.
Secondary Outcome	The secondary outcome includes 1. The development of any persistent confirmed beta-cell autoantibody (defined as at least one confirmed autoantibody in two consecutive samples, including GADA, IA-2A, IAA, ZnT8A, or TS7A, OR the presence of a confirmed beta cell autoantibody together with or immediately followed by diabetes)

	2. Persistent confirmed IAA. 3. Persistent confirmed GADA 4. Abnormal glucose tolerance (AGT=dysglycemia) or diabetes.
Timeline	Recruitment: 3.17 years (projected 3.5 years) intended start (FPFV): February 2018 (projected January 2018) Intervention period: 29 to 32 months per participant Follow-up after intervention: 6-46 months Intended End (LPLV): June 2024

Study Methods

Trial Design

The GPPAD-POInT Study is designed as a randomized, placebo-controlled, double blind, multicentre, multinational primary prevention phase IIb study.

Randomization

Participants are randomized to the trial arms in a 1:1 ratio.

Framework

Primary and secondary endpoints are tested for superiority.

Sample Size

The study was supposed to provide 80% power to detect a 50% risk reduction in the hazard rate of the event “persistent confirmed multiple beta-cell autoantibodies or diabetes” using a two-sided log-rank test at the 0.05 level. A total of 1,050 infants were allocated in a 1:1 ratio to the two groups.
In the placebo group, at 3.5 years (approximate age of participants, 4 years), an event probability of 7.5% was assumed. Based on the exponential distribution, this led to a hazard of 0.02227.
Based on an estimation of the total hazard during a blinded sample size re-estimation in the course of the study, the hazard rate in the placebo groups was changed to be 0.02456.
It was expected that the hazard is halved by the treatment.
Accrual time was 3.17 years (February 2018 until March 2021)
Follow-up time will be 3.25 years
A dropout rate of 20% was expected.
Sample size and power, respectively, were estimated with PROC POWER, SAS version 9.4.
The final analysis will take place after the end of the observation period as defined by 6 months after the last day of treatment in a participant (expected June 2024) and after final data export and quality control. Primary and secondary outcomes will be analysed collectively.

Timing of outcome assessment

There will be 8 intervention visits (from baseline visit up to the age of three years) and up to nine follow-up visits after age three. The minimum follow-up period will be six months (*Table 1*).

Blood will be drawn at every visit to assess autoantibody status (*Figure 2*).

Oral glucose tolerance tests will be performed from age 3 years in children with persistent confirmed beta-cell autoantibodies.

Statistical Analysis Plan

GPPAD-03

Table 1: GPPAD-POInT STUDY: VISIT –SCHEDULE (STUDY FLOW CHART)

POInT Trial	**Trial**												
	Screening Phase	**Intervention**											
Visits		baseline visit (age 4 - 7 months)	visit 2 months post baseline	visit 4 months post baseline	visit 8 months post baseline	call	visit at age 1.5 years	call	visit at age 2.0 years	call	visit at age 2.5 years	call	visit at age 3.0 years
Visit window	below 7 months of age		± 10d	± 10d	± 10d		± 10d		± 14d		± 14d		± 14d
Study visit	0	1	2	3	4		5		6		7		8
Study call						1		2		3		4	
Informed consent	X												
Review Incl./Excl. Criteria	X												
Randomization		X											
Medical History	X												
Psychological impact Questionnaire (mother&father)				X			X						X
Antibodies measurement (IAA; GADA; IA-2A; ZnT8RA; ZnT8WA; TS7A)		X	X	X	X		X		X		X		X
Vitamin D (25OHD) [B]		X	X	X	X		X		X		X		X
Intervention													
dispense medication (+ compliance data sheet)		X	X	X	X		X		X		X		
Treatment		daily with 7,5 mg Insulin OR Placebo	daily with 22,5 mg Insulin OR Placebo	daily with 67,5 mg Insulin OR Placebo									
Investigations													
Physical examination (height, weight)		X	X	X	X		X		X		X		X
Assessment of AEs and SAEs [E]		X	X	X	X		X		X		X		X [E]
Blood glucose (0/30/60/120) [C]		X	X	X	X								
Blood glucose							X		X		X		X
Differential blood count		X											(X) [A]
OGTT (0/30/60/90/120) [D]													X [D]
Storage													
storage: serum samples		X	X	X	X		X		X		X		X
storage: plasma samples		X			X				X				X
PBMC		X			X				X				X
blood volumes for protocol parameters (mL):		5.0	3.8	3.8	3.8		3.8		3.8		3.8		5-11
blood volumes for protocol parameters (%):		0.8-1.0	0.5-0.6	0.5-0.6	0.4-0.5		0.4		0.4		0.3		0.4-0.9
additional biobank blood volumes (mL):		7.5	10.1	10.1	17.6		17.6		22.5		17.6		17.6-22.5
Total blood volumes (mL):		**12.5**	**13.9**	**13.9**	**21.4**		**21.4**		**26.3**		**21.4**		**27.5-28.6**
Total blood volume (%):**		**1.9-2.4**	**2.0-2.2**	**1.8-2.0**	**2.5-2.8**		**2.4**		**2.7**		**2.0**		**2.3-2.4**

**Blood volumes are < 5% NIH/WHO allowance and in accorance to the Pre-POINT (Bonifacio et al.: Effects of high dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-1549, 2015), Pre-POINT Early (EudraCT number: 2014-005287-15, NCT02547519, BfArM no. 4040595), and Fr1da-Intervention (EudraCT number: 2015-003028-30, NCT02620072, BfArM no. 4040830) studies

[A] In autoantibody positive children who will have an OGTT, a separate blood glucose sample is **not** taken.

[B] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with 1000 IU vitamin D daily

[C] Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo)

[D] If the participant developed beta-cell-autoantibodies during trial. OGTT (0/30/60/90/120) will be performed and samples measured in laboratory
Children who seroconverted to beta-cell-autoantibodies should have a confirmation sample within 4 – 12 weeks (interim visit)

[E] AEs/SAEs/SUSARs will be noted and reported as under intervention phase for 60 days after end of treatment day

Statistical Analysis Plan

GPPAD-03

POInT Trial	Trial															
	minimum 6 months FU		Follow-up variable with maximum up to 54 months FU													
Visits	call	visit at age 3.5 years	call	visit at age 4.0 years	call	visit at age 4.5 years	call	visit at age 5.0 years	call	visit at age 5.5 years	call	visit at age 6.0 years	call	visit at age 6.5 years	call
Visit window		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d		± 30d	
Study visit		9		10		11		12		13		14		15	
Study call	5		6		7		8		9		10		11		12
Informed consent Review Incl./Excl. Criteria Randomization Medical History															
Psychological impact Questionnaire (mother&father)		(X)[f]		(X)[f]		(X)[f]		(X)[f]		(X)[f]		(X)[f]		(X)[f]	
Antibodies measurement (IAA; GADA; IA-2A; ZnT8RA;		X		X		X		X		X		X		X	
Vitamin D (25OHD) [b]															
Intervention dispense medication (+ compliance data sheet) Treatment															
Investigations															
Physical examination (height, weight)		X		X		X		X		X		X		X	
Blood glucose (0/30/60/120) [c]															
Blood glucose		(X)[a]		(X)[a]		(X)[a]		(X)[a]		(X)[a]		(X)[a]		(X)[a]	
Differential blood count															
OGTT (0/30/60/30/120) [b]		X[b]		X[b]		X[b]		X[b]		X[b]		X[b]		X[b]	
Storage															
storage: serum samples		X		X		X		X		X		X		X	
storage: plasma samples				X				X				X			
PBMC				X				X				X			
blood volumes for protocol parameters (mL):		3.8-3.8		3.8-3.8		3.8-3.8		3.8-3.8		3.8-3.8		3.8-3.8		3.8-3.8	
blood volumes for protocol parameters (%):		0.3-0.8		0.3-0.7		0.3-0.7		0.3-0.6		0.2-0.6		0.2-0.6		0.2-0.5	
additional biobank blood volumes (mL):		25.1		25.1		25.1		30		30		30		30	
Total blood volumes (mL)*:		**28.9-34.9**		**28.9-34.9**		**28.9-34.9**		**33.8-39.8**		**33.8-39.8**		**33.8-39.8**		**33.8-39.8**	
Total blood volumes (%)*:		**2.5-3**		**2.1-2.6**		**2.1-2.6**		**2.2-2.6**		**2.2-2.6**		**2.0-2.3**		**2.0-2.3**	

**Blood volumes are < 5% NIH/WHO allowance and in accordance to the Pre-POINT (Bonifacio et al.: Effects of high dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-1549, 2015), Pre-POINT Early (EudraCT number: 2014-005287-15, NCT02547519, BfArM no. 4040595), and Fr1da-Intervention (EudraCT number: 2015-

[a] In autoantibody positive children who will have an OGTT, a separate blood glucose sample is **not** taken.

[b] If a vitamin D level < 75 nmol/L will be assessed during intervention, family pediatrician will be advised to supplement patient with vitamin D

[c] Blood glucose measurements before (0) and 30, 60 and 120 min after administration of the study drug (oral insulin or placebo)

[b] If the participant developed beta-cell-autoantibodies during trial. OGTT (0/30/60/90/120) will be performed and samples measured in laboratory
Children who seroconverted to beta-cell-autoantibodies should have a confirmation sample within 4 – 12 weeks (interim visit)

[f] hand out of the Psychological Impact Questionnaire only if this visit is childs last follow-up visit (i.e. only at End of Study Visit)

Statistical Principles

Confidence intervals and P values

Testing will be two-sided and on the 0.05 level of significance. All calculated confidence intervals will be 95%-confidence intervals. No adjustment for multiple testing will be performed. Accordingly, only the test for the primary outcome will be confirmatory hypothesis testing, any further hypothesis testing is exploratory.

All randomized children, including those who drop out of the study before their last visit will be included in the primary and secondary analyses where appropriate and possible.

Analysis populations

The intention-to-treat population will include all randomized children who received at least one dose of study medication, according to the treatment they were randomized to receive. Children, who are already multiple beta-cell autoantibody positive at baseline will be excluded from the full analysis data set for the analysis of the primary outcome (modified ITT). Children, who are already multiple beta-cell autoantibody positive at baseline, will be included in a sensitivity analysis of the primary outcome (ITT).

Children who are already single or multiple beta-cell autoantibody positive at baseline, will be excluded from the analysis of the secondary outcome 'any beta cell autoantibody' and for the secondary outcome 'IAA' or 'GADA' if the respective autoantibody was detected at baseline.

Children, who are single or multiple beta-cell autoantibody positive at baseline will be included in the secondary outcome 'dysglycemia/T1D' analysis.

The safety population will consist of all randomised children who received at least one dose of study medication and will be analysed according to the treatment they actually received.

The per-protocol population will be defined as all randomised children who were administered at least 85% of the expected number of capsules until either age 3 years or, for children who reached the study primary outcome prior to age 3 years, until the study visit when the child was defined as primary outcome positive (persistent confirmed multiple islet autoantibodies or diabetes). Adherence will be calculated as absolute or relative adherence (see below).

Adherence

Adherence is assessed by counting the capsules returned. The two records will be checked for major deviations. Adherence will be calculated as a percentage for each child in the ITT population.

% adherence = (number of capsules taken / number of capsules supposed to have been taken)*100%

We will use two different definitions of the number of capsules supposed to have been taken and thus of adherence:

1. Absolute adherence: The number of capsules that should have been taken will be calculated as the duration of pre-scheduled treatment (third birthday of child or day of primary outcome diagnosis – start of study medication + 1).

2. Relative adherence: The number of capsules that should have been taken will be calculated as the duration of actual treatment (end of treatment – start of study medication + 1).

As participants are supposed to take one capsule per day, the number of days and number of capsules to be taken are identical. Adherence will be analysed overall as well as stratified by study arm.

The absolute and relative adherence of each participant will be plotted for each study arm. The number and percentage of participants taking at least 85% of the scheduled capsules in each study arm will be calculated.

Protocol deviations

Protocol deviations and violations will be classified prior to unblinding of treatment arm. The number and percentage of children with minor and major protocol deviations will be reported by treatment arm. The number of children included in the ITT analysis population will be used as the denominator to calculate the percentages.

The following pre-defined protocol violations will be reported as number and percentage of the ITT population:

1. Missed visits
2. Missing blood samples for measurement of beta-cell autoantibodies
3. Errors in applying inclusion or exclusion criteria
4. Administration of expired IMP

Trial Population

Screening data

Children participating in POInT will be recruited from the GPPAD-02 screening program. The percentage of children eligible for participation in POInT who participated in POInT will be assessed as the percentage of children who participated in POinT and were assessed for eligibility by participating in a consulting visit. Sex and family history of type 1 diabetes (yes, no) will be compared between participating and non-participating eligible children.

Eligibility

Inclusion criteria of POInT are

1. Infant between the ages of 4 months and 7 months at the time of randomization.
2. A high genetic risk (>10%) to develop beta-cell autoantibodies by age 6 years:
    a. For infants without a first-degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype, and a genetic risk score that is >14.4. These represent close to 1% of all newborns.
    b. For infants with a first-degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, and none of the following protective alleles: DRB1*1501, DQB1*0503. These represent around one third of infants with a first-degree family history of T1D.
3. Solid foods introduced into diet of infant.
4. Written informed consent signed by the custodial parent(s).

Exclusion criteria for POInT are:

1. Concomitant disease or treatment that may interfere with the assessments, as judged by the investigators.
2. Any condition that could be associated with poor compliance.
3. Any medical condition or medical condition coexisting, which, in the opinion of the investigator, may jeopardize the participant's safe participation in the study.
4. Diagnosis of diabetes at the time of recruitment.
5. Participation in another clinical trial.

The number of ineligible though randomized participants will be reported with reasons for ineligibility.

Recruitment, withdrawal and follow-up

To comply with the CONSORT statement, a CONSORT 2010 Flow Diagram to visualize participant flow within the study will be prepared as required below. Withdrawals of consent and losses to follow-up will be reported per study arm including the respective time points and reasons.

CONSORT 2010 Flow Diagram

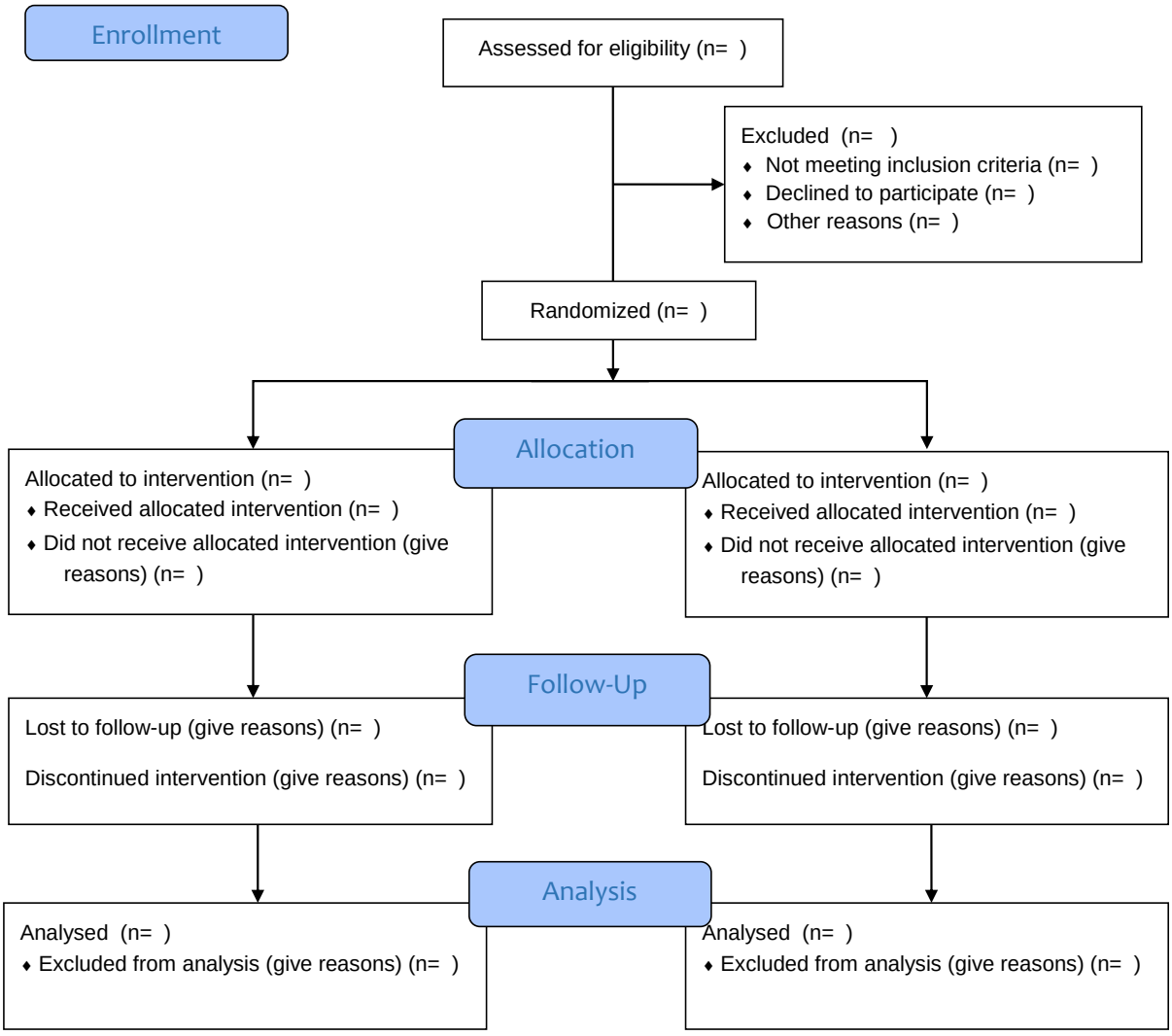


Figure 1: The CONSORT flowchart

Baseline participant characteristics

Participant baseline characteristics will be described using percent of ITT population for:

- sex,
- GRS (Genetic Risk Score)
- INS genotype for SNPs rs3842753 and rs1004446,
- HLA-DR4-DQ8/non-DR4-DQ8 and non DR3,
- HLA-DR3/DR4-DQ8,
- HLA-DR4-DQ8/DR4-DQ8,
- Family history of type 1 diabetes (yes/no for any first-degree relative),
- Sibling with T1D (yes/no)
- Mother with T1D (yes/no)
- Father with T1D (yes/no),
- Country
- Study site

Continuous data will be summarized using mean, SD and range or median, IQR and range for

- Age at baseline visit,
- Weight (age and sex adjusted Z-score),
- Height (age and sex adjusted Z-score),
- BMI (age and sex adjusted Z-score)
- values of the laboratory assessment.

Analysis

The primary outcome is the elapsed time from random treatment assignment to the development of **persistent confirmed multiple beta-cell autoantibodies** or **diabetes**.

The secondary outcome (1) is the elapsed time from random treatment assignment to the development of any **persistent confirmed beta-cell autoantibody**.
The secondary outcome (2) is the elapsed time from random treatment assignment to the development of **persistent confirmed IAA**.
The secondary outcome (3) is the elapsed time from random treatment assignment to the development of **persistent confirmed GADA.**
The secondary outcome (4) is the elapsed time from random treatment assignment to the development of **persistent dysglycemia** or **diabetes**.

In children with no primary or secondary outcome, the elapsed time is the time from random treatment assignment to the last available sample.

Definition of Outcomes

Criteria for persistent confirmed beta-cell autoantibodies:
Persistent confirmed beta-cell autoantibodies are assessed according to *Figure 2*. Each blood sample is tested for beta-cell autoantibodies against insulin (IAA), GAD65 (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8A) in the GPPAD central autoantibody laboratory and, if positive, tested in the GPPAD confirmatory laboratory. If confirmed, the autoantibody result of the next sample (a persistency sample or the next visit sample) is assessed and, if the same antibody is positive in both the central and confirmatory laboratory, the criteria for persistent confirmed beta-cell antibodies are fulfilled. Antibodies to tetraspanin-7 are tested at study end in last visit samples and if positive, prior samples are assessed to define the date of seroconversion.
If a child develops confirmed beta-cell autoantibodies and subsequently develops diabetes prior to the collection of a sample for beta-cell autoantibody persistence, this will fulfil the criteria for persistent confirmed beta-cell autoantibodies.
Cut-offs for autoantibody positivity are defined in the Manual of Operation and in the eCRF. The date of outcome is defined below.

Criteria for persistent confirmed multiple beta-cell autoantibodies:
Persistent confirmed **multiple** beta-cell autoantibodies are defined as confirmed IAA, confirmed GADA, confirmed IA-2A, or confirmed ZnT8A in two consecutive samples, AND a confirmed second antibody from these four antibodies in one sample.

If a child develops confirmed multiple beta-cell autoantibodies and subsequently develops diabetes prior to the collection of a sample for beta-cell autoantibody persistence, this will fulfil the criteria for persistent confirmed beta-cell autoantibodies.
It is expected that beta-cell autoantibodies will be detected prior to diabetes onset; however, the presence of diabetes in the absence of beta-cell autoantibodies is also considered as an outcome, and in this case situation, the date of diagnosis is the time of the outcome.

Persistent confirmed beta-cell autoantibodies that are considered maternally derived GADA or IA-2A are NOT included as positive for the primary outcome (see definition below).

Definition of maternally derived autoantibodies:
In children who are positive for any of the four beta-cell autoantibodies in the first sample taken and where there is no negative sample prior to this sample, the likelihood that they have maternally derived autoantibodies will be considered. The status of the autoantibodies will be classified as maternally derived beta cell autoantibodies if they become negative in a subsequent sample taken before age 3 years. Maternally derived beta cell autoantibodies are not a primary outcome endpoint and are not considered as a positive outcome in the statistical analysis.
If children are positive for beta-cell autoantibodies at their first sample and therefore potentially have maternally derived beta cell autoantibodies, they are still eligible for randomization and treatment. The elapsed time from randomization to primary outcome in children with maternally derived beta-cell autoantibodies will be determined as:
For children who become beta-cell autoantibody negative before age 3 years, the primary outcome is defined as the first confirmed multiple beta-cell autoantibody positive sample after the negative sample.
Children who are positive for a beta-cell autoantibody from the start of sample collection and remain positive for the same autoantibody until age 3 years will be classified as beta-cell autoantibody positive from baseline.
Children who are positive for two or more beta-cell autoantibodies from the start of sample collection and remain positive for only one of these autoantibodies until age 3 years will be classified as multiple beta-cell autoantibody positive from when they subsequently develop multiple beta cell autoantibodies.
Children who are positive for multiple beta-cell autoantibodies from the start of sample collection and remain positive for the same autoantibodies until age 3 years will be classified as multiple beta-cell autoantibody positive from baseline.

Criteria for type 1 diabetes onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis).

One of the following criteria must be met on two occasions as soon as possible but no less than 1 day apart for diabetes to be defined:

1. Symptoms of diabetes and a casual plasma glucose ≥200 mg/dL (11.1mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

OR

2. Fasting plasma glucose (FPG) ≥126 mg/dL (7 mmol/L), fasting is defined as no caloric intake for at least 8 hours

OR

3. Two-hour plasma glucose (PG) ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75g anhydrous glucose dissolved in water.

It is preferred that at least one of the two testing occasions involve an OGTT. Cases identified will be confirmed as having diabetes if the glucose values to make these determinations were obtained in a GPPAD central autoantibody laboratory as part of an OGTT. Cases diagnosed with diabetes by symptoms and casual glucose ≥ 200mg/dL or by other criteria than the above will be adjudicated by the GPPAD Endpoint Adjudication Committee. Trial treatment will be terminated if T1D is reached.

Although children who develop persistent confirmed multiple beta-cell autoantibodies will have reached the primary study outcome, these children will continue to receive assigned treatment and will be followed in the study for continued monitoring of glucose tolerance and diabetes development and safety assessments.

Criteria for Dysglycemia:
• impaired fasting plasma glucose of ≥110 mg/dL (6.1 mmol/L), OR
• impaired 2-hour glucose of ≥140 mg/dL (7.8 mmol/L), OR
• high glucose levels at intermediate time points on OGTT (30, 60, 90 min levels of ≥200mg/dL (11.1 mmol/L)) at two consecutive occasions OR at one occasion followed by diabetes onset in the next sample.

Date of persistent confirmed beta-cell autoantibody positive
= date of first occurrence of a positive antibody confirmed in two laboratories and in two consecutive samples
OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the beta-cell autoantibody

Date of persistent confirmed IAA positive
= date of first occurrence of IAA positivity confirmed in two laboratories and in two consecutive samples and by RBA and LIPS

OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the IAA

Date of persistent confirmed GADA positive
= date of first occurrence of GADA positivity confirmed in two laboratories and in two consecutive samples
OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the GADA.

Date of persistent confirmed IA-2A positive
= date of first occurrence of IA-2A positivity confirmed in two laboratories and in two consecutive samples
OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the IA-2A.

Date of persistent confirmed ZnT8A positive
= date of first occurrence of ZnT8A positivity confirmed in two laboratories and in two consecutive samples
OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the ZnT8A.

Date of persistent confirmed TS7A positive
= date of first occurrence of TS7A positivity confirmed in two consecutive samples
OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the TS7A.

Date of persistent confirmed multiple beta-cell autoantibody positive
= date of first occurrence of a second positive antibody confirmed in two laboratories, in addition to a persistent confirmed first positive antibody
OR in one sample followed by diabetes onset if diabetes is diagnosed prior to a sample to evaluate persistency of the multiple beta-cell autoantibodies

Date of primary outcome
= date of persistent confirmed multiple beta cell AAB positive (as defined above)
OR date of diabetes onset, whichever comes first

Date of secondary outcome 'any beta-cell autoantibody positive'
= date of any persistent confirmed beta-cell autoantibody (=at least one autoantibody as defined above, including IAA, GADA, IA-2A, ZnT8A, or TS7A),

OR, for those children who develop diabetes prior to collection of a sample to determine persistency, it is the date of diabetes onset.

Date of secondary outcome 'dysglycemia or diabetes onset'
= date of first occurrence of persistent dysglycemia on two consecutive time points OR on one time point followed by diabetes onset
OR date of diabetes onset, whichever comes first

Definition of censoring time for children without reaching primary or secondary outcomes:

For children not reaching the primary outcome will be censored at the time of their last tested blood sample that was beta-cell autoantibody negative.

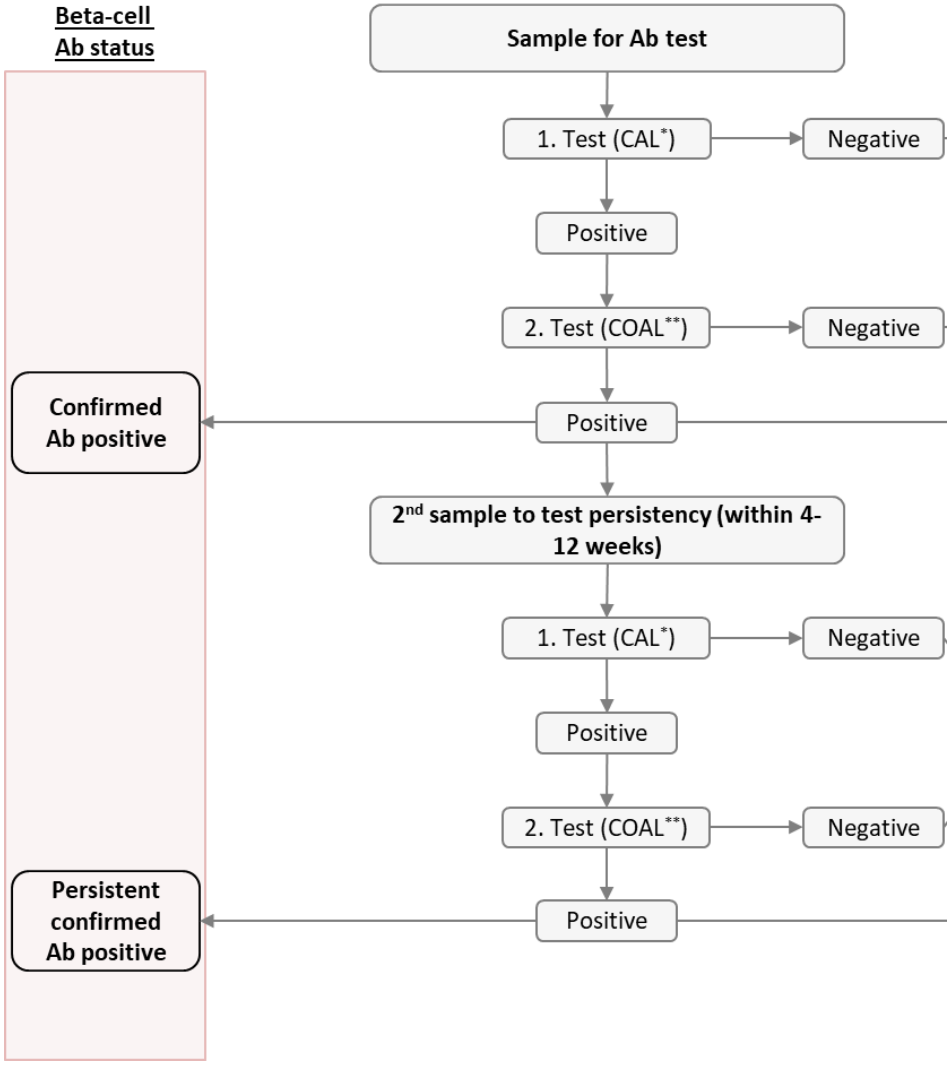


Figure 2: Flowchart for endpoint definition

Analysis methods

Primary outcome

The cumulative incidence of beta-cell autoantibodies over time since randomization within each treatment group will be estimated from a Kaplan-Meier function. The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.05 level, two-sided, using Cox regression and the Wald test.

Results will be presented as hazard ratios and cumulative incidences at 1, 2, 3, 4, 5, 6 and 7 years including the respective 95% confidence intervals, and will be visualized using cumulative incidence curves also showing the confidence intervals.

The proportional hazards assumption will be checked visually by plotting the Schoenfeld residuals against observation time and will be tested by including a time-varying covariate as an interaction between the predictor and the observation time.

Secondary outcomes

In addition to the primary outcome of multiple islet autoantibodies, four secondary outcomes will be included for analysis.

1. Any persistent confirmed beta-cell autoantibody

2. Persistent confirmed IAA.

3. Persistent confirmed GADA

4. Dysglycemia or diabetes.

The treatment arms will be compared on the corresponding cumulative incidence rates of each secondary outcome using the log rank statistic.

Additional analyses

A variety of secondary analyses are planned after completion of the trial.

The following variables are considered relevant for such analyses:
Sex
First degree relative with type 1 diabetes
Country/site
INS genotype for SNPs rs3842753 and rs1004446
HLA genotype
GRS
Maternal antibodies at baseline
Weight, height, BMI at baseline and throughout the intervention period

Subgroup analyses will be conducted comparing the effects of oral insulin versus placebo on the risk of multiple beta-cell autoantibodies with a test of the group by subgroup factor interaction in a Cox proportional hazard (PH) Model. Subgroups of the population classified by sex, country, first degree relative status (none, mother, father, sibling), maternally transferred beta cell autoantibody status at baseline, genetic risk score tertiles, and INS genotype. Differences in the treatment effect between subgroups will be tested using a covariate by treatment group effect in a Cox PH model.

Similar analyses may be conducted using the values of quantitative baseline factors including weight (z score), height (z score), BMI (z score), and genetic risk score. The dependence of the treatment

effect on the quantitative levels of a covariate will also be assessed by a covariate of treatment group interaction in a PH model.

Analysis will also be performed considering outcomes that occur during the treatment period vs the non-treatment period. Incidence rates will be determined for each of these periods within the oral insulin and placebo groups and compared in a PH model.

Additional factors may be defined before unmasking of the study data to the investigators. The analyses will distinguish between factors specified prior to unmasking, and those identified post-hoc during analysis. If the assumption of proportional hazard is not appropriate, the data will be examined to determine the cause of non-proportional hazard, such as the presence of a decaying, diverging, or crossing effect of hazard ratios over time. Based on the cause of the non-proportional hazard, post-hoc analyses such as frailty models, parametric models, or models with interactions and time-dependent covariates may be employed.

Exploratory analyses after the main study analysis (and not included into the initial trial report) may comprise the following: Longitudinal analyses, that will assess the effects of oral insulin versus placebo treatment on immunologic, transcriptomic, and metabolic markers and selected viral antibodies over time up to the onset of beta-cell autoantibodies. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors if the assumption of multivariate normal random errors is violated.

Immunologic, transcriptomic, and metabolic markers and viral antibodies will be modelled to determine the effects of oral insulin versus placebo treatment while adjusting for subject characteristics for each follow-up time point of interest. For continuous endpoints that lend themselves to normal error linear models, ANOVA and ANCOVA models will be employed. Generalized linear modelling will be employed for dichotomous and categorical endpoints by using the most appropriate link functions. Longitudinal analyses may be employed in order to characterize the relationship among the repeated measures during the treatment period and possibly beyond. Due to the exploratory nature of the longitudinal modelling, treatment effect hypothesis testing will not be conducted.

The association of demographic, genetic, immunologic, transcriptomic, metabolic, viral antibodies, and other factors, both at baseline and over time, with the risk of beta-cell autoantibodies onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on beta-cell autoantibodies risk will be assessed using time-dependent covariates for these factors. Analyses will also be conducted separately within the oral insulin and placebo groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Models will then be assessed within the two groups combined, taking account of any group by covariate interactions.

Subgroup analyses analogous to those described for the beta-cell autoantibodies endpoint will be conducted on the primary and secondary outcome endpoints. For these exploratory analyses, the date of the first confirmed antibody will be the date of the outcome.

Harms

There are no expected adverse events associated with the treatment in this trial, thus number and percentage of all adverse events will be presented for each treatment arm categorized according to MedDRA and by severity. Study arms will be compared descriptively. Also, their classification as definitely, probably and possibly related to treatment will be reported.

Glucose values before and after study drug intake at visits 1, 2, 3 and 4 will be compared between treatment arms.

Height (z-score), weight (z-score) and BMI (z-score) during treatment and after treatment will be compared between treatment arms.

Statistical Software

All analyses will be performed using SAS, preferably the latest version at the time of analysis but at least version 9.4.

PRIMARY ORAL INSULIN TRIAL

Statistical Analysis Plan Amendment 1.0 / 15.10.2024

EudraCT-No.: 2017-003088-36

Prevention of Diabetes Autoimmunity with Oral Insulin Therapy

A study of the Global Platform for the Prevention of Autoimmune Diabetes

Andreas Weiss, Anette-G. Ziegler, Ezio Bonifacio

GPPAD
GLOBAL PLATFORM FOR THE PREVENTION OF AUTOIMMUNE DIABETES

Primary Oral
Insulin Trial

Statistical Analysis Plan
Amendment 1.0
GPPAD-03

Signatures

15.10.2024

Prof. Dr. med. Anette-G. Ziegler — Date
Protocol Chair, Forschergruppe Diabetes, Klinikum rechts der Isar,
Technische Universität München, Germany, Institute of Diabetes Research, Helmholtz
Munch, Germany

15.10.24

Andreas Weiss — Date
Trial Statistician, Institute of Diabetes Research, Helmholtz Munch, German, Helmholtz
Munich, Germany

15/10/24

PD Dr. Markus Pfirrmann — Date
Independent Trial Statistician, IBE, LMU Munich

Primary Oral
Insulin Trial

Statistical Analysis Plan Amendment 1.0

GPPAD-03

With this amendment the following pre-defined exploratory analyses have been added to the Statistical Analysis Plan version 1. 0 dated 22 January 2024:

- An additional exploratory analysis will be done for the outcome type 1 diabetes as this is clinically relevant. The definition of censoring time for children not reaching this outcome is last contact.

- An additional exploratory analysis will be done for progression from islet autoantibody seroconversion to clinical T1D. Seroconversion is the date of the primary outcome or the date of the secondary outcome 1. The definition of censoring time for children not reaching this outcome is last contact

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Klinikum rechts der Isar

Technische Universität München

Lehrstuhl für Diabetes und Gestationsdiabetes

PRIMARY ORAL INSULIN TRIAL

Note to File

Subject: Children that are already AAB-positive at Baseline

Children, that are already multiple AAB-positive at baseline have to be considered differently for different analysis. This should be done as following:

- These children **will be excluded** from the full analysis data set for the analysis of the primary outcome (modified ITT).
- These children **will be included** in a sensitivity analysis of the primary outcome (ITT).
- These children **will be excluded** from the over-morbidity assessment, as for these children treatment cannot have an impact on the outcome.
- These children **will be included** in the secondary outcome T1D analysis.
- These children **will be included** in the SAE- and AE-frequency analysis.

Munich, 08.12.2021

Prof. Dr. A.-G.Ziegler