Anti-viral action against Type 1 diabetes autoimmunity

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PROTOCOL APPROVAL

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

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Date

Study Title:

Anti-viral action against Type 1 diabetes autoimmunity

Protocol Date and Version: **Version 2.0, 05. March 2024**

Protocol Signature Page

The undersigned has read and understood the study protocol detailed above and agrees to conduct the study in compliance with the protocol.

Investigator (Please print name)	**Signature**	**Site name or ID number**	**Date**

Following any amendments to the protocol, this page must be updated with the new protocol version number and date and re-signed by the site PI.

TABLE OF CONTENTS

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2. SYNOPSIS

Trial Title	Anti-viral action against Type 1 diabetes autoimmunity
Internal ref. no. (or short title)	GPPAD-05 AVAnT1A
Sponsor	Klinikum rechts der Isar of Technical University Munich
Funder	Helmsley Charitable Trust
Clinical Phase	Phase IV
Trial Design	Investigator initiated, randomised, controlled, multicentre, multinational, primary prevention trial
Population/Indication	Infants 3 months of age at enrolment with a high genetic risk (>10%) to develop islet autoantibodies by age 6 years
Sample Size	2252 (1:1 randomisation to vaccine or placebo)
Planned Trial Period	expected FPFV: 04/2024 expected LPFV: 07/2027 expected LPLV: 10/2029 study duration per participant: minimum from age 4.00 months to age 2.5 years; maximum from age 3.00 months to age 6.0 years (last visit if recruitment period exceeds 39 months)
Planned Recruitment Period	39 Months
Inclusion Criteria	1. Ages between 3.00 and 4.00 months at the time of enrollment. 2. A high genetic risk (>10%) to develop islet autoantibodies by age 6 years as determined by a HLA DR/DQ genotype, polygenic risk score and first-degree family history of type 1 diabetes status. 3. Written informed consent signed by the custodial parent(s).
Exclusion Criteria	1. Previous hypersensitivity to the excipients of the vaccine. 2. Any medical condition, concomitant disease or treatment that may interfere with the assessments or may jeopardize the participant's safe participation in the study. These include immune deficiencies, and conditions or treatments that lead to immune suppression. 3. Likely poor compliance due to expected change in residency. 4. Diagnosis of diabetes prior to recruitment or randomisation 5. Current use of any other investigational drug
Primary Objectives	To determine whether vaccination of children with elevated genetic risk for type 1 diabetes against COVID-19 from 6 months of age reduces the cumulative incidence of islet autoantibodies or type 1 diabetes in childhood.
Primary Outcome for vaccination trial	The primary efficacy outcome is the elapsed time from random treatment assignment to the development of persistent confirmed islet autoantibodies or type 1 diabetes.
Secondary Objectives	1. To determine whether vaccination against COVID-19 similarly reduces the cumulative incidence of multiple islet autoantibodies in childhood. 2. To determine whether vaccination against COVID-19 similarly reduces the cumulative incidence of type 1 diabetes in childhood.

	3. To determine whether vaccination against COVID-19 similarly reduces the cumulative incidence of celiac disease-associated transglutaminase autoantibodies in childhood.
Secondary Outcomes	Secondary outcomes are the elapsed time from random treatment assignment to the development of persistent confirmed multiple islet autoantibodies; the development of type 1 diabetes; the development of persistent confirmed transglutaminase autoantibodies.
Exploratory Objectives (outside of this clinical trial)	**Vaccine intervention:** To determine whether COVID-19 vaccination influences the frequency and features of islet autoreactive T cells. To determine the effects of COVID-19 vaccination on glucose metabolism and pancreatic function. To describe how immune parameters alter in response to COVID-19 vaccination. **Ancillary Infection surveillance:** To determine whether COVID-19 infection and/or the severity of infection is associated with the development of islet autoimmunity. To determine whether maternally acquired SARS-CoV-2 or other anti-viral antibodies reduce islet autoantibody risk. To determine the rate of specific viral infections (SARS CoV-2, Enteroviruses, Rotavirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus, Norovirus, Astrovirus) over the first 2 years of life over the study period. To determine whether there is a temporal association between specific viral infections (Enteroviruses, Rotavirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus, Norovirus, Astrovirus) and the development of islet autoimmunity. **Ancillary Immune surveillance** To describe how immune parameters alter in response to perturbations such as infection and vaccination. To determine whether the development of islet autoantibodies is associated with immune parameters and T cell response profile to islet autoantigens. To determine whether COVID-19 and other selected viral infections are associated with specific profiles of islet autoimmunity. To determine whether COVID-19 vaccination is associated with specific profiles of islet autoimmunity. **Ancillary Metabolic and General Analyses** To determine whether COVID-19 infection and other viral infections are associated with blood glucose levels and beta cell function in children. To identify metabolic and transcriptomic correlates of immunological changes, autoimmunity, COVID-19 and other virus infection and vaccination in children.
Intervention IMP Active ingredient	Comirnaty® 3 µg Omicron XBB.1.5 or future new variant developments replacing current Comirnaty vaccines for children Suspension for injection, for intramuscular use Manufacturer: BioNTech /Pfizer Dosing: three doses 1st dose at age 6.0 to 7.0 months 2nd dose at least 3 weeks through to 6 weeks after 1st dose 3rd dose at least 8 weeks after 2nd dose (around age 8.5 to 11 months)
Comparator	0.9 % Sodium Chloride Solution (saline) for injection

Reference Placebo	

3. ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
FPFV	First Patient First Visit
FU	Follow-Up
GCP	Good Clinical Practice
GPPAD	The Global Platform for the Prevention of Autoimmune Diabetes
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IMP	Investigational Medicinal Product
LPFV	Last Patient First Visit
LPLV	Last Patient Last Visit
PI	Principal Investigator
OGTT	Oral Glucose Tolerance Test
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
T1D	Type 1 Diabetes

4. BACKGROUND AND RATIONALE

4.1. Type 1 Diabetes

Type 1 diabetes is an immune-mediated disease in which the insulin-producing beta cells are destroyed, resulting in life-long dependence on exogenous insulin [1, 2]. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients diagnosed with type 1 diabetes each year is increasing and is approaching an epidemic level in some countries that track this information [3, 4].

Multiple factors such as genetic predisposition [5] and environmental factors [6] have been shown to be involved in the pathogenesis of type 1 diabetes and to influence the risk of developing the disease. In particular, an association between viral infections and type 1 diabetes development has been shown in epidemiological, clinical, and pathological studies in humans [7-10].

The immune-mediated destruction of the pancreatic islet beta cells is clinically silent and can be identified by circulating autoantibodies to islet antigens (GADA [11], IA-2A [12, 13], IAA [14] and ZnT8A [15]). The presence of autoantibodies can be used to diagnose type 1 diabetes and to differentiate it from the more common form of diabetes, type 2 diabetes. Compared to individuals with type 2 diabetes, (where individuals retain endogenous insulin production that is inadequate to maintain normal glucose and lipid metabolism), patients with type 1 diabetes have a more severe metabolic impairment and a more complete loss of insulin production [16]. 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, 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 hypoglycaemia [17]. While there have been significant improvements in insulin analogues 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 type 1 diabetes 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 [18]. They are also at high risk for acute complications such as severe hypoglycaemia, recurrent ketoacidosis, and others [18]. Prevention of type 1 diabetes would clearly represent a significant advancement.

4.2. Natural History of Type 1 Diabetes

Type 1 diabetes 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 the appearance of circulating autoantibodies to islet antigens (GADA, IA-2A, IAA and ZnT8A). Almost all children who develop the stage of multiple islet autoantibodies progress to clinical diabetes [19] (Figure 1). The earlier the process of islet autoimmunity is initiated, the more rapid is the progression to type 1 diabetes.

Islet autoantibody seroconversion has a clear peak incidence period between age 9 months and 3 years demonstrated in German [20], Finnish [21], and TEDDY studies [22] (Figure 2). In an analysis of over 13,000 prospectively followed children, 80% of the children who developed type 1 diabetes before the age of 20 years already developed islet autoantibodies before the age of 5 years [19]. Therefore, therapy given as a primary prevention strategy must be started early in life.

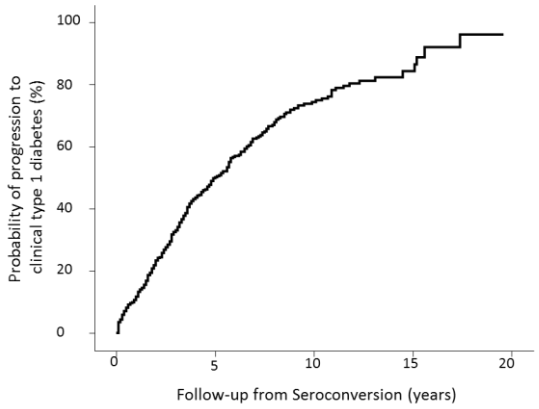


Figure 1: Children with multiple autoantibodies progress to symptomatic T1D (19)

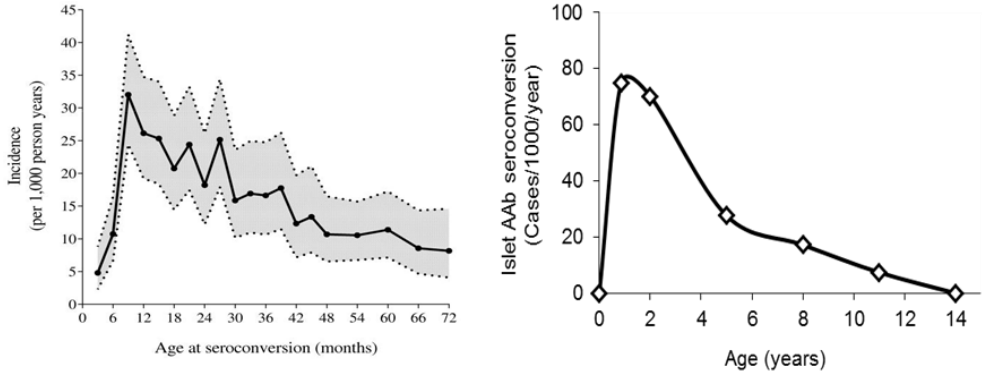


Figure 2: The incidence of islet autoantibodies peaks in early childhood in children including general population at genetic risk for T1D (left) (22) and with a first-degree relative with T1D (right) (20)

Data from the Primary Oral Insulin Trial (POInT) suggest that pancreatic islet function undergoes substantial changes during the first years of life which may be responsible for an increased susceptibility of beta cells to autoimmunity during this period [23, 24]. Notably, blood glucose concentration declines during the first year of life to a nadir at 1–1.5 years of age and increases after this age. This not only coincides with the peak incidence of islet autoimmunity but also follows the adiposity peak typically seen at 8–9 months of age, suggesting growth pressure on islets during the susceptible period. Thus, it is reasonable to propose that age 1 year may be a period of islet cell activity, increased beta cell stress and greater vulnerability to insults. Also, respiratory infections are most frequent during the first 2 years of life [25, 26, 27]. It is thought that while some viruses directly infect beta cells by entering through receptors, such as the coxsackie- and adenovirus receptors (CXADRs), others may indirectly induce beta cell damage and immune activation via systemic inflammation.

4.3. Identification of children at increased risk for islet autoimmunity and type 1 diabetes

Type 1 diabetes has a multifactorial aetiology, which is determined by genetic and environmental factors [28]. Risk in a European population is around 0.4%. A first-degree family history of type 1 diabetes is associated with a 5% risk for type 1 diabetes [29]. There are also at least 50 known regions of the genome where genetic variation is associated with type 1 diabetes risk [30].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 type 1 diabetes. Notably, infants who have the HLA DR3/DR4-DQ8 or the DR4-DQ8/DR4-DQ8 genotype have a risk of around 5% [31, 32]. Children with type 1 diabetes susceptible genotypes also have a marked risk for autoimmunity found in celiac disease as shown in the TEDDY study [33].

Combination of HLA risk genotypes with multiple single nucleotide polymorphisms (SNPs) at type 1 diabetes susceptibility regions has led to polygenic risk scores that identify children with greater than 10% risk

to develop multiple islet autoantibodies by age 6 years in the TEDDY cohort [34, 35]. This score together with the selection of infants who have a first-degree relative with type 1 diabetes and have at least one HLA DR4-DQ8 haplotype and no protective HLA DR and DQB1 alleles has been implemented as eligibility criteria in in primary prevention trials [36]. We have recently made further attempts to refine the score and to define sex-specific polygenic risk scores. New sex-specific scores based on a total of 47 SNPs improve screening efficiency yielding both a higher frequency of high-risk children identified by the thresholds and a risk increase in those identified. This is achieved using sex-specific score thresholds corresponding to the 98.75th centile of general population children without a first-degree type 1 diabetes family history, These scores identify 1.25% of such children and 23.1% of children with stage 1, 2 or 3 type 1 diabetes (Odds Ratio, 23.8). In comparison, previous thresholds and scores identified less than 20% of cases with an odds ratio of 22.6. The new sex-specific scores will be used in GPPAD-05.

4.4. Viral infections and type 1 diabetes risk and rationale for using COVID-19 vaccination

Previous epidemiological and genetic data have associated viral infections with type 1 diabetes. Infections in the first year of life increase the risk of islet autoimmunity and type 1 diabetes [26, 27, 37]. A large study of claims data showed that children with two or more infections by 6 months of age were more than twice as likely to develop type 1 diabetes by 8 years of age [10]. Furthermore, it was reported that children who develop islet autoimmunity experience their first viral infection earlier than children who do not develop islet autoimmunity or type 1 diabetes [37]. An increased frequency of viral infections in the time window before the first appearance of islet autoantibodies was also reported [26, 27]. These data suggest that early childhood infections and infections shortly before the onset of autoimmunity may promote autoreactivity towards islet cells. Furthermore, multiple virus response genes have been linked to the risk of developing islet autoimmunity [38].

Repeated attempts have been made to identify which viruses are responsible for the increased risk of autoimmunity and diabetes. Possible candidates are enteroviruses, particularly coxsackie B virus. Evidence supporting this include an increased prevalence of coxsackie B virus infection prior to islet autoimmunity [39], the expression of CXADR on beta cells that provides an entry point for coxsackie B virus [40], and the presence of coxsackie B virus antigen in pancreases from people with islet autoantibodies or type 1 diabetes [41, 42]. A large sequencing study of stool samples from more than 700 children suggested that prolonged shedding of enterovirus B may be involved in the development of islet autoimmunity [43]. However, only 11.8% of children with islet autoantibodies (vs 6.5% of children without islet autoantibodies) exhibited prolonged shedding of enterovirus B, suggesting that it may be one of many aetiological causes of islet autoimmunity. Rotavirus and cytomegalovirus have also been implicated in islet autoimmunity [44–46], and a decrease in type 1 diabetes incidence was observed after the introduction of rotavirus vaccination in some, but not all studies [47–50]. Taken together, the available data corroborate the notion that viruses, especially those capable of infecting islet cells in young children, contribute to the development of islet autoimmunity in childhood.

Recent studies have also suggested a contribution of the **severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)** to the development of type 1 diabetes. In addition to reports of cases with type 1 diabetes that occurred after COVID-19 [51], an increased incidence rate of childhood diabetes since the start of the pandemic has been observed in most [52–53] but not all studies [54], together with increased severity of disease measured by diabetic ketoacidosis [53–57]. Like other viruses associated with type 1 diabetes, the SARS-CoV-2 virus can enter and infect islet beta cells [58–60]. Therefore, it is plausible that COVID-19 may also increase the susceptibility for islet autoimmunity, which has potentially important implications for future type 1 diabetes incidence. In a study of over 1.1 million statutorily insured children born between

2010 and 2018 in Bavaria, Germany, a temporal association between COVID-19 and the development of type 1 diabetes was observed [61]. A 50% increase in the incidence rate of type 1 diabetes was observed in 2020-2021 as compared to 2018-2019. Importantly, the likelihood of developing type 1 diabetes in 2020 to 2021 was increased by over 50% in children who had a diagnosis of COVID-19 as compared to children without a COVID-19 diagnosis. The increase in type 1 diabetes incidence occurred in the same quarter as the COVID-19 diagnosis and also in later quarters. These data show an association between a COVID-19 diagnosis and the development of type 1 diabetes that may be mediated by an acceleration of disease progression (Figure 3).

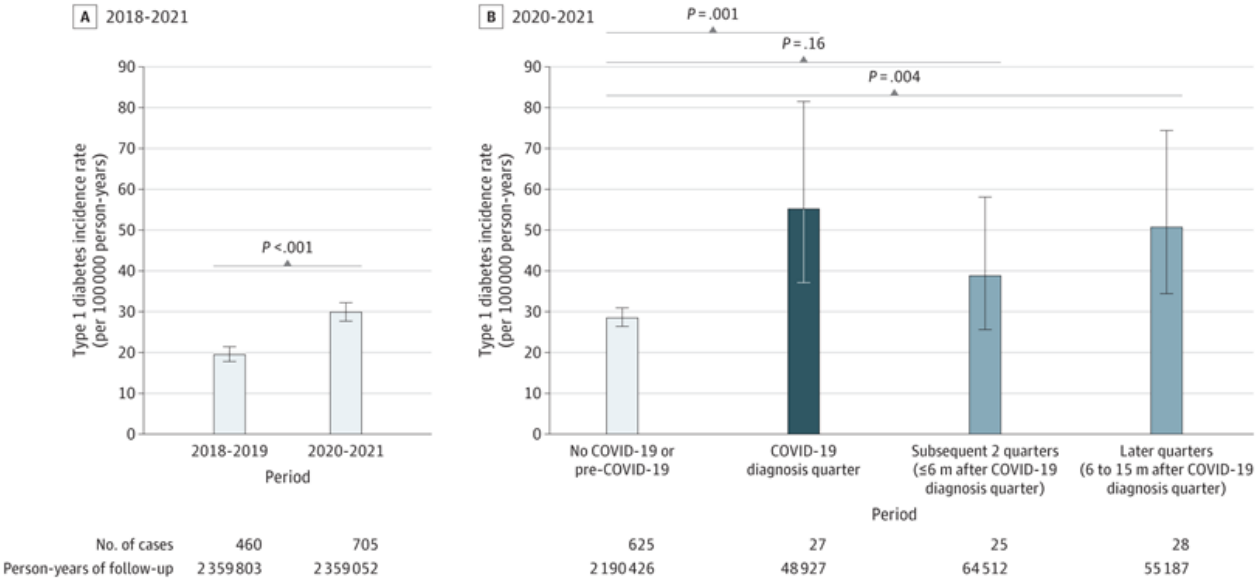
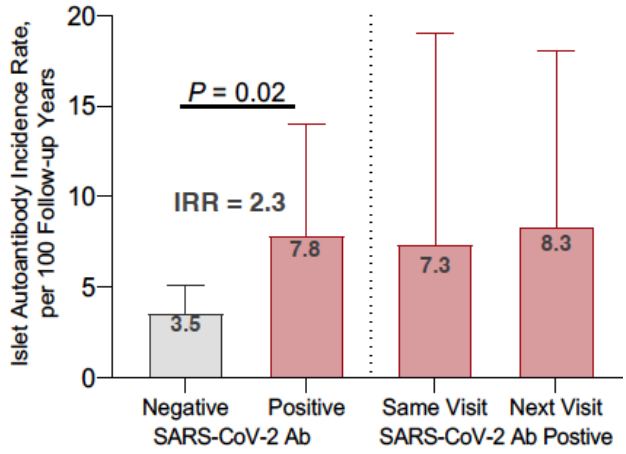


Figure 3. Incidence Rates for Type 1 Diabetes in Children With and Without a COVID-19 Diagnosis
A, Incidence rate of type 1 diabetes before the pandemic (2018-2019) and during the pandemic (2020-2021) for 1 181 096 children with medical insurance claims data. B, For children in the pandemic period, the incidence rate of type 1 diabetes is shown from January 2020 to December 2021 in the absence of a preceding or concurrent COVID-19 diagnosis (light blue bar), during the quarter with the COVID-19 diagnosis (dark blue bar), and for the 2 quarters (6 calendar months) and subsequent quarters (6 to 15 calendar months) after the COVID-19 diagnosis quarter (blue bars). The number of cases of type 1 diabetes and the person-years of follow-up per group are indicated. Error bars indicate 95% binomial CIs of the respective incidence rate estimate.

A recent study found an association between COVID-19 and the development of islet autoantibodies [62]. By investigating a cohort of prospectively followed children from the POInT study (ClinicalTrials.gov number NCT03364868) the timing of COVID-19 in relation to the appearance of islet autoantibodies was examined [62]. The POInT study recruited 1050 babies with a genetically defined risk of at least 10% for developing multiple islet autoantibodies from 2018 to 2021 [63]. The genetic selection represents around 1% of all babies born and captures up to 25% of those who will develop type 1 diabetes during childhood [64]. The enrolled children are followed from four to seven months of age with blood samples collected longitudinally every 2 to 6 months for the detection of islet autoantibodies.
SARS-CoV-2 antibodies developed in 170 children at a median age of 1.5 years (range 0.5–2.1 years) from the first positive sample in July 2020 until June 2022. None of the children had received COVID-19 vaccination. The adjusted hazards ratio for developing islet autoantibodies when children were SARS-CoV-2 antibody positive was 3.5 (95% CI 1.6–7.7; *P*=.002). The incidence rate of islet autoantibodies was 3.5 (2.2-5.1) in children without SARS-CoV-2 antibodies and 7.8 (5.3–19.0) in children with SARS-CoV-2 antibodies (*P*=.02). The risk for developing islet autoantibodies in the SARS-CoV-2 antibody positive children was increased in children infected prior to age 18 months (HR 5.3, 95% CI, 1.5-18.3; *P*=.009, Figure 4) and islet autoantibody incidence rate when children were SARS-CoV-2 antibody-positive was highest at age 12 to

16 months (36.5), which was 10-fold higher than the incidence rate in the children who were not infected by this age (*P*<.001).



Covariate	HR (95% CI)	*P* Value
Sex, Girls	1.2 (0.4-3.9)	.77
HLA DR3/DR4-DQ8	0.6 (0.2-2.0)	.43
First-degree Relative with Type 1 Diabetes	0.6 (0.2-2.1)	.44
Age of SARS-CoV-2 Antibody <18 mo	5.3 (1.5-18.3)	.009

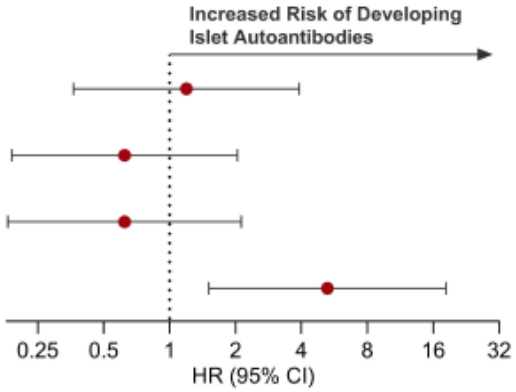


Figure 4. A. Incidence rate of islet autoantibodies from July 2020 in 735 children for the time until their last SARS-CoV-2 antibody-negative sample (open bar, 27 islet autoantibody positive cases) and for 165 SARS-CoV-2 antibody-positive children (red bar, 12 islet autoantibody positive children, left). For the 165 SARS-CoV-2 antibody positive children, the incidence rates are also shown from the last SARS-CoV-2 antibody negative sample to the SARS-CoV-2 antibody positive sample (red, same visit, 6 islet autoantibody positive cases), and from the SARS-CoV-2 positive sample to the next study visit (red, next visit, 6 islet autoantibody positive cases). B. Univariable Cox proportional hazards model performed on 165 SARS-CoV-2 antibody positive children who were islet autoantibody negative at the time of SARS-CoV-2 antibody seroconversion. Each covariate was binary with the risk category indicated.

These data show a temporal association between COVID-19 and the development of islet autoantibodies in children genetically at risk for type 1 diabetes, particularly when infection occurred at young age. In particular, COVID-19 infection before 18 months of age increased the risk of islet autoantibodies more than 5-fold. COVID-19 infections in teenagers were not associated with an increased risk to develop islet autoantibodies, consistent with the notion that infections earlier in life are critical [65]. These findings differ from those reported in cross-sectional screening of more than 50,000 youths in Colorado (USA) and Bavaria (Germany), which found no association between COVID-19 and islet autoimmunity [66, 67]**.** It should be noted, however, that the cross-sectional design in that study did not allow the researchers to determine whether the autoantibodies developed before or after COVID-19 and because of the age of those screened (from 1 to 17 years) it is likely that the majority of islet autoantibody positive cases occurred prior to the pandemic.

To help validate the findings from the POInT study, SARS-CoV-2 antibodies were examined in children participating in the SINT1A study. These children are also at increased genetic risk and are followed for the development of islet autoantibodies with study visits at age 6 months, 12 months and yearly thereafter. Enrolment into SINT1A commenced in 2021. COVID-19 was assessed by family reporting or SARS-CoV-2

antibodies in 453 children. After adjusting for maternally derived antibodies, COVID-19 was observed in 300 of the children with a cumulative infection incidence of 87% by age 2 years. This far, 11 children have developed islet autoantibodies. The risk for developing islet autoantibodies at the same or subsequent visit in the children with SARS-CoV-2 antibodies was 4.5% as compared to 0.7% in children who were SARS-CoV-2 antibody negative (*P* = .09). The incidence rate of islet autoantibodies when SARS-CoV-2 antibody positive was 11 per 100 person-years. These preliminary findings corroborate those reported in the POInT study.

4.5. Celiac disease-associated transglutaminase autoantibodies

Children with increased risk for islet autoimmunity and type 1 diabetes also have an increased risk of developing celiac disease-associated transglutaminase autoantibodies [68, 69]. There is no clear evidence that COVID-19 infection leads to an increase in celiac disease incidence, though more research on this topic with longer-term follow-up is necessary to make this assessment [70]. Viral infections have been reported to trigger increased immune reaction to dietary antigens. Development of celiac disease has been associated with viral gastrointestinal infections [71], and early reports of COVID-19 included significant abdominal symptoms such as vomiting and diarrhea. Similar to islet beta cells, intestinal enterocytes express the ACE2 receptor, and a theoretical risk of an increased incidence of celiac disease as a consequence of COVID-19 has been hypothesized.

4.6. COVID-19 vaccination in young children

The safety and efficacy of vaccines against coronavirus disease 2019 (COVID-19) have been demonstrated in young children [72, 73], and consequently the vaccines have been broadly approved by EMA and FDA for children from age 6 months and older. In the study using the BNT162b2 vaccine (Pfizer-BIONTECH), 1178 children 6 months to less than 2 years of age received 3 doses of 3µg. BNT162b2 reactogenicity events were mostly mild to moderate, with no grade 4 events. Low, similar incidences of fever were reported after receipt of BNT162b2 (7% among children 6 months to <2 years of age and 5% among those 2 to 4 years of age) and placebo (6 to 7% among children 6 months to <2 years of age and 4 to 5% among those 2 to 4 years of age). The observed overall vaccine efficacy against symptomatic COVID-19 in children 6 months to 4 years of age was 73.2% (95% confidence interval, 43.8 to 87.6) from 7 days after dose 3 (on the basis of 34 cases). It was concluded, that a three-dose primary series of 3-µg BNT162b2 was safe, immunogenic, and efficacious in children from 6 months of age. (ClinicalTrials.gov number, NCT04816643.) In the study using mRNA-1273 (Moderna), 1762 children 6 to 23 months of age were randomly assigned to receive two 25-µg injections of mRNA-1273, and 593 children 6 to 23 months of age were randomly assigned to receive placebo. Adverse events were mainly low-grade and transient, and no new safety concerns were identified. At day 57, neutralizing antibody geometric mean concentrations were 1781 (95% CI, 1616 to 1962) among 6-to-23-month-olds, as compared with 1391 (95% CI, 1263 to 1531) among young adults, who had received 100-µg injections of mRNA-1273, findings that met the noninferiority criteria for immune responses for both age cohorts. The estimated vaccine efficacy against COVID-19 was 50.6% (95% CI, 21.4 to 68.6) among 6-to-23-month-olds, at a time when B.1.1.529 (omicron) was the predominant circulating variant (ClinicalTrials.gov number, NCT04796896).

Comirnaty COVID-19 vaccine for children from 6 months of age:

Comirnaty® 3 µg Omicron XBB.1.5 and Comirnaty® 3 µg are approved in GPPAD-participating countries from 6 months of age. For primary vaccination it is given in three doses of 3 µg each; the first two doses are given three to six weeks apart, followed by a third dose given at least 8 weeks after the second dose.

For children within these age groups, the vaccine is given as injections in the muscles of the upper arm or the thigh. The most common side effects in children aged from 6 months to 4 or 5 years were comparable to those seen in older age groups. Irritability, sleepiness, loss of appetite, rash and tenderness at the injection site were also common side effects in children aged 6 to 23 months. These effects were usually mild or moderate and improved within a few days of vaccination. A recent study performed in Germany showed that the symptoms reported after BNT162b2 administration were comparable overall to those for on-label non–SARS-CoV-2 vaccines in a cohort of children younger than 5 years [74]. Although there are fewer studies in young children, data from adults suggest that these vaccines do not offer long-term high levels of protection against infection.

5. OBJECTIVES, ENDPOINTS AND HYPOTHESIS

5.1. OBJECTIVES

5.1.1. Primary objective

To determine whether vaccination of children with elevated genetic risk for type 1 diabetes against COVID-19 from age 6 months reduces the cumulative incidence of islet autoantibodies or type 1 diabetes in childhood.

5.1.2. Secondary objectives

1. To determine whether vaccination against COVID-19 similarly reduces the cumulative incidence of multiple islet autoantibodies in childhood.
2. To determine whether vaccination against COVID-19 similarly reduces the cumulative incidence of type 1 diabetes in childhood.
3. To determine whether vaccination against COVID-19 similarly reduces the cumulative incidence of celiac disease-associated transglutaminase autoantibodies in childhood.

5.1.3. Exploratory objectives (Analyses outside of this clinical trial)

Exploratory objectives will be analysed outside of the clinical trial. They will be assessed in subgroups of participants and measurements may be performed and analysed also after completion of the trial.

Vaccine intervention:

To determine whether COVID-19 vaccination influences the frequency and features of islet autoreactive T cells.

To determine the effects of COVID-19 vaccination on glucose metabolism and pancreatic function.

To describe how immune parameters alter in response to COVID-19 vaccination.

Ancillary Infection surveillance:

To determine whether COVID-19 infection and/or the severity of infection are associated with the development of islet autoimmunity.

To determine whether maternally acquired SARS-CoV-2 or other anti-viral antibodies reduce islet autoantibody risk.

To determine the rate of specific viral infections (SARS CoV-2, Enteroviruses, Rotavirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus, Norovirus, Astrovirus) over the first 2 years of life over the study period.

To determine whether there is a temporal association of specific viral infections (Enteroviruses, Rotavirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus, Norovirus, Astrovirus) with the development of islet autoimmunity.

Ancillary Immune surveillance:

To describe how immune parameters alter in response to perturbations such as infection and vaccination.

To determine whether the development of islet autoantibodies is associated with immune parameters and T cell response profile to islet autoantigens.

To determine whether COVID-19 and other selected viral infections are associated with specific profiles of islet autoimmunity.

To determine whether COVID-19 vaccination is associated with specific profiles of islet autoimmunity.

Ancillary Metabolic and General Analyses:

To determine whether COVID-19 and other viral infections are associated with blood glucose levels and beta cell function in children.

To identify metabolic and transcriptomic correlates of immunological changes, autoimmunity, infection and vaccination in children.

5.2. OUTCOMES

5.2.1. Primary Efficacy Outcome

The primary efficacy outcome for the vaccination trial is the elapsed time from random treatment assignment to the development of persistent confirmed islet autoantibodies or type 1 diabetes. For subjects who develop persistent confirmed islet autoantibodies, the elapsed time will be from the random treatment assignment to the first confirmed autoantibody positive sample used in defining the persistent confirmed islet autoantibody positive status. A diagnosis of type 1 diabetes is realized by OGTT criteria for diabetes or clinical criteria for diabetes. If type 1 diabetes develops before a persistency sample for islet autoantibodies is obtained, the presence of confirmed islet autoantibodies followed by type 1 diabetes is also considered as a primary outcome, and in this case the elapsed time will also be from the random treatment assignment to the first confirmed autoantibody positive sample used in defining the primary outcome status. It is expected that islet autoantibodies will be detected prior to diabetes onset; however, the presence of type 1 diabetes in the absence of islet autoantibodies is also considered as a primary outcome endpoint, and in this case, the date of diagnosis is the time of the end point.

5.2.2. Secondary outcomes

Secondary outcomes for the vaccination trial are the elapsed time from random treatment assignment to the development of persistent confirmed multiple islet autoantibodies;-the development of type 1 diabetes; the development of persistent confirmed transglutaminase autoantibodies. For subjects who develop persistent confirmed multiple islet autoantibodies the elapsed time will be from the random treatment assignment to the first confirmed multiple islet autoantibody positive sample used in defining the autoantibody positive status. For subjects who develop persistent confirmed transglutaminase autoantibodies, the elapsed time will be from the random treatment assignment to the first confirmed autoantibody positive sample used in defining the autoantibody positive status. The study secondary outcome type 1 diabetes is realized by OGTT criteria for diabetes or clinical criteria for diabetes.

5.2.3. Exploratory Measures (outside of this clinical trial)

- Anti-viral antibodies in blood measured by multiplexed anti-viral antibody panel (including SARS CoV-2, Enteroviruses, Rotavirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus, Norovirus, Astrovirus)
- Saliva virus qPCR panel (SARS CoV-2, Enterovirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus)

- Stool virus qPCR panel (Enterovirus, Rotavirus, Adenovirus, Rhinovirus, Norovirus, Astrovirus)
- Islet autoantigen specific T cell response and single cell RNAseq of the responsive cells
- Whole blood multiparameter flow counts
- inflammatory markers
- in vitro immune cell response to viral and autoantigens and general stimuli
- Whole blood transcriptomics
- Whole blood DNA methylation
- Single cell multiomics
- Pre-prandial and post-prandial blood glucose
- C-peptide, proinsulin, insulin
- HbA1c
- Serum Metabolomics
- Plasma vitamin D3 concentration
- OGTT in islet autoantibody positive children
- Weight-for-length, BMI

5.3. HYPOTHESES

The primary hypothesis to be tested in this study is that vaccination against COVID-19 is superior to placebo in preventing development of islet autoantibodies in childhood.
Additional secondary and exploratory (outside of this study) hypotheses include:

- COVID-19 vaccination prevents the development of celiac disease-associated transglutaminase autoantibodies in childhood.
- Infections with specific viruses influence the risk for beta cell autoimmunity.
- Infection influences immune trajectories in early childhood.

5.3.1. Criteria for persistent confirmed islet autoantibodies

Criteria are based on the measurement of islet autoantibodies against insulin (IAA), GAD65 (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8A) tested in the GPPAD central autoantibody laboratory.

- Confirmed IAA is defined as sample positive for IAA in both a screening and confirmatory assay that has a different format to the screening assay.
- Confirmed GADA is defined as sample positive for GADA in both a screening and confirmatory assay that has a different format to the screening assay.
- Confirmed IA-2A is defined as sample positive for IA-2A in both a screening and confirmatory assay that has a different format to the screening assay.
- Confirmed ZnT8A is defined as sample positive for ZnT8RA or ZnT8WA in both a screening and confirmatory assay that has a different format to the screening assay.

The status persistent confirmed islet autoantibody-positive is defined as confirmed IAA, confirmed GADA, confirmed IA-2A, or confirmed ZnT8A in two consecutive samples. Persistence of at least one confirmed islet autoantibody until the last follow-up sample is required for an outcome of persistent confirmed islet autoantibody. The rate of progression to type 1 diabetes in children in the POInT trial who develop islet autoantibodies with these criteria was >10% per year, which is the same as previously reported in children with persistent confirmed multiple islet autoantibodies [19,75.76].
Islet autoantibodies that are considered maternally derived are NOT positive for the primary outcome. Maternally derived islet autoantibodies are expected in a minority of infants at baseline and expected to

be randomly distributed between treatment groups. The presence of maternal islet autoantibodies decreases rapidly over the first year of life and is only present after 12 months in a minority of those who have maternally transferred autoantibodies at birth. Maternally derived autoantibodies are defined as follows:

In children who are positive for any of the four islet 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 islet autoantibodies if they are positive from their first sample and decline in titre in subsequent samples or become negative in a subsequent sample taken before age 2.5 years. Maternally derived islet autoantibodies are not a primary outcome endpoint and are not considered as a positive outcome in the statistical analysis.

The elapsed time from randomisation to primary outcome in children with maternally derived islet autoantibodies will be determined as:

- For children who become islet autoantibody negative before age 2.5 years and subsequently develop persistent confirmed islet autoantibodies, the primary outcome is defined as the first confirmed islet autoantibody positive sample after the negative sample.
- Children who are positive for an islet autoantibody from the start of sample collection and remain positive for the same autoantibody until age 2.5 years will be classified as islet autoantibody positive. They will be outcome positive from the first measurement that shows an increase in antibody titres.

5.3.2. Criteria for persistent confirmed multiple islet autoantibodies

Persistent confirmed multiple beta-cell autoantibodies 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.

5.3.3. Criteria for persistent confirmed transglutaminase autoantibodies

Criteria are based on the measurement of transglutaminase autoantibodies in the GPPAD central autoantibody laboratory and in a second laboratory. Confirmed transglutaminase autoantibodies is defined as sample positive for transglutaminase autoantibodies in both a screening and confirmatory assay that has a different format to the screening assay. The status persistent confirmed transglutaminase autoantibodies positive is defined as confirmed transglutaminase autoantibodies in two consecutive samples.

5.3.4. Criteria for type 1 diabetes onset

Criteria for type 1 diabetes onset are based on criteria defined by the American Diabetes Association (ADA) [75] which include glucose testing, or HbA1c or the presence of unequivocal hyperglycaemia with acute metabolic decompensation (diabetic ketoacidosis):

1. Fasting plasma glucose (FPG) ≥126 mg/dl (7 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.***** OR

2. 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.***** OR

3. HbA1c ≥6.5% (48mmol/mol). The test should be performed in a certified laboratory. ***** OR

4. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l). OR

5. In a patient without classic symptoms of hyperglycaemia, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l).*

*In the absence of unequivocal hyperglycaemia, diagnosis requires two or more abnormal test results from the same sample or at least one in two separate test samples (not the same date).
It is preferred that at least one of the two testing occasions involve 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 Endpoint Committee. Study participation will be terminated if type 1 diabetes is reached.

6. TRIAL DESIGN

The GPPAD-05-AVAnT1A Study is a randomised, multicentre, multinational, primary prevention trial for children at increased risk of type 1 diabetes starting from age 3.00 to 4.00 months. A target of 2,252 children will be randomly assigned in a 1:1 design to receive COVID-19 vaccination or placebo from age 6 months. The study will be conducted with the currently approved vaccine Comirnaty® 3 µg Omicron XBB.1.5. If, during the course of the study, other BIONTECH-COVID-19 variant vaccines replace the current vaccine and become available for the study, it is intended that these will then be used for newly enrolled children. 0.9% Sodium Chloride solution for injection (saline) will be used as the placebo in this study. Injection Syringes containing either the vaccine or placebo will look similar and will be prepared by an unblinded pharmacist. Further details are described in the IMP Manual.
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 type 1 diabetes is offered at delivery (cord blood) or together with the regular newborn screening. GPPAD is a network of collaborating Investigators from European countries. The network was created to allow for a coordinated, multi-disciplinary approach to prevent type 1 diabetes by early intervention. Study sites in Belgium, Germany, Poland, Sweden, and UK are part of this network.
Visits will be carried out at the study site. Alternative options including home visits, telemedicine, phone calls, video chats or other ways of communication may also be used as an exception if needed to substitute or supplement on-site visits and to reduce the burdens of study participation for the child and parents/guardians. Blood draws may also be delegated to a local physician of the child.
The study duration for individual children will depend upon when they are enrolled into the study. The minimum duration of study participation for individual children will be from age 3.00 to 4.00 months until age 2.5 years. This will apply to last child enrolled into the study. The first child enrolled into the study will have a study duration from age 3.00 to 4.00 months until age 6 years. From age 6 months, children will be randomised to COVID-19 vaccine or placebo. Children will be administered three vaccinations from age 6 to 7 months, 3 to 6 weeks after 1st vaccination and 8 weeks after the 2nd vaccination with either Comirnaty (Comirnaty® 3 µg Omicron XBB.1.5) or Placebo followed by a blinded follow-up (FU) period. The period to enrol all 2252 subjects is expected to take 39 months. Children for whom consent is withdrawn prior to randomisation or develop diabetes before randomisation will be replaced.
Study Procedures are summarized in Appendix A. In case of unforeseen circumstances, e.g. lockdowns, every endeavour will be made to safeguard the study conduct including data collection without compromising the participants' safety.
An independent Data Safety Monitoring Board (DSMB) will be commissioned to continually assess the conduct of study. Periodic review of clinical data will also be conducted by a medical monitor.

7. PARTICIPANT IDENTIFICATION

7.1. Trial Participants

In the GPPAD-02 study, blood is collected using filter paper cards shortly after birth. Children are tested for genetic risk of type 1 diabetes based on risk scores derived from SNPs that define HLA DR3, HLA DR4, HLA DQ8 and HLA DQ7 alleles as well as SNPs from the HLA region, including HLA class II protective alleles and non-HLA type 1 diabetes susceptibility genes. Children with a predicted risk of >10% to develop islet autoimmunity by age 6 years and who fulfil the inclusion criteria as stated below will be asked to participate in the GPPAD-05 AVAnT1A Study.

7.2. Inclusion Criteria

Each potential subject must satisfy all of the following criteria in order to permit enrollment in the study:

1. Age between 3.00 and 4.00 months at the time of enrolment.
2. A high genetic risk (>10%) to develop islet autoantibodies by age 6 years:
   a. For children without a first-degree family history of type 1 diabetes, high genetic risk is defined as a DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8 or DR3/DR4-DQ7 rs6901541 C/T genotype and an elevated sex-specific genetic risk score that is at the 98.75th centile, in other words identifies around 1.25% of newborns without a first-degree family history with type 1 diabetes.
   b. For children with a first-degree family history of type 1 diabetes, high genetic risk is defined as having HLA DR4 and DQ8, none of the following protective alleles: DRB1*1501, DQB1*0503, DRB1*1303, and a sex-specific genetic risk score >50th centile of the background population. These represent around 25% of children with a first-degree family history of type 1 diabetes.
3. Written informed consent signed by the custodial parent(s).

7.3. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study:

1. Previous hypersensitivity to the excipients of the vaccine. These include:
   ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315)
   2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159)
   1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC)
   cholesterol
   trometamol
   trometamol hydrochloride
   sucrose
2. Any medical condition, concomitant disease or treatment that may interfere with the assessments or may jeopardize the participant's safe participation in the study. These include immune deficiencies, and conditions or treatments that lead to immune suppression.
3. Likely poor compliance due to expected change in residency.
4. Diagnosis of diabetes prior to recruitment or randomisation.
5. Current use of any other investigational drug.

8. TRIAL PROCEDURES

8.1. Screening and Eligibility Assessment

Prior to enrolment, children with genetic risk for type 1 diabetes will be identified in the GPPAD-02 study. The screening results of the GPPAD-02 study will be discussed with the custodial parents of these children. This can be conducted as on-site visit, home visit or remotely (e.g. by phone call or video conference).

During this discussion, inclusion and exclusion criteria of the GPPAD-05 AVAnT1A Study will be assessed and the opportunity to participate in the study will be discussed.

8.2. Informed Consent

The GPPAD-05 AVAnT1A Study will be described to the custodial parent(s) of potential participants by qualified GPPAD study personnel according to national regulations. The custodial parent(s) will have the opportunity to read the consent document and to discuss any questions concerning the consent or study participation. If participation in the study is considered by the family, they may review the informed consent form at home to discuss with family or whomever they trust. 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 form prior to or at the baseline visit. The signature of the custodial parent(s) indicates that he/she understands the meaning, the potential risks and benefits of study participation.

As statement of the information process and assurance that the custodial parent(s) have understood all obligations and study procedures, date and signature of the study Investigator (or according to national regulations other authorized study personnel, if applicable) will also be obtained on the consent form.

A copy of the informed consent form will be handed out to the families. The custodial parent(s) of the prospective participant will be told that being in the study is voluntary and that the participant may withdraw from the study at any time, for any reason.

The trial is performed for infants aged 3 to 4 months with treatment administered between age 6 months to 12 months and follow-up to a maximum age of 6 years. In assessing whether the trial could be conducted in older children or adults, it was considered that attempts to prevent islet autoimmunity should start prior to the peak incidence of islet autoimmunity at 1 to 3 years of age. While islet autoimmunity can develop in older children, the risk for seroconversion is greatly reduced after age 3 years [79], making a primary prevention trial necessitating a considerably larger number (>5-fold) of participants if performed in older children. Furthermore, in this specific case, the potential benefits are expected to be greatest for children before age 2 years (62). As a result, the trial is specifically conducted in young children, and assent for participation in the trial will not be obtained because it is not possible in this age group.

The informed consent form must be revised whenever any new information becomes available that may affect participation in the study.

According to the EU regulations, national laws and regulations information regarding data processing and privacy rights has to be provided and consent must be achieved.

Legal consenting requirements currently vary between countries. In any case, study sites must adhere to country specific requirements and the requirements of the sponsor.

8.3. Randomisation

Subjects will be randomised in a 1:1 ratio to COVID-19 vaccine or placebo. Enrolment and randomisation should only occur if the subject meets the inclusion criteria and none of the exclusion criteria and after written consent has been obtained by the custodial parent(s). Siblings within one household will be randomised to the same intervention arms. Participants for whom consent is withdrawn prior to randomisation will be replaced.

8.4. Blinding and code-breaking

The participant and the treating physician and the central research team will be blinded for the vaccination arms of the trial. Injection syringes will not indicate whether the content is Comirnaty or placebo, but random numbers. They will be prepared by an unblinded pharmacist. A randomisation system will assign

the appropriate numbers for each participant following a randomisation list. For further details please refer to the IMP manual.
Although SARS-CoV-2 antibodies will be measured during the study, these will be performed by operators who are not directly involved in the AVAnT1A study. Results for individual participants will only be made available to the independent study statistician. These results will be analyzed by the independent study statistician and a summary presented to the DSMB members. Individual results and summaries will not be seen by participants of the study or their family members and will not be seen by other study personnel, including the study sponsor and principal investigator until completion of the study. Therefore, the risk of unblinding of treatment arms to the participating family or study site through the SARS-CoV-2 antibody measurements will be negligible.
Emergency unblinding will be available through the randomisation system or emergency envelopes. In case of temporary unintended unavailability of the system a 24h hotline is available likewise.
Unblinding of treatment assignment is planned to be conducted upon completion and verification of the study, closure of database and completion of outcome and confounder parameter measurements. It will occur upon approval of the Sponsor and Protocol Chairs. There are special provisions for the DSMB.

8.5. Baseline Assessments

Visit 1 will occur at the age of 3.00 to 4.00 months.
The clinical assessment at Visit 1 includes the following procedures:

- Review of inclusion-/ exclusion criteria
- Medical history including birth weight
- Physical examination
- Height & body weight measurement
- Capillary blood draw for
    - Virus antibodies (see 5.1.3 Exploratory objectives for list of virus antibodies measured in multiplexed panel)
    - Inflammatory protein panel
- Saliva collection for detection by qPCR of SARS-CoV-2 virus and other viruses (see 5.1.3 Exploratory objectives for list of viruses measured in multiplexed qPCR panel, which will be analysed outside of this trial).

All families will be instructed to look for symptoms of infection and how to complete the e-diary entries.
All families will receive saliva collection packages for weekly collection as well as instructions for storage and transport to study site.
All families will receive stool collection packages for monthly collection as well as instructions for storage and transport to study site.

8.6. Subsequent Visits

Visit 2

Visit 2 will occur at age 6 months (in a window of 6 months + 28 days). Visit 2 may not occur before the child has reached age 6 months.

The clinical assessment at visit 2 includes the following:

- Review of exclusion criteria
- Physical examination
- Height & body weight measurement

- Discussion of electronic questionnaires (related to infections & COVID-19)
- Venous Blood draw for
    - Islet autoantibody test
    - Virus Antibodies including SARS-COV-2 antibodies*
    - Inflammatory protein panel
    - Multiparameter flow
    - Cell isolation (freezing/RNAseq)
    - RNA sequencing
    - Pre-prandial and 30min post-prandial glucose, insulin, C-peptide, proinsulin
    - Metabolomics

Children will be randomised to COVID-19 vaccine or placebo.
The first vaccination will be administered after the clinical assessment and blood draw.
There will be close observation for at least 15 minutes following the administration of the vaccination.
All families will receive saliva collection packages for weekly collection as well as instructions for storage and transport to study site.
All families will receive stool collection packages for monthly collection as well as instructions for storage and transport to study site.
* SARS-CoV-2 antibody measurements at this and subsequent visits will be performed by operators who are not otherwise involved in the AVAnT1A study. The individual participant results will not be reported to families or AVAnT1A study personnel except for the independent study statistician.

Visit 3

Visit 3 will occur 3 to 6 weeks after visit 2 (+4 days).
The clinical assessment at visit 3 includes the following:

- Physical examination
- Height and body weight measurement
- Assessment of AEs and SAEs
- Discussion of electronic questionnaires (related to infections & COVID-19)
- Capillary Blood draw for
    - Virus antibodies
    - Inflammatory protein panel
    - Pre-prandial glucose

The second vaccination will be administered at visit 3. Vaccination will be performed after the clinical assessment and capillary blood draw.
There will be close observation for at least 15 minutes following the administration of the vaccination.
All families will receive saliva collection packages for weekly collection as well as instructions for storage and transport to study site.
All families will receive stool collection packages for monthly collection as well as instructions for storage and transport to study site.

Visit 4

Visit 4 will occur 8 weeks (+14 days) after visit 3 at approximately age 8.5 to 11 months.
The clinical assessment at visit 4 includes the following:

- Physical examination
- Height & body weight measurement
- Assessment of AEs and SAEs

- Discussion of electronic questionnaires (related to infection & COVID-19)
- Venous Blood draw for
    - Islet autoantibody test
    - Virus antibodies
    - Inflammatory protein panel
    - Pre-prandial and 30min post-prandial glucose, insulin, C-peptide, proinsulin

The third vaccination will be administered at visit 4. Vaccination will be performed after the clinical assessment and blood draw.
There will be close observation for at least 15 minutes following the administration of the vaccination.
All families will receive saliva collection packages for weekly collection as well as instructions for storage and transport to study site.
All families will receive stool collection packages for monthly collection as well as instructions for storage and transport to study site.

Further study visits at the study sites will take place at age 12 months (+/- 14 days), at age 15 months ((+/- 14 days), at age 18 months (+/- 14 days), at age 21 months (+/- 14 days) , at age 24 months (+/- 14 days), at age 30 months (+/- 14 days), at age 36 months (+/- 14 days) and then every 12 months (+/- 30 days) until the end of the study.
The assessment at these visits includes the following procedures:

- Physical examination
- Height & body weight measurement
- Assessment of AEs and SAEs (until 1 month after last vaccination)
- Discussion of electronic questionnaires (related to infections & COVID-19 until age 36 months
- Blood draw* for
    - Islet autoantibody test (all visits)
    - Virus antibodies (all visits)
    - Inflammatory protein panel (all visits except visit 8)
    - Pre-prandial glucose (until visit 11)
    - Post-prandial glucose (only visits 5, 6. 7, 9, 10 and 11)
    - Pre-prandial and 30min post-prandial insulin, C-peptide, proinsulin (only visits 5, 6, 7, 9, 10 and 11)
    - Transglutaminase autoantibody test (only visits 5, 7, 9, 10, 11, 12 and subsequent visits)
    - Whole blood multiparameter Flow (only visits 5, 9 and 11)
    - Whole blood for cell isolation (Freezing/RNAseq) (only visits 5, 7, 9 and 11)
    - Whole blood for RNA sequencing (only visits 5, 7, 9 and 11)
    - Fresh cell assays, selected sites (only visits 5, 9 and 11)
    - DNA for methylation (only visits 5, 9 and 11)
    - HbA1c (only visits 5, 7, 9, 11 and 12+)
    - Vitamin D (only visits 6, 9, 11)
    - Metabolomics (only visits, 5, 7, 9, 11 and 12+)

  *Venous at all visits except visit 8 (age 21 months) which can be a capillary blood draw.
- A psychological assessment questionnaire will be given to the parents at visits 5 and 9 (see section 8.7).

Weekly saliva sample collection and monthly stool sample collection will continue until age 24 months.

An oral glucose tolerance test with venous blood collection at 0 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes will be performed in children who have persistent confirmed islet autoantibodies from age 3.0 years (visit 11).

8.7. Psychological impact of study participation on families

The psychological effect of study participation will be monitored by a questionnaire at visit 5 and visit 9. The questionnaire is preferably completed by each of both parents or custodial parent(s). The questionnaire was previously used the GPPAD-03 POInT study [78]. 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 [78].

8.8. For participants who develop positive islet autoantibodies

If the participant develops one or more confirmed islet autoantibody, information on persistence of a positive islet autoantibody status will be obtained at the next study visit. If the participant develops one or more confirmed islet autoantibodies at age 3.0 years or later, when follow-up visits only occur yearly, the participant should have a persistency sample drawn within 4-12 weeks. The collection of venous or capillary blood for the persistency sample can be obtained by a local physician and shipped to the study site.
Custodial parent(s) will be informed when a child has developed persistent confirmed islet autoantibodies. The parents will be asked to participate in an educational program informing about the diagnosis of islet autoantibody positivity. Contents of the education will be:

- Information what the diagnosis “islet autoantibodies” means
- How to recognize clinical symptoms of type 1 diabetes
- How to self-monitor blood glucose

Home monitoring of blood glucose will be recommended if a child has clinical symptoms of type 1 diabetes, or any infection or fever; Home monitoring will also be recommended routinely every 2-months if a child is considered at risk for a rapid progression to diabetes (eg IA-2A positive, very high titers of antibodies, or impaired blood glucose values). Children who develop persistent confirmed islet autoantibodies will have oral glucose tolerance tests including assessments of C-peptide from age 3 years at each study visit. They will remain in the study until they develop clinical type 1 diabetes.

8.9. For participants who developed positive transglutaminase autoantibodies

If the participant develops confirmed transglutaminase autoantibodies, information on the persistence of a positive transglutaminase autoantibody status will be obtained at the next study visit. If the participant develops confirmed transglutaminase autoantibodies at age 2 years or later, the participant should have a persistency sample drawn within 4-12 weeks. The collection of venous or capillary blood for the persistency sample can be obtained by a local physician and shipped to the study site.
Custodial parent(s) will be informed when a child has developed persistent confirmed transglutaminase autoantibodies. A consultation of the treating physician or specialist for celiac disease will be recommended to them in this case. Children with transglutaminase autoantibodies or celiac disease will remain in the study.

8.10. Sample Handling

<u>Sample handling for study purposes:</u>
Samples for determination of islet autoantibodies, transglutaminase autoantibodies, and RBD and NP antibodies to SARS-CoV-2, and C-peptide and insulin are shipped to the central laboratory at the Institute of

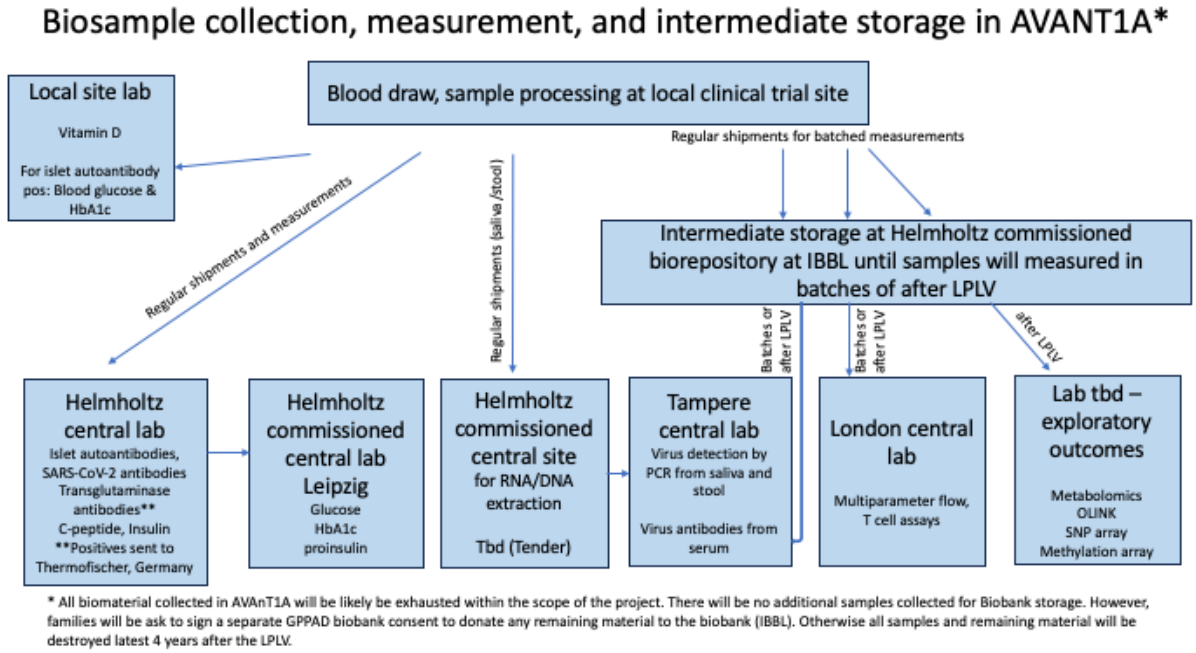
Diabetes Research, Helmholtz Munich, Germany. Autoantibody measurements will be performed in real time. For confirmation of positive transglutaminase autoantibodies, samples will be shipped to a second central laboratory, Thermo Fisher Scientific, Freiburg, Germany. Samples for glucose, proinsulin, and HbA1c will be determined centrally at the laboratory at the Universitätsklinikum Leipzig, Institut für Laboratoriumsmedizin, Klinische Chemie und molekulare Diagnostik, Leipzig, Germany.

Saliva and stool sample RNA and DNA will be extracted and sent to the Faculty of Medicine and Health Technology, Tampere University of Applied Sciences, Finland for virus detection by qPCR. Saliva and stool samples will be discarded after extraction.

Blood for whole blood multiparametric flow will be collected and frozen at -80°C in medium containing DMSO following a standard operating procedure and sent to the Guy's Hospital Campus of King's College London for analysis. Blood for the isolation and freezing of peripheral blood mononuclear cells (PBMC) will be processed locally following a standard operating procedure. The frozen PBMC along with aliquots of serum and samples for future transcriptomic, DNA methylation, metabolomics, viral antibodies, and inflammation markers are first stored locally until shipping to the Helmholtz commissioned biorepository at IBBL (Integrated BioBank of Luxembourg), Luxembourg Institute of Health for intermediate storage. These exploratory measurements will be measured centrally and either in batches throughout the study or at the end of the study after all samples have been obtained. The measurement of virus antibodies (Enteroviruses, Rotavirus, Human Coronaviruses-NL63, -229E, -OC43, and -HUK1, influenza A, Rhinovirus, Adenovirus, Bocavirus, Norovirus, Astrovirus) will be performed at the Faculty of Medicine and Health Technology, Tampere University of Applied Sciences, Finland.

Laboratories for assessments of other exploratory outcomes will be selected according to the best performance and costsbefore the end of the trial. Samples will be stored for a maximum of 4 years after LPLV for measurements to be completed. The samples will then be destroyed.

All biomaterial collected in AVAnT1A will be likely be exhausted within the scope of the project. There will be no additional samples collected for Biobank storage. Nevertheless, separate to the study protocol, families will be asked to donate for research purposes any remaining sample after the specified measurements have been performed. These samples will be stored at the Helmholtz-commissioned biobank, IBBL.



* All biomaterial collected in AVAnT1A will be likely be exhausted within the scope of the project. There will be no additional samples collected for Biobank storage. However, families will be ask to sign a separate GPPAD biobank consent to donate any remaining material to the biobank (IBBL). Otherwise all samples and remaining material will be destroyed latest 4 years after the LPLV.

8.11. Summary of known and potential risks and benefits

8.11.1 Benefits

The potential benefit for a participating child would be the prevention of emerging islet autoantibodies that occur during or after a COVID-19 infection. A benefit would also be a marked delay in the development of islet autoantibodies, dysglycaemia, or diabetes. Because all participating children, including children who receive placebo, have a high risk (>10% and almost 20% for children who have a COVID-19 infection) of developing islet 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 islet autoantibody positive, will be invited to receive education and teaching to learn about the risk of hyperglycaemia and means to prevent diabetic ketoacidosis. Participation in other studies that have a positive influence on the course of T1D may be possible, and can be discussed if suitable for the child. If a participating child develops type 1 diabetes 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 [80, 81] 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. A benefit for the children who are assigned to the COVID-19 vaccine arm of the trial is a relative protection against COVID-19 infection and the potential consequences of a COVID-19 infection.

8.11.2 Risks

Comirnaty, BioNTech's mRNA vaccine against COVID-19 used in this study, has been shown to be safe and effective in infants and is approved for children 6 months of age and older. Treatment with Comirnaty may cause adverse effects or discomfort. In children aged 6 to 23 months, very common side effects include irritability, drowsiness, loss of appetite, tenderness or redness at the injection site and fever. Further details can be found in the SmPC.

Other viral infections such as Coxsackie B virus infection are associated with the development of islet autoantibodies. Therefore, an efficacy of COVID-19 vaccination in preventing islet autoimmunity associated with COVID-19 infection will not prevent the occurrence of islet autoimmunity associated with other etiology. The development of islet autoantibodies or type 1 diabetes may alter the behavior of parents or guardians to the child and may affect further family planning or might cause psychological stress. Parental/guardian well-being is monitored via a psychological questionnaire (Appendix D). The development of islet autoantibodies may also affect insurance options of the participant.

Study-related measures such as blood sampling may be associated with risks or lead to discomfort. 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. It is very rare for infections, blood clots or bleeding to occur in the punctured vein and, in extremely rare cases, nerve damage. It is possible that blood may also have to be taken from the head (in the case of difficult vein conditions). This is not more painful or associated with greater risks than taking blood from a vein in the arm or hand.

The study involves a large number of procedures, especially up to age 2 years. These include frequent study visits and blood draws, up to weekly collection of saliva samples, monthly collection of stool sample and responding to questions fortnightly. This is considerably more than what is standard care during this

age and may be burdensome for children and participating families. The extent of the procedures as compared to standard care will be explained during the consent process. In a previous GPPAD conducted study (POInT), which enrolled children from age 4 months for a study duration and with a blood draw schedule similar to that of AVAnT1A, the study drop-out rate was low (<10%). Therefore, the study procedures are considered tolerable to the majority of participants and families. The psychological questionnaire will monitor and identify families requiring attention due to participation demands and solutions to reduce the demands such as less frequent sampling or questionnaires will be discussed with these families.

Children randomized to the placebo arm of the trial will be denied access to the benefits of a COVID-19 vaccine, including the possible prevention of serious early and late complications from a COVID-19 infection. The current number of children below age 5 years who are provided this benefit through vaccination is extremely low (<0.1%). Further mitigation of the risk includes 1. the exclusion of children with diseases or treatments that lead to immune deficiency. This exclusion helps minimize the potential risk for this specific group of participants. 2. including the benefits and risks of a COVID vaccination in the consent process as well as informing those families who intend to and can pursue the benefits of vaccination in their country of their options to do so, including declining participation in the trial. For those unsure, the option to withdraw at any time is emphasized.

8.11.3 Benefit/risk ratio summary

Benefits:

- Regular monitoring and care of the child at high risk of type 1 diabetes
- Prevention of diabetic ketoacidosis, symptoms, and hospitalization through early care
- If islet autoimmunity is diagnosed, possible availability of other disease-modifying therapies
- Protection against COVID-19 infection and its early and late complication in children randomized to vaccine
- Potential protection from islet autoimmunity and diabetes in children randomized to vaccine

Risks and measures:

- Side effects of IMP. These are minimal in comparison to the benefits of the IMP, which is approved.
- Frequent blood draws. Discomfort is minimized by the use of an anaesthetic cream.
- Intense follow-up as compared to standard care. Information material will be developed to explain to families and children why we are collecting the material.
- Behavioural changes and psychological impact. This will be monitored by questionnaires. Parents with a high level of anxiety or distress will be contacted and may be advised to consult a psychologist. Potential impedance to benefits of COVID-19 vaccination in children randomized to the placebo arm. Mitigation of this risk by excluding children with conditions or treatment leading to immune deficiency and by informing families intending to vaccinate their children of the risk of receiving placebo and their options with respect to declining participation or withdrawal. Because of the current infrequent (<0.1%) practice of vaccinating children below age 5 years in the participating countries the overall risk of impedance is considered low and considerably less than the benefit of providing vaccination to 50% of the participants.

With the incorporated mitigation of risks, the overall benefits of participation are considered to outweigh the risk.

8.12. Early Discontinuation/Withdrawal of Participants

During the course of the trial a participant may choose to withdraw at any time. This may happen for a number of reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable AE.
- Participant decision
- Inability to comply with trial procedures

Participants may choose to stop treatment and/or study assessments but may remain on study follow-up. Participants may also withdraw their consent, meaning that they wish to withdraw from the study completely.

In addition, the investigator may discontinue a participant from the trial treatment at any time if the Investigator considers it necessary for any reason including, but not limited to:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures

The type of early discontinuation and reason for withdrawal will be recorded in the eCRF. Every effort will be made to follow all participants enrolled in the study (including those that do not complete the treatment period).

Families who decide to pause participation but do not explicitly end participation, are welcome to re-join active participation at any time. They will be regularly contacted to ensure the timely capture of the development of clinical type 1 diabetes in the child.

If families withdraw consent but agree to further assessments and/or collection of data in the study database after official withdrawal of the study, they will be asked to confirm this in writing by signing a re-consent form.

8.13. Definition of End of Trial

The end of study is the point when the last enrolled participant has completed the follow-up visit at age 2.5 years (this will be LPLV (Last Patient Last Visit)), and the aim is that at this point, the data have been entered as complete as possible and queries resolved – by the latest 6 months after the last patient had the last visit.

Follow-up for other participants, who are enrolled before the last participant, will be terminated either on the LPLV date or when that particular individual turns 6 years of age – whichever occurs first.

The trial may be terminated earlier for the following reasons:

- Observed efficacy of a COVID-19 vaccination in preventing islet autoantibody development
- Evidence of adverse events associated with COVID-19 vaccination or participation in the trial
- Futility (the likelihood of failing to reject the null hypothesis). This may be due to changes that would necessitate the inclusion of more than 2,400 participants to test the hypothesis. These changes include a much lower than predicted COVID infection rate by age 2.5 years, a larger than predicted dropout rate, a lower than predicted primary outcome in the placebo group or low treatment efficacy, and a low efficacy of the COVID-19 vaccine against COVID-19 infection from future variants. Futility will be assessed by the independent study statistician.
- A need to reallocate COVID-19 vaccines for national safety. In this unlikely event, the decision lies in the provider of the vaccine (German government).

9. TRIAL INTERVENTIONS

Subjects will be randomly assigned in a 1:1 ratio to receive Comirnaty (Comirnaty® 3 µg Omicron XBB.1.5 or future new variant developments replacing current Comirnaty vaccines) or placebo.
The IMP will be administered as intramuscular injection – 3 injections in total (of 3 µgs each):
1st dose at age 6 months*
2nd dose 3 to 6 weeks after 1st dose*
3rd dose at least 8 weeks after 2nd dose (around age 8.5 to 11 months)*
* The vaccination schedule will be modified if the family reports a COVID-19 infection (PCR-proven) in the participant with prior to the first vaccine or between vaccinations. These participants will receive 2 vaccine or placebo injections. Administration of vaccine or placebo will occur at least 3 months after infection.

No further dose will be given to those participants that have experienced anaphylaxis following a prior dose of the vaccine.

9.1. Investigational Medicinal Product(s) (IMP) Description

The IMP (mRNA vaccine against COVID-19) will be distributed by University Hospital Heidelberg, Pharmacy
Initial Active vaccine:

Name:	Comirnaty Omicron XBB.1.5 3 µg/dose
Manufacturer:	BioNTech/Pfizer Manufacturing GmbH
Active ingredient:	3 µg Raxtozinameran (Comirnaty Omicron XBB.1.5)
Formulation:	concentrate for dispersion for injection after dilution
Dose strength:	3 µg / dose

For further information also refer to the Summary of Product Characteristics (SmPC).
0.9% Sodium Chloride solution for injection (saline) will be used as the placebo in this study. .

9.1.1. Blinding of IMPs

The comparison of Comirnaty with placebo is double-blind; therefore, the injection syringes containing either the vaccine or placebo will look similar and will only show random numbers but not indicate whether the content is Comirnaty or placebo. The injection syringes will be prepared by an unblinded pharmacist. Further details are described in the IMP Manual.
The use of placebo in relation to Regulation No. 536/2014 was extensively considered and discussed within the protocol committee and with the advisory board including members of STIKO prior to finalizing the study design. The decision to include a placebo was guided by two primary rationales. First, the COVID-19 vaccine is available from age 6 months in all participating countries, albeit not currently recommended at this age (with the exception of Poland the Ministry of Health announcements). In the other participating countries, the vaccine is recommended only for at-risk children. Second, the current utilization of the COVID-19 vaccine in children is notably low across all participating countries, estimated at <0.1% of children aged 6 months to 4 years. For example, in Germany, the Robert Koch Institute reports only 2,083 fully COVID-19 vaccinated children in this age range since approval of use. In our currently ongoing trial (SINT1A) none of nearly 1,000 children aged less than 2 years have been vaccinated. This includes over 200 children from Poland. Consequently, the study is anticipated to enhance COVID-19 vaccination rather than impede access to the vaccine, a characteristic viewed positively by vaccination experts. The informed consent process will communicate that families intending to vaccinate their child against COVID-19 may choose not to participate in the trial or may withdraw from the study. The ICF has been revised accordingly. Throughout

the deliberations, alternative study designs and in particular an active control design were considered but ultimately dismissed. These alternatives included randomization to COVID-19 vaccination or another suitable vaccine. The influenza vaccine was contemplated, but its seasonal administration in two doses rendered it impractical. Notably, children who would be randomized to a flu vaccine in March to August would have little or no protection against flu in the following winter, but may appear to be protected and the appropriate seasonally-adapted vaccine dismissed. A MenACWY vaccine was also evaluated as a comparator, but its recommendation varies significantly in the participating countries. In Germany, it can be administered as a single dose for infants and in primarily healthy children it is recommended in the second year of life. In Poland, it is recommended earlier. Furthermore, recommendations for this vaccine across European countries are undergoing changes. Consequently, using it as a comparator poses a potential risk to impede access to a vaccine that is or becomes both generally recommended and utilized. Other vaccinations are impractical as a comparator as they are fully integrated in the in the respective state-recommended vaccination schedule. The prospect of a non-randomized open trial was discussed but discarded due to concerns about enrollment bias and the inability to draw conclusive findings on the efficacy of the treatment in reducing the incidence of islet autoimmunity and type 1 diabetes from an open, non-randomized trial. Additionally, participating in a six-year study that produces inconclusive results would not be advantageous for families.

9.1.2. Storage of IMP

IMP (active ingredient) will be stored in a closed, limited access area at the pharmacy at -90°C to -60°C and protected from light until use.

9.1.3. Accountability of the Trial Treatment

Full accountability will be performed and documented on a drug accountability form.

9.1.4. Prohibited Concomitant Medications

None.

9.1.5. Restricted Medications

No research has been conducted regarding the interaction of the IMP (active ingredient) and other medicinal products. Therefore caution should be taken in this regard.

10. SAFETY REPORTING

The investigators are responsible for the recording and reporting of adverse events (AE) observed from randomisation until 1 month after administration of the study medication. SAE's will be recorded and reported from the time of consent until 1 month after administration of the study medication. All adverse events should be followed until event resolution or stabilisation or participant completion of the trial, whatever occurs first.

10.1. Adverse Event Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • results in a congenital anomaly or birth defect Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out in the approved SmPC

10.2. Assessment of Causality

The relationship of each adverse event to the investigational medicinal product will be determined by the site investigator. The site investigator will also record the assessment of causality in the 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 following definitions:

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).
SAEs reported as possibly, probably or definitely related will be managed as related i.e., may be assessed as a SUSAR.

10.3. Procedures for Reporting Adverse Events

All AEs occurring during the safety window for the trial as defined above that are observed by the Investigator or reported by the participant, will be reported on the eCRF, whether or not attributed to the study medication.
The following information will be reported on the eCRF: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.
The severity of events will be assessed according to the FDA - Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trial (September 2007)*.
It will be left to the investigator's clinical judgment to decide whether or not an AE requires the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE.

10.4. Reporting Procedures for Serious Adverse Events

All SAEs other than those defined in this protocol as not requiring reporting must be reported on the SAE Reporting Form to Dr. Nibler & Partner immediately or within 24 hours of site study team becoming aware of the event being defined as serious.

10.4.1. Events exempt from immediate reporting as SAEs

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.

10.4.2. Procedure for immediate reporting of SAEs

- Site study team will complete an SAE report form for all reportable SAEs.
- The SAE report form will be faxed or scanned and emailed to Dr. Nibler & Partner, within 24 hours of site study team becoming aware of the event.
- Site study team will provide additional, missing or follow up information in a timely fashion.
- All SAE Reports will be received, processed and assessed by Dr. Nibler & Partner as outlined in the safety management plan and applicable SOPs. This includes assessment of reported serious adverse events with respect to:
  - seriousness
  - causality
  - expectedness
  - impact on current risk-benefit assessment and study procedures.

10.5. Expectedness

Expectedness will be determined according to the approved SmPC.

10.6. SUSAR Reporting

All SUSARs will be reported to the relevant Competent Authority and to the ethics committees and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days

after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

10.7. Development Safety Update Reports

DSURs will be submitted once a year throughout the clinical trial, or on request to the Competent Authority, Ethics Committee and Sponsor.

11. STATISTICS

A general description of the statistical methods to be used to analyse the efficacy and safety data is outlined below. Specific details will be provided in the Statistical Analysis Plan (SAP) developed before the LPLV. Descriptive statistics (e.g. mean, median, standard deviation [SD], minimum, and maximum) will be used to summarize continuous variables. Counts and percentages will be used to summarize categorical variables. Graphic data displays may also be used to summarize the data. Efficacy analyses will be based on the modified intent-to-treat (mITT) population (i.e. all randomised subjects who have received at least 1 dose of study drug). Subjects included in the efficacy analyses will be summarised according to their assigned treatment group regardless of whether or not they received the assigned treatment. Safety analyses will include all subjects who received at least 1 dose of study treatment. The baseline measurement is defined as the closest measurement taken at or before the time of the week 0 administration of study drug for the double-blind period. The analyses will be performed using R (version 4.1.2) and SAS 9.4.

11.1. Subject Information

For all subjects who receive at least 1 dose of study drug, descriptive statistics will be provided. Subject baseline data, demographic, and baseline clinical disease characteristics will be summarized for all mITT subjects.

11.2. Sample Size Determination

The sample size calculation is based on the numbers needed to test the hypothesis that COVID-19 vaccination will reduce the incidence rate of islet autoantibodies in children with a high genetic risk to develop type 1 diabetes.

The trial numbers have been determined for 70% power to detect a 70% reduction in islet autoantibodies in the treated group who will have been exposed to COVID-19 at a two-tailed significance of 0.05. The expected infection rate until age 2.5 years used to determine the trial numbers is 60%. The trial numbers are based on a 2-fold increase in the risk for islet autoantibodies in infected versus non-infected children. Therefore, the risk by age 4 years is expected to be 8.75% in uninfected children and 17.5% in infected children. A vaccination is supposed to reduce the additional risk of 8.75% in infected children by 70% to 2.625%, resulting in a total risk of 11.375%. The Hazard ratio with these assumptions is: **Hazard in placebo group / hazard in vaccination group** = **1.38397**. This is derived from the exponential distribution assumption and the following calculations.

- 0.4 x 8.75% + 0.6 x 17.5% = 14% probability of islet autoantibody occurrence in the placebo group. The time-invariant hazard is then calculated to be 0.04309. Accordingly, median time in the placebo group for freedom from islet autoantibody occurrence is 16.08603 years.

- 0.4 x 8.75% + 0.6 x 11.375% = 10.325% probability of islet autoantibody occurrence in the vaccination group. The time-invariant hazard is then calculated to be 0.03114. Accordingly, median time in the vaccination group for freedom from islet autoantibody occurrence is 22.25906 years.

With an accrual time of 3.25 years, an additional follow-up of 2 years and accounting for an assumed 15% rate of loss to follow-up, a total of 2252 children would have to be included into the study. The sample size calculations were performed with the program "PS Power and Sample Size Calculations, Version 3.1.2" (Dupont WD, Plummer WD. PS power and sample size program available for free on the internet. Controlled Clinical Trials. 1997; 18:274). Sample size calculation in this program is based on the work of Schoenfeld and Richter (Schoenfeld DA, Richter JR. Nomograms for Calculating the Number of Patients Needed for a Clinical Trial with Survival as an Endpoint. Biometrics. 1982; 38 (1):163-170).

Statistical power will depend on the actual recruitment rate and the infection rate in the placebo group. Therefore, the recruitment rate and an estimate of the actual exposure based on SARS-CoV-2 antibodies and PCR on saliva will be measured during the trial and independently monitored in the placebo group so that number of recruited trial participants or the length of follow-up may be adjusted if infection deviates substantially from the expected 60%. IgG and IgA antibodies to SARS-CoV-2 RBD and NP will be measured in each participant during the study. Results will be interpreted as consistent with infection (RBD and NP antibody positive, IgA antibody positive), consistent with vaccine (RBD antibody positive, NP antibody negative, IgA antibody positive), consistent with only maternally transferred antibodies (declining titres, IgA antibody negative) or negative. These antibody results will not be reported to the families of study participants. These and the weekly obtained saliva SARS-CoV-2 virus results (positive or negative) for each participant will only be provided to the independent statistician. The independent statistician will tabulate for each of the children who receive placebo the occurrence and the sample date(s) or infection defined as positive PCR in saliva and/or SARS-CoV-2 antibody profile that is 'consistent with infection'. From the 2-year time point of the study and at 12-monthly intervals, the independent statistician will provide a Kaplan-Meier curve summary of the frequency of children treated with placebo who have a COVID-19 infection over time to the DSMB who will assess the cumulative frequency against the predicted frequency, the rate of recruitment, the primary outcome rate, and the frequency of drop-outs. The independent statistician will compare the cumulative incidences of the primary outcome with the probabilities to be expected according to the assumptions of the sample size calculation. Furthermore, the number of observed events with respect to the primary outcome will be compared with the number of events according to the formula of Lakatos (Lakatos E, Richter JR. Sample Sizes Based on the Log-Rank Statistic in Complex Clinical Trials. Biometrics. 1988; 44 (1):229-241). Futility will be judged if more than 2,400 children followed for more than 2 years after LPFV are required for the trial to have at least 70% power to achieve its specified outcome. For example, this corresponds to an infection rate of around 57% by age 2.5 years. In case of a higher than predicted rate of COVID-19 infection, the trial may be adjusted to include a lower total number of participants or shorter follow-up. The guiding principles for such a decision will be that the reduced trial numbers should allow for at least 80% power to achieve the specified outcome. Calculations for the adjustment of trial numbers will be performed by the independent study statistician and discussed within the Data Monitoring Committee. Adjustments will require approval by the GPPAD steering committee.

11.3. Analysis Populations

All efficacy analyses will be performed on the mITT population unless otherwise specified. The mITT population includes all randomised subjects who have received at least 1 dose of study drug. An analysis will also be performed on the per protocol population that includes all participants who complete the 3 vaccination visits.

Children, who are already islet autoantibody positive at baseline after exclusion of maternally acquired islet autoantibodies will be excluded from the full analysis data set for the analysis of the primary outcome (mITT). Children, who are already islet autoantibody positive at baseline after exclusion of maternally acquired islet autoantibodies will be included in a sensitivity analysis of the primary outcome (ITT).
Children, who are islet autoantibody positive at baseline after exclusion of maternally acquired islet autoantibodies will be included in the secondary outcome 'development of type 1 diabetes' 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.

11.4. Primary Endpoint

The primary efficacy endpoint for the vaccination trial is the elapsed time from random treatment assignment to the development of persistent confirmed islet autoantibodies or type 1 diabetes as detailed in section 5. For the development of persistent confirmed islet autoantibodies, the observation time will be censored at the last time when no persistence of a confirmed islet antibody could be verified in the corresponding blood sample. Regarding type 1 diabetes, the observation time will be censored for children who are free from diabetes at the time of their last contact with the trial centre. For the primary endpoint considering either the development of a persistent confirmed islet autoantibody or type I diabetes – whatever occurs first – observation time will be censored at the last time when neither the persistence of a confirmed antibody nor type I diabetes was recorded, taking the minimum of both times into account. The cumulative incidences of islet autoantibodies over time since randomisation within each treatment group will be estimated by the Kaplan-Meier method. The overall difference between the groups in the cumulative incidence functions will be tested by the log-rank test at the two-sided significance level of 0.05. The hazard ratio of the two groups and its 95% confidence interval will be determined by the Cox model. As a sensitivity analysis, the hazard ratio of the two groups will be assessed using the Cox regression when including site as covariate. The estimates of cumulative incidence and the log-rank test will adjust for periodic outcome assessment visits to assess islet autoantibody status.
While the trial is ongoing, the current vaccine intended for use may be substituted with an updated version, ensuring at least non-inferiority in reducing the occurrence of SARS-CoV2 infection. Consequently, the positive vaccination effect on the primary outcome is likely to be enhanced, and no adverse impact is anticipated on the final trial outcome due to the introduction of a new vaccine. In the event of vaccine replacement during the study, a sensitivity analysis will be performed, evaluating the hazard ratio of the two groups through Cox regression. The sensitivity analysis will include the vaccine variant as a covariate, with children assigned to the vaccine category for which they received the most numbers of doses. If children only receive two doses, they will be assigned to their second vaccine category.
In line with real-life scenarios, children acquiring a SARS-CoV2 infection during the 3-4 months vaccination period will remain part of the modified intention-to-treat (mITT) population. As part of a sensitivity analysis, the hazard ratio for the two groups will be assessed, considering children who have completed all three injections with the vaccine or placebo without a prior COVID-19 infection. This analysis will use the date of the last injection as the starting point for follow-up.

11.5. Handling of missing and spurious data

Reasons for missing data include missed visits, withdrawal of consent, loss-to-follow-up, or the unlikely event of death. Missed visits will not be counted or imputed in the assessment of the primary or secondary outcomes or in defining SARS-CoV-2 antibody status. It is expected that either an event or complete follow-up until the end of the trial will be documented for at least 85% of the children (drop-out rate 15%), which

is considered in the sample size. To address potential issues, consistent monitoring of the centers, including their recruitment, dropout rates, and timely documentation of data in the eCRF, will aid in early identification of problems and mitigating the incidence of missing data. Emphasizing the advantages of trial participation for children and providing support to parents will help maintain high motivation and compliance. Furthermore, recruitment, dropout rates, and the completeness of data documentation are reported and assessed at 6-monthly DSMB meetings. The monitoring manual, along with reviews by a monitor, support correctness, plausibility, and completeness of data. Unusual or potentially spurious data undergo queries, and the occurrence of unused data is not expected.

11.6. Secondary Endpoints

1. The elapsed time from random treatment assignment to the development of multiple islet autoantibodies.
2. The elapsed time from random treatment assignment to the development of type 1 diabetes.
3. The elapsed time from random treatment assignment to the development of persistent confirmed transglutaminase antibodies.

The secondary endpoints will be analysed using the same statistical methods as the primary endpoint.

11.7. Safety Endpoints and Analyses

Safety assessments will include the examination of the incidence rates of AEs, and physical examinations. Safety analyses will be conducted on the safety analysis set, which is defined as all subjects who have received at least a 1 dose of study agent. When analysing AEs, also time at risk will be considered.

11.8. Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Treatment-emergent AEs are AEs with onset during the treatment period or that are a consequence of a pre-existing condition that has worsened since baseline. All reported treatment-emergent AEs will be included in the analysis. For each AE, the percentage of subjects, who experience at least 1 occurrence of the given event, will be summarised by system organ class and preferred term for treatment group. Treatment-emergent AEs will also be summarised by a maximum severity and relationship to study agent. Separate summaries will be provided for SAEs and AEs leading to study agent discontinuation. In addition, summary tables will be presented by treatment group.

11.9. Physical Examination

Proportion of subjects with abnormal physical examination findings will be summarised at each scheduled timepoint. Subjects with any post-treatment abnormalities will be listed.

11.10. Exploratory Analyses

Exploratory analyses (inflammatory markers, immune cell populations, metabolomics, islet function markers, vitamin D3 concentrations, and virus exposure) may be assessed after completion and unblinding of the study and may be conducted later. Results of exploratory analyses are not part of the clinical study report. A separate analysis plan on exploratory outcomes will be developed before these analyses are performed.

12. DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

12.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, records from birth charts and well baby visits, microfiches, radiographs, and correspondence.

Corrections on the source documents must be made so that the initial record is still readable (i.e. whiteout is not permitted). Furthermore, corrections have to be dated and signed.

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. All documents will be stored safely in confidential conditions and in accordance with regulatory requirements.

12.2. Access to Data

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12.3. Data Recording and Record Keeping

Encrypted data will be transferred by the designated study personnel of the respective site to eCRFs. The participants will be identified by a unique study specific number in any database. The name and any other identifying detail will NOT be included in any electronic study data file.

Corrections on eCRFs will be recorded and tracked through an approved audit trail database system. Missing data or data, that was not collected, has to be indicated as such (i.e. "n.a." for not applicable or "n.d." for not done), where appropriate reasons for missing data should be documented.

Further details, e.g. how to keep records, are described in the Data Management Plan.

12.4. Data collection and documentation

The documentation of the clinical trial data in adherence to the GCP-guidelines and the trial protocol is the responsibility of the investigator. All essential documents will be kept in the Investigator Site File (ISF), which will be stored at the Trial Site in accordance with ICH GCP. Original data (source documents) remain in local medical records and information on the eCRF must be traceable and consistent with the original data. Source documents are e.g. laboratory results. No information in source documents about the identity of the patients will be disclosed. Data collected in this clinical trial (see Appendix A – Schedule of Procedures) except data for exploratory analysis must be entered in an eCRF which has to be completed by the investigator or authorized trial personnel and signed by the investigator. This also applies for those patients who do not complete the clinical trial. If a patient withdraws from the clinical trial, the reason should be recorded on the eCRF. The principal investigator is responsible for ensuring the accuracy, completeness, and timeliness of all data reported to the sponsor in the eCRFs and in all required reports.

After database lock, the principal investigator will receive data for archiving.

12.5. Measures in case of data security breach

The conduct of the study and the handling of personal data comply with the provisions of the applicable data protection regulations, in particular the GDPR.
Breaches of the protection of personal data processed as part of the study will be handled and investigated in accordance with the provisions of Articles 33 and 34 GDPR. If the legal requirements of Art. 33 GDPR are met, the controller shall report the personal data breach within the specified 72-hour period.
If it is determined that the requirements of Art. 34 GDPR have been met, the data subjects are notified in accordance with the established processes.
If such a breach occurs at a processor used as part of the study, the processor shall inform the controller without undue delay.
The sponsor as data controller and all data processors used shall take appropriate technical and organizational measures to prevent possible data breaches.

13. QUALITY ASSURANCE PROCEDURES

13.1. Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

13.2. Monitoring

Risk Based Monitoring will be performed according to the study specific monitoring plan. Monitoring activities will include central monitoring and on-site monitoring visits. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the study specific monitoring plan. Following the monitoring plan, the monitors will verify that the study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13.3. Trial committees

13.3.1. Data Safety Monitoring Board (DSMB)

The Data Safety Monitoring Board (DSMB) is an independent group of experts that advises the sponsor. The major responsibility of the DSMB is to monitor enrolment, compliance, COVID-19 exposure rates, and safeguard the well-being of the study participants and to provide pertinent advice to the sponsor and the protocol committee.
Functions and responsibilities:

- Monitors safety parameters during the study
- Monitor over-morbidity
- Monitors the proper conduct of the study, e.g. recruitment rate, rate of drop-outs and lost to follow ups, compliance with the study protocol
- Monitor COVID-19 exposure rates in the placebo group in the event of required revision of numbers and trial duration to reach outcome
- Reviews the DSMB reports and advises pertinent recommendations
- 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. Conference calls are permitted. Before each DSMB meeting, the DSMB will receive a report with all relevant information on recruitment rate, data completeness and safety data. It will also receive a report on SARS-CoV-2 antibody data for surveillance of infection rates. The DSMB will be asked to examine these data and comment on these data, and eventually advise on trial size and duration.
If the DSMB has concerns of over-morbidity, the DSMB will be able to ask for unblinding at any stage of the study and will advise the protocol committee and the sponsor as to whether the study should be continued, modified, suspended, or terminated.

13.3.2. Medical monitor

The functions of the medical monitor are:

- Periodic review of all adverse event reports, masked to treatment assignment
- Periodic review of SAEs
- Periodic review of protocol compliance

13.3.3. Protocol Committee

The protocol committee consists of the protocol authors.
Significant changes that occur to this protocol during the course of the study require the formal approval of the sponsor, protocol chair and statistician.
Functions of the committee may include:

- 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

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1. Declaration of Helsinki

The investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

14.2. Guidelines for Good Clinical Practice

The investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice (GCP).

14.3. Approvals

Prior to study initiation, the protocol and other relevant study documents will be submitted to the ethics committees and regulatory authorities for approval as required. Any substantial modification must also be approved before they are implemented.

14.4. Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all

study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

15. FINANCE AND INSURANCE

15.1. Funding

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

15.2. 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-Gerling Industrie Versicherung AG
Ganghoferstrasse 37-39
80339 München, Germany
Study participants are insured for all adverse events resulting from study participation according to legal requirements (see also respective insurance policy). 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.

15.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

16. PUBLICATION POLICY

Any publications resulting from the trial will be agreed between the principal investigators and co-authors prior to submission. Patient names or other identifiers will not be disclosed.
For further details refer to https://www.gppad.org/de/publication-guidelines-de/.

17. ARCHIVING

Following closure of the study, all study related records and source documents must be stored in a safe and secure location according to regulatory requirements.

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19. APPENDIX A: SCHEDULE OF PROCEDURES

Visits	Baseline Visit age 3 months	Visit age 6 months	Visit visit 2 plus 3-6 weeks	Visit visit 3 plus 8 weeks	Visit age 12 months	Visit age 15 months	Visit age 18 months	Visit age 21 months	Visit age 24 months	Visit age 30 months	Visit age 36 months	Visit every 12 months until maximum age 6 years
Visit window	+28d	+28d	+4d	+14d	± 14d	± 14d	± 14d	± 14d	± 14d	± 14d	± 14d	± 30d
Study visit	1	2	3	4	5	6	7	8	9	10	11	12+
Informed consent, Review Incl./Excl. Criteria	X X	X										
Medical History (including birth weight)	X											
Randomization (vaccine / placebo)		X										
Intervention												
Vaccination		X	X	X								
Primary and Secondary Outcome measurements												
IAA; GADA; IA-2A; ZnT8RA; ZnT8WA		X		X	X	X	X	X	X	X	X	X
Transglutaminase antibodies					X (from islet autoantibody aliquot)		X (from islet autoantibody aliquot)		X (from islet autoantibody aliquot)	X (from islet autoantibody aliquot)	X (from islet autoantibody aliquot)	X (from islet autoantibody aliquot)
Local investigations & measurements												
Physical examination (incl. weight / height)	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of AEs and SAEs			X	X	X							
Psychological Questionnaire					X				X			
Exploratory Outcomes												
Infection surveillance												
Stool sample (at home) and brought to visits		monthly from age 3 months to 24 months										
Saliva (at home) and picked up	X	weekly from age 3 months to 24 months										
Serum for virus antibodies	X	X	X	X	X	X	X	X	X	X	X	X
e-diary (related to infection & SARS-COV-2)	X	X	X	X	X	X	X	X	X	X	X	
Immune surveillance												
OLINK inflammatory panel	X	X	X	X	X	X	X		X	X	X	X
Whole blood multiparameter Flow		X			X				X		X	
Whole blood for cell isolation (Freezing/RNAseq)		X			X		X		X		X	
Whole blood for RNAseq		X			X		X		X		X	
Fresh cell assays (selected sites)					X				X		X	
DNA for methylation					X				X		X	
Metabolic markers												
Pre-prandial and 30min post-prandial glucose, insulin, C-peptide, proinsulin		X	X only Glucose pre-prandial	X	X	X	X	X only Glucose pre-prandial	X	X	X	
HbA1c					X		X		X		X	X
OGTT (at 0/30/60/90/120 min.) for glucose, c-peptide, insulin, proinsulin											X If islet autoantibody positive	X If islet autoantibody positive
Vitamin D						X			X		X	
Metabolomics		X			X		X		X		X	X
Sample collection												
Total serum/ blood for serum requirements	capillary blood		capillary blood					capillary blood				
Other blood												
Total												
Blood volumes												
Blood volumes for protocol parameters (mL):	0.2	13.6	0.2	4	19.8	5.2	14.5	0.2	21	4	21	5.5
Maximum blood volume (3% of total blood volume) in	13.51	17.64	***	20.33	22.32	22.46	23.83	***	26.48	28.96	31.37	35.66
Blood volumes per protocol %												

*** max. 300µl of capillary blood

20. APPENDIX B: 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

Page 1/5

Serious Adverse Event Report Form

DR. NIBLER & PARTNER

1. Study Information

Short Title:	**GPPAD-AVANT1A**
Clinical Trial Code:	**GPPAD-05-AVANT1A**
EU Trial Number:	**2023-507348-35-00**
Indication:	**Type 1 Diabetes**

2. Subject Information

Patient ID: - - ☐
Country Site Subj Case No

Age (month):

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

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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 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**
	☐	please en	plea		please enter		please enter
	☐	please en	plea		please enter		please enter
	☐	please en	plea		please enter		please enter

* 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:

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6. Study Treatment Information

Study treatment	Kit No.	Treatment Dates		Dosage		Study Treatment Action because of SAE			Restart of Study Treatment	
						1. No change 2. Drug interrupted 3. Stopped permanently 4. Not applicable 5. Unknown		Mark with (x) if event improved		Mark with (x) if event reoccurred
	Kit Number	Date dose first received (dd/mm/yyyy)	Date of last dose prior to SAE (dd/mm/yyyy)	Frequency	Route	1-5	Date of Action (dd/mm/yyyy)		Restart of Study treatment date (dd/mm/yyyy)	
						pl		☐		☐
						pl		☐		☐
						pl		☐		☐

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

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

☐ none

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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)
		pleas	
		Pleas	
		Pleas	
		Pleas	
		Pleas	

☐ 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*
				Please
				Please
				Please
				Please
				Please

☐ 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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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

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21. Appendix C: E-diary

ELECTRONIC DIARY

To be completed by parents every 2 weeks until age 36 months ± 14 days

This part will be pre-filled electronically:

GPPAD-ID: __ __ __ __ __ __ __

Period: from __ __ / __ __ / __ __ __ __ until __ __ / __ __ / __ __ __ __

DD MM YYYY DD MM YYYY

1. Did your child have any of the following respiratory infections during the last 2 weeks?

- ☐ COVID-19
- ☐ Middle Ear Infection
- ☐ Eye infection
- ☐ Common cold, Sinusitis, Laryngitis, Pharyngitis, Influenza, Bronchitis
- ☐ Tonsillitis
- ☐ Pneumonia
- ☐ Other (please discuss with your study doctor)

- ☐ No

*Was the diagnosis confirmed by a medical doctor?

☐ Yes ☐ No

*Was the diagnosis confirmed by a laboratory test?

☐ Yes ☐ No

*Did your child experience fever

☐ Yes ☐ No

(temperature above 38 degrees during the infection)?

#If your child had CoViD-19, were there symptoms?

☐ Yes ☐ No

#Which ones and how many days did the symptoms last?

Fever	__ __ days
Cough	__ __ days
Rhinorrhoea	__ __ days
Sore throat	__ __ days
Dyspnoea	__ __ days
Headache and malaise	__ __ days
Gastrointestinal symptoms (diarrhoea, nausea, vomiting, and /or abdominal pain)	__ __ days
Other (fatigue, myalgia, arthralgia, rash, conjunctivitis, disturbances of smell or taste)	__ __ days

**for each diagnosis ticked the additional questions, if diagnosis was confirmed by MD, if the diagnosis was confirmed by a laboratory test and if the child experienced fever should appear*

 # for COVID-19 diagnosis, the additional question about symptoms should appear – if "yes" is ticked for symptoms, the additional questions on which and for how many days should appear

2. Did your child have a gastrointestinal infection during the last 2 weeks?	☐ Yes ☐ No * Was the diagnosis confirmed by a medical doctor? ☐ Yes ☐ No * Did your child experience fever? ☐ Yes ☐ No (temperature above 38 degrees during the infection)?	*if yes, the additional questions, if diagnosis was confirmed by MD and if the child experienced fever should appear
3. Did your child have any other infections during the last 2 weeks?	☐ Yes ☐ No * Was the diagnosis confirmed by a medical doctor? ☐ Yes ☐ No * Did your child experience fever? ☐ Yes ☐ No (temperature above 38 degrees during the infection)?	*if yes, the additional questions, if diagnosis was confirmed by MD and if the child experienced fever should appear
4. Did your child have fever (temperature above 38 degrees) without other signs of infection during the last 2 weeks?	☐ Yes ☐ No	
5. Did your child develop a chronic disease in the last 2 weeks?	☐ Yes ☐ No *If yes, please discuss this with your study doctor.	*if yes, the additional question "which chronic disease" should appear

6. Is your child currently breast-fed?	☐ Yes ☐ No, Date of last breast-feeding: __ __ / __ __ / __ __ __ __ DD MM YYYY ☐ The child was never breast-fed If Yes, Does your child receive any additional formula milk? ☐ Yes ☐ No	This question will no longer appear, if the child was not or no longer breast-fed be-fore
7. a). Did you in-troduce solid food during the last 2 weeks?	☐ Yes ☐ No If Yes, Date of first food: __ __ / __ __ / __ __ __ __ DD MM YYYY Or at the age of __ __ weeks	This question will no longer appear, if solid food was already intro-duced before
7. b) Did you in-troduce food contain-ing glu-ten (such as wheat, rye, bar-ley, oats, spelt, green spelt, e.g. in the form of semo-lina, oat-meal, baby biscuits, rusks in milk for-mula) during the last 2 weeks?	☐ Yes ☐ No If Yes, Date of first food containing gluten: __ __ / __ __ / __ __ __ __ DD MM YYYYJ Or at the age of __ __ weeks	This question will only appear, if solid food was already intro-duced before. It should no longer appear, if food contain-ing glu-ten was already intro-duced before-

22. Appendix D: Psychological Questionnaire

Well-being Questionnaire: mother, father, other guardian

Today's date:

_____ / _____ / _____

(day) (month) (year)

Filled out by the study team:

☐ V5 ☐ V9

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 ☐ extremely 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-AVAnT1A-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-AVAnT1A-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-AVAnT1A-Study to other parents?

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

Thanks for taking time to complete this questionnaire!