Supplementary information – Ver-A-T1D Protocol appendix

Legal status of the investigational medical product (IMP)/ placebo:

Verapamil SR is an L-type calcium channel blocker, that has been approved by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA).

Supply of IMP/ Placebo

IMP supply to sites will be overseen by the Medical University of Graz and distributed by ABF Pharmaceutical Services GmbH, Vienna. Upon initial authorisation by the sponsor, an initial supply will be sent to sites; supplies thereafter will be requested and distributed as detailed in the pharmacy manual.

Accountability of the trial treatment:

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study medication accountability, reconciliation, and record maintenance (receipt, reconciliation, and final disposition records). Only participants enrolled in the study may receive IMPs and only authorised site staff may supply or administer study intervention. Used and unused study medication will be destroyed at site after written confirmation from the sponsor. Destruction of IMPs will be documented and carried out according to local procedures after accountability is finalised and reconciled by the monitor.

Definitions for assessment of safety in Ver-A-T1D:

Clinical assessment of severity is classified as Mild, the participant is aware of the event or symptom, but the event or symptom is easily tolerated, Moderate, the participant experiences sufficient discomfort to interfere with or reduce his or her usual level of activity or Severe, significant impairment of functioning; the subject is unable to carry out usual activities and / or the participant's life is at risk from the event.

Recording of all adverse events starts from the point of informed consent regardless of whether a participant has yet received a medicinal product. Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

The IDMC will assess safety from reported adverse events and will have the right to suspend or stop the trial at any point for safety concern. The trial may be suspended by the chief investigator and/or the sponsor following SUSAR.

Adverse reaction to an investigational medicinal product (AR) is an untoward and unintended response to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected adverse reaction is an adverse reaction, the nature, or severity of which is not consistent with the applicable reference safety information (RSI) (e.g. summary of product characteristics

(SmPC) for an authorised product). When the outcome of the adverse reaction is not consistent with the applicable RSI this adverse reaction should be considered as unexpected. The term "severe" is often used to describe the intensity (severity) of a specific event. This is not the same as "serious," which is based on participant /event outcome or action criteria.

Serious adverse event or serious adverse reaction (SAE / SAR) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is an important medical event - Some medical events may jeopardise the participant or may require an intervention to prevent one of the above characteristics/ consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious'

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the participant was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Suspected Unexpected Serious Adverse Reaction (SUSAR) is a serious adverse reaction, the nature and severity of which is not consistent with the information set out in the Reference Safety Information. All expected Adverse Reactions are listed in the latest MHRA approved version of the RSI. This must be used when making a determination as to the expectedness of the adverse reaction. If the adverse reaction meets the criteria for seriousness, this must be reported as per section.

A list of medical events that defines which reactions are expected for the IMP within a given trial and thus determining which serious adverse reactions (SARs) require expedited reporting. The RSI is contained in a clearly identified section (section 4.8) of the Summary of Product Characteristics (SmPC).

Procedures for the management of AV-Block are outlines in Figure 1.

Study Samples:

Ver-A-T1D will utilise the INNODIA consortium established SOPs for sample collection, shipments, storage and analysis. All Ver-A-T1D sample shipments from participating sites to central laboratories will be recorded via the eCRF. Samples sent to central laboratories will be pseudo-anonymised. The following central INNODIA consortium laboratories will be used for analysis and storage of Ver-A-T1D biological samples: 1) Core Biochemical Assay Laboratory (CBAL) at the Cambridge University Hospitals NHS Foundation Trust, UK led by Mr Keith Burling, 2) Paediatric Diabetes Research Group (PEDIA) Laboratory at the University of Helsinki, Helsinki, Finland led by Prof Mikael Knip, 3) Peter Gorer Department of Immunobiology at the School of Immunology and Microbial Sciences, King's College London, Guy's Hospital, London, UK led by Dr Tim Tree, 4) Diabetes and Autoimmunity Research (DeAR) Lab at the Institut Cochin, INSERM U1016, Paris, France led by Prof Roberto Mallone, 5) Diabetes Research Lab at the Leiden University Medical Centre, Leiden, The Netherlands led by Prof Bart Roep, 6) Centre for Regenerative Therapies Dresden at the Technische Universität Dresden, Dresden, Germany led by Prof Ezio Bonifacio, 7) Department of Medical Sciences, Surgery and Neurosciences at the University of Siena, Siena, Italy led by Dr Guido Sebastiani, 8)

JDRF/Welcome Trust Diabetes and Inflammation Laboratory at the University of Oxford, Oxford, UK led by Prof John Todd with 2)-7) being INNODIA immune hubs. The Ver-A-T1D Trial Manual details sample collection, processing and shipment requirements in more detail.

All other samples collected for exploratory studies (blood, urine and stool for Omics analysis) will be processed locally at participating sites according to the respective INNODIA SOPs and stored locally in <-69°C in freezers with temperature monitoring until shipped in batches to the University of Cambridge. Until required for exploratory analysis, samples will be sorted and stored by the University of Cambridge, at the University of Cambridge or at the National Institute of Health Research National Biosample Centre. When requested, samples will be sent to selected analytical laboratories. At every location, samples will be kept at <-69°C in freezers with temperature monitoring. For transport, samples will be shipped on dry ice. All shipments will be recorded in the INNODIA data warehouse which tracks the location of the samples (Table 1).

Toxicology and normal standard of care samples:

Samples collected in the trial that are part of the normal standard of care or for screening/safety (for example HbA1c, FBC, biochemistry, HIV, hepatitis and SARS-CoV-2 serology and PCR) will be analysed locally by the local hospital accredited laboratory and samples discarded once analysed as per normal local protocols.

Mixed Meal Tolerance Test (MMTT) measurements:

At baseline and at 3, 6-, 9-, 12- and 24-months trial participants will have an initial 120-minute MMTT, during which blood will be collected for measuring glucose and C-peptide. The MMTT will be performed according to the following protocol. Long-acting insulins or basal rates (in case of using an insulin pump) will be continued. The use of rapid-acting insulin is acceptable up to 2 hours before the MMTT and the use of short-acting insulin up to 6 hours before the MMTT to correct hyperglycaemia. The test will be only performed if the glucose level is between 4 and 11.1 mmol/l.

Participants will be given 6 ml/kg of Ensure Plus meal solution (up to a maximum of 360 ml) orally which needs to be ingested within 10 minutes. Blood samples for the measurement of C-peptide and glucose will be collected 10 minutes prior to the meal (-10 mins), at the time of ingestion (0 minutes), and at 15, 30, 60, 90 and 120 minutes thereafter. Participants who are not able to tolerate Ensure Plus will be advised to eat a standardised breakfast with a defined content of carbohydrates, proteins and lipids, which will be the same for all visits during the study.

If the glucose level at t=120 minutes is >8 mmol/l, a subcutaneous insulin correction dose will be given, either via injection or pump, according to the participant's own insulin sensitivity factor. If the glucose level at t=120 is >14 mmol/l, ketones will be tested by finger prick. If ketones are >0.6 mmol/l, glucose and ketones will be repeated until ketones have decreased <0.6 mmol/l and the participant can be discharged from the clinical centre.

Dried Blood Spot (DBS) measurements:

The first DBS and blood glucose measurements will be carried out in parallel with the MMTT. Only the first DBS is collected on-site (V0). Following the baseline DBS test, fasted participants will be requested to collect DBS samples and record capillary blood glucose measurements at home, collection will be monthly. Each time, a blood glucose measurement will need to be recorded and a DBS sample collected, immediately before and 60 min after starting a liquid meal (Ensure Plus) solution (replacing breakfast), whilst omitting their morning/pre-breakfast insulin.

Continuous Glucose Monitoring (CGM):

Dexcom G6 devices will be provided by Dexcom for use in Ver-A-T1D (use Dexcom Order Form). Blood glucose variability will be studied through subcutaneous glucose variation, using data de-rived from glucose monitoring for the 2 weeks prior to each clinic visit. All participants will be provided with a continuous glucose monitoring system (Dexcom G6) during visit V0. Suitable training will be provided to the participants so they can place the device and collect data at home. Only the Dexcom G6 can be used for the trial assessments.

Training will be provided to site staff on how the device is to be used in Ver-A-T1D. This training should be recorded on a training log. Trained individuals may then be added to the Log of Staff Delegation. The Dexcom G6 device is commercially available and User Guides from the manufacturer are provided with each kit in the local language. Please refer to these documents for additional in-formation on how to apply the Dexcom G6. Data will be downloaded in the Clarity software from the receiver by the trial team once 14 days of home CGM is complete.

Patient diary:

The participants will be provided with a diary at visit V0 and at all subsequent visits as applicable. The participants should be trained in the correct use of the diary. Detailed instructions are outlined in the patient diary itself. Mean daily insulin use will be calculated over 7 consecutive days during the 2 weeks preceding all visits and participants will be asked to record all insulin usage in their diary during those 2 weeks. This value will be calculated in units of IU/kg/day and combine doses of all different types of insulin administered over this study period. Where data from consecutive days are not available, the three days closest together will be used.

The investigator or delegate should review the diary during the in-house visits. Completed diaries will be collected at each in-house visit and filed in the participant's study records. New diaries should be handed out (in sufficient quantity to cover longer intervals between the study vis-its) to the participants whenever the previous one is collected or completed by the participants. The patient diary is version controlled and must be approved by the local or national ethics committee prior to implementation. The patient diary must be kept as they are source data.

Table 1. Study Sample storage and analysis methods

Sample For	Sample	Before analysis	Analysis	Storage after analysis
Screening eligibility criteria	Random plasma C-peptide	Plasma collected for C-peptide analysis will be sent on dry ice to University of Cambridge	CBAL	Samples will be kept at <-69°C in freezers with temperature monitoring by University of Cambridge
	Diabetes-related autoantibodies (GADA, IAA, IA-2A or ZnT8A)	Serum will be sent for analysis fresh within 24 hours to the PEDIA Laboratory (Helsinki, Finland)	PEDIA Laboratory	Samples will be kept at <-69°C in freezers with temperature monitoring by the PEDIA Laboratory
Primary outcome	AUC stimulated C-peptide over first 2h of MMTT at 12 months follow-up	Plasma collected for C-peptide analysis will be sent on dry ice to the University of Cambridge, where they will be stored until analysis at <-69°C in freezers with temperature monitoring	Analysis in batches CBAL	Before and after analysis, samples will be kept at <-69°C in freezers with temperature monitoring by University of Cambridge
Secondary outcomes	AUC stimulated C-peptide over first 2h of MMTT at baseline, 3, 6 and 12-months follow-up	Plasma collected for C-peptide analysis will be sent on dry ice to the University of Cambridge, where they will be stored until analysis at <-69°C in freezers with temperature monitoring	Analysis in batches by CBAL	Samples will be stored at <-69°C in freezers with temperature monitoring by University of Cambridge
	DBS C-peptide at observed times	DBS cards collected for C-peptide analysis will be stored locally at participating sites (frozen <-69°C) until shipment on dry ice to the University of Cambridge	Analysis by CBAL	After analysis by CBAL, samples will be stored by the University of Cambridge
	HbA1c (all time points)	Whole blood collected for HbA1c measurement will be sent to local hospital accredited laboratory routinely performing this analysis as standard of care	Analysis by local hospital accredited laboratory. HbA1c results will be entered into the eCRF by participating sites.	Samples will be discarded after analysis as per normal local protocols

Sample For	Sample	Before analysis	Analysis	Storage after analysis
	T1D-associated autoantibodies at baseline and 12 months	Serum will be sent for analysis fresh within 24 hours to the PEDIA Laboratory	Analysis by the PEDIA Laboratory	After analysis samples will be kept at <-69°C in freezers with temperature monitoring by the INNODIA central laboratory by the PEDIA Laboratory
Exploratory studies	Biomarkers related to immunological changes and β-cell death/ survival	Whole blood will be sent fresh following collection to an INNODIA immune hub	Fresh blood immune assays and PBMC isolation performed by INNODIA immune hubs	Isolated PBMCs will be stored in liquid nitrogen by an INNODIA immune hubs
	Diabetes-related genotyping	Blood cells collected for genotyping will be stored locally at participating sites (frozen <-69°C) until shipment on dry ice to the University of Cambridge where they will be further forwarded to the genotyping lab for DNA extraction and analysis	JDRF/Welcome Trust Diabetes and Inflammation Laboratory	Remaining cells and extracted DNA samples will be stored at <-69°C in freezers with temperature monitoring by JDRF/Welcome Trust Diabetes and Inflammation Laboratory