TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	Exploration of the peripheral immune system in subjects with New Onset T1 Diabetes (NOT1D)

Compound Number: NONE

Development Phase: NA

Effective Date: 06-MAR-2017

Protocol Amendment Number: 03

Author (s): PPD

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2015N246275_00	2015-DEC-11	Original
2015N246275_01	2016-APR-04	Amendment No. 1
• Objective "To assess the biochemical fingerprint derived from metabonomic analyses of peripheral blood in HVs and NOT1D subjects" & associated endpoint removed in Section 3. References to this objective and endpoint in Section 6.1; and Section 6.8 were removed accordingly. This objective and associated endpoint were removed because they should be addressed in separate studies in the future. • New exploratory objective ("To assess the phenotype and transcriptomic fingerprint of stromal cells from iLN core biopsy samples in HVs and NOT1D subjects") and associated endpoint added in Section 3, Section 6.8 and Section 8.1 were updated accordingly. This objective and associated endpoint were added to provide additional insights into the exploration of the peripheral immune system in subjects with New Onset T1 Diabetes, which were not envisaged during the finalisation of the original protocol. • The Questionnaire in Appendix 1 was modified to avoid any duplication with Informed Consent procedures and duplication of data collection in eCRF. The entire questionnaire has been moved from the Study protocol to the Study Reference Manual (SRM) and is therefore not present in the amended protocol anymore. • All appendix numberings and in-text references to appendices have been updated to reflect that the Lymph Node Questionnaire is no longer located in Appendix 1 of the Study Protocol. • Clarifying wording was added and typos were corrected in Section 6.2; Section 6.7.5 and Section 6.8. • Appendix 1 - Abbreviations and Trademarks lists were updated.		
2015N246275_02	2016-OCT-06	Amendment No. 2
• Time and Event table: Duplicate assesment removed and updated (medical history/past current medical conditions and laboratory assessments). Clarified when telephone call for Follow Up Period 1 would be collected. Clarified genetics testing was optional in consenting subjects. Added SAE/AE review. • Added HbA1c to other screening tests and removed wording 'levels' to fasted C-peptide.		

2015N246275_03	2017-MAR-06	Amendment No. 3

- Extended the interval between the initial diagnosis and Day 1, from 6 weeks to 8 weeks following recruitment challenges. 8 weeks is the upper limit for the NOTID population and does not affect the scientific rationale for the study. The 8 week change was updated throughout the protocol.

- Clarified exclusion criteria, vaccination ≤ 28 days before day 1.

- Interim Analysis: Added additional information for the Interim Analysis after the recruitment of 5 evaluable HVs and 5 evaluable NOTID subjects. This will facilitate the decision making of the study and prioritization of the exploratory assays.

- Clarified fasting at screening in the Time and Event table and added footnote to Table 1 to clarify fasted glucose at screening and non fasted glucose on Day 1

SPONSOR SIGNATORY

PPD

6th March 2017

Caroline O Savage, FRCP, FMedSci Date

VP & Head, Experimental Medicine Unit

PPD

MEDICAL MONITOR/SPONSOR INFORMATION PAGE

Medical Monitor/SAE Contact Information:

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SAE contact information	= Medical monitor				

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In some countries, the clinical trial sponsor may be the local GlaxoSmithKline Affiliate Company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number(s): Not applicable

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number

I confirm agreement to conduct the study in compliance with the protocol, as amended by this protocol amendment.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

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1. PROTOCOL SYNOPSIS FOR STUDY OTX203158

Rationale

This study will assess and compare the molecular immune profile of cells derived from the inguinal lymph nodes (iLN) in healthy volunteers (HVs) and New Onset Type 1 Diabetes Mellitus (NOT1D) subjects, to better understand the immunological processes that may lead to beta cell destruction. It is hypothesised that early changes in the immune system in NOT1D subjects can be detected in immune cells from the iLNs that will be distinct from changes observed in peripheral blood derived immune cells, such as for example Peripheral Blood Mononuclear Cells (PBMCs). Novel biomarkers of disease activity may be identified, potentially supporting the early readout of therapeutic efficacy in future Type 1 Diabetes (T1D) trials.

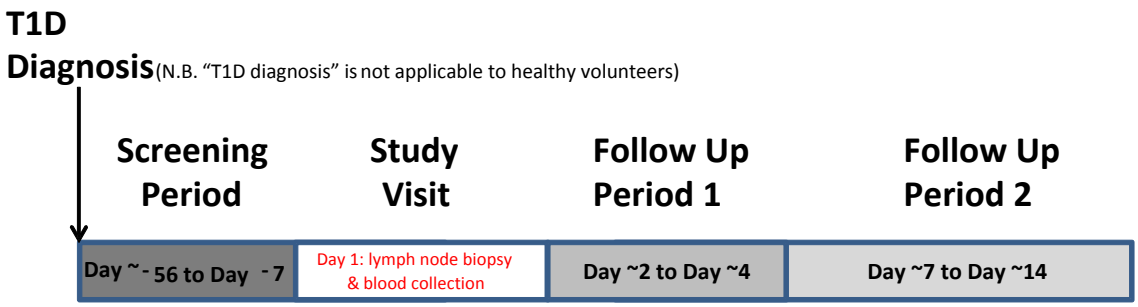
Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
1) To assess the frequency and phenotype of leukocyte subsets in iLN and peripheral blood in HVs and NOT1D subjects	• Absolute number and/or proportion of leukocyte subsets in iLN and peripheral blood
Secondary	
1) To assess the frequency and phenotype of leukocyte subsets in iLN core biopsies and iLN fine needle aspirates in HVs and NOT1D subjects	• Absolute number and/or proportion of leukocyte subsets in iLN core biopsies and iLN fine needle aspirates.
2) To assess the safety and tolerability of iLN biopsy as well as expectations and experience of the biopsy procedure.	• Number of AEs and SAEs following lymph node biopsy procedure • Descriptive data obtained by a questionnaire on the acceptability of iLN biopsy in research setting.
Exploratory*	
1) To assess suppression activity of T regulatory lymphocytes from the iLN and peripheral blood in HVs and NOT1D subjects	• Relative levels of T lymphocyte suppressive activity of cells from iLN and peripheral blood respectively
2) To assess the frequency of cytokine-producing lymphocytes in HVs and	• Proportion of lymphocyte populations producing pro-inflammatory and/or anti-

Objectives	Endpoints
NOT1D subjects	inflammatory cytokines in iLN and peripheral blood
3) To assess clonal expansion of T and B lymphocyte populations in HVs and NOT1D subjects	• Comparison of TCR and BCR usage in lymphocytes in iLN and peripheral blood
4) To assess the gene expression fingerprint of auto-antigen specific lymphocytes in HVs and NOT1D subjects	• Gene expression levels of sorted auto antigen stimulated T lymphocyte populations in iLN and peripheral blood
5) To assess the phenotype and transcriptomic fingerprint of stromal cells from iLN core biopsy samples in HVs and NOT1D subjects	• Absolute number and/or proportion of stromal cell subsets and gene expression levels of stromal cell subsets from iLN core biopsy samples

* The Exploratory endpoints are listed in order of priority and the conduct of any experimental assays will be dependent on the material available (i.e. number of cells obtained) from iLN biopsies and peripheral blood.

Overall Design



- A multi-centre, non-drug treatment study to compare differences in immune cells derived from iLN and peripheral blood of NOT1D subjects and HVs.
- After informed consent, each subject will attend a screening visit followed by a study visit for iLN biopsy and peripheral blood collection (Day 1). Follow up phone calls after the study visit will be carried out at approximately Day 2-4 and Day 7 to 14. The total duration of this study will be up to 10 weeks, including the screening period.
- A data look will be carried out after the recruitment of a cohort of up to 5 HVs to determine if the quality and quantity of cells derived from either fine needle aspirate or core biopsy of the inguinal lymph node and from peripheral blood are likely to be sufficient to continue the study to meet its primary objective. Full details on this

feasibility analysis will be provided in the Study Reference Manual (SRM) and Reporting and Analysis Plan (RAP).

- An interim analysis will be carried out after the recruitment of 5 evaluable HVs and 5 evaluable NOT1D subjects. The primary purpose of this interim analysis will be to facilitate decision making and study design for a potential follow-up interventional study.

Type and Number of Subjects

Up to 15 evaluable NOT1D subjects and up to 15 evaluable HVs will be enrolled. If subjects prematurely discontinue the study, additional replacement subjects (up to 5 subjects) may be enrolled at the discretion of the sponsor in consultation with the investigator. Evaluable subjects are defined as subjects that provide both iLN biopsy (at least sufficient amounts of cells by one of the two biopsy methods employed to enable the conduct of assays related to at least the primary endpoint) AND at least sufficient amounts of peripheral blood to enable the conduct of assays related to at least the primary endpoint).

Analysis

Descriptive statistics will be calculated for each of the primary flow cytometry cell types by group and sample type (blood / biopsy). Generalized linear models with appropriate link functions will be used to analyze data separately for each flow cytometry cell type to provide estimates for comparisons.

2. INTRODUCTION

Lymph nodes (LNs) are important sites in the body where either an immune response to pathogenic antigens or tolerance is initiated [Sainte-Marie, 2010]. In T1D there is a break down in tolerance against self antigens resulting in immune attack of the beta cells of the pancreas.

In the non-obese mouse model of T1D (NOD mouse) islet specific T-cells can be detected in the pancreatic LN before the onset of disease [Höglund, 1999] and removal of these LNs rescues NOD mice from developing T1D [Gagnerault, 2002]. In addition, in subjects with T1D, auto-reactive T cells isolated from the blood are capable of destroying islet cells when co-cultured *in vitro*, suggesting that immune activation is not only occurring at the pancreas but also in other lymphoid tissues [Unger, 2012]. However, identification of clonal signatures unique to T1D subjects has proven to be difficult due to the low frequency of antigen specific cells in the blood of these subjects. To date, researchers have studied pathogenic T cells in response to insulin-specific antigens in the peripheral blood but the results have shown no significant differences between HVs and T1D subjects [Herold, 2009], most likely due to lack of response to antigen by low affinity T-cell Receptors (TCRs). In addition, emerging data suggest that post-translational modification of insulin-related antigens is playing a role in break of tolerance and destruction of beta cells [van Lummel, 2014].

2.1. Study Rationale

This study will assess and compare the molecular immune profile of cells derived from LNs in HVs and NOT1D subjects, to better understand the processes that may lead to beta cell destruction. Since retrieving peri-pancreatic LN cells is not feasible in a research setting and immune cells circulate quickly throughout the body, we will address our questions using cells from the LNs in the inguinal region. This method has been utilised in subjects with rheumatoid arthritis, subjects at risk of rheumatoid arthritis and HVs [van Baarsen, 2013].

It is hypothesised that early changes in the immune system in subjects with NOT1D can be detected in LNs that will be distinct from changes observed in cells derived from peripheral blood, such as PBMCs. As a result, novel biomarkers of disease activity may be identified, potentially supporting the early readout of therapeutic efficacy in future T1D trials.

2.2. Brief Background

2.2.1. Type 1 Diabetes Mellitus

T1D represents 5-10% of all subjects with Diabetes Mellitus [Maahs, 2010] and is primarily a result of the T cell-mediated destruction of beta cells in the pancreas [Atkinson, 2014]. The disease occurs most often in children, adolescents, and young adults, but can present at any age. T1D has strong Human leukocyte antigen (HLA) associations, particularly with linkage to DR and DQ genes. Autoreactive antibodies precede the onset of disease and have been used for risk stratification in susceptible

subjects [Wasserfall, 2006], however, they cannot predict disease progression. Due to the varying length of time between autoantibody seroconversion and clinical symptoms in subjects, it is postulated that the environment also plays a role in the development of the disease. Environmental factors such as viruses [Yeung, 2011], lack of vitamin D [Hyppönen, 2001], weaning and improved hygiene [Knip, 2010] have all been implicated as triggers for T1D, but their roles remain controversial. Major steps to address this are being undertaken through the longitudinal 'TEDDY' study (The environmental determinants of diabetes in the young), which is monitoring genetically susceptible children from birth (https://teddy.epi.usf.edu/).

During the preclinical period between seroconversion and disease onset there is progressive loss of beta cells in the pancreatic islets, which impairs the ability of an individual to maintain blood glucose levels within a physiologic range [Rowe, 2011]. A complex interplay between the innate and adaptive arms of the immune system, in response to islet-specific antigens, is responsible for the pathogenesis of T1D. The precise events that trigger the development of an autoimmune attack on and destruction of the beta cells are unclear, but may be driven primarily by cytotoxic T cells. Indeed, islet-derived CD8+ T cells expressed T-cell receptors bound to MHC class 1 tetramers with the beta-cell autoantigen Islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) and other target peptides in NOT1D subjects [Skowera, 2015]. In addition, autoreactive CD8+ and CD4+ T lymphocytes can be detected in peripheral circulation and are directed to many of the same epitopes in recent-onset diabetes subjects [Velthuis, 2010; Unger, 2011] Interestingly, analyses of pancreata from subjects diagnosed with diabetes within 1 year suggest that subjects under the age of 14 have higher incidence of insulitis (68%) compared to those between 15 and 39 (39%) In't Veld, 2014]. This suggests that a more vigorous autoimmune attack occurs in children. As a consequence of this autoimmune attack, autoantibodies against: glutamic acid decarboxylase (GAD), protein tyrosine phosphatase-related islet antigen-2 protein (IA 2), IA 2β, islet-cell antigen (ICA), zinc transporter 8 (ZnT8) and/or insulin (IAA) are detectable in approximately 85% to 90% of subjects with T1D [Vermeulen, 2011]. But as discussed earlier, these autoantibodies can neither predict absolute disease progression nor be used to monitor therapeutic efficacy.

The net effect of autoimmune destruction of beta cells coupled to little or no regeneration marks a progressive deterioration of glucose homeostasis, culminating in an absolute dependence on exogenous insulin replacement therapy. It is estimated that only 10-20% of beta-cells are still functioning at the time of clinical diagnosis [Knip, 2002]. The rate of progression from normo-glycaemia, through pre-clinical impairment of glucose handling to insulin dependence is variable. Indeed, even 1-5 years after diagnosis, about 50% of T1D subjects show clinically meaningful beta-cell function [Palmer, 2009].

The destruction process of beta cells is not a continuous phenomenon and it has been argued that T1D is a relapsing-remitting disease [Von Herrath, 2007]. Therefore in order to prevent further deterioration of the pancreas it is vital that subjects are treated as close to or prior to diagnosis if possible.

Clinical trials in T1D/NOT1D usually rely on metabolic measurements as endpoints, most commonly C-peptide, HbA1c, mixed meal tolerance test and insulin usage

[Aronson, 2014]. Reliable biomarkers of immune dysfunction and beta cell destruction are not available at the moment and it is hypothesized that analysis of lymphocyte phenotypes and additionally the deep phenotyping of T and B cells in the earliest stages of the autoimmune process in T1D / NOT1D may provide a window on the immune system, which will eventually enable the characterisation of biomarkers and allow an early evaluation of the efficacy of immunomodulatory regimes in future studies.

2.2.2. Lymph node biopsy immunophenotyping

iLN biopsies will be carried out by two related methods in this study: fine-needle aspirate biopsy (referred to as "fine needle aspirate" in this protocol) and core needle biopsy (referred to as "core biopsy" in this protocol).

Immunophenotyping of cells obtained by iLN core biopsies in autoimmune disease was described by van Baarsen [van Baarsen, 2013]. The authors examined the cellular composition of iLNs in HVs compared to subjects with early rheumatoid arthritis (RA) and subjects at risk of developing RA and showed significant changes in B and T cell composition by flow cytometry analysis between groups. This difference was not detected in PBMCs, indicating that studying the composition of immune cells of the iLN has additional value in understanding molecular events in the early phases of an autoimmune disease. Furthermore, biopsy by fine needle aspirate of lymph nodes has been described as a method to immunophenotype cells derived from axillary lymph nodes following skin antigen delivery [Tatovic, 2015].

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
1)To assess the frequency and phenotype of leukocyte subsets in iLN and peripheral blood in HVs and NOT1D subjects	• Absolute number and/or proportion of leukocyte subsets in iLN and peripheral blood
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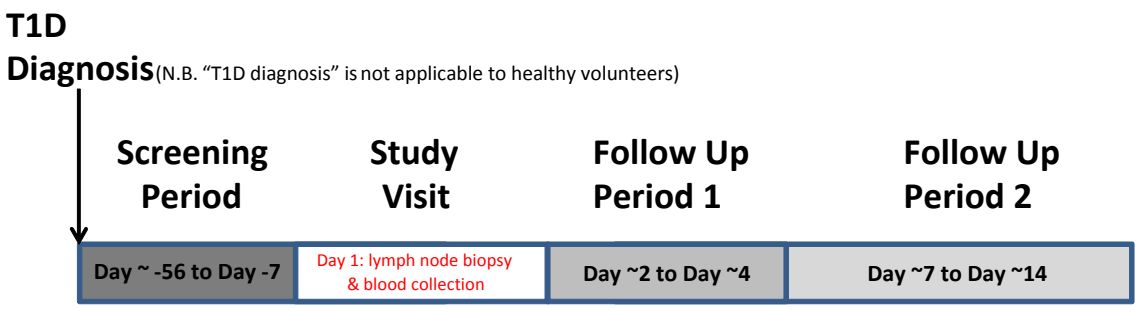
* The Exploratory endpoints are listed in order of priority and the conduct of any experimental assays will be dependent on the material available (i.e. number of cells obtained) from iLN biopsies and peripheral blood.

See Section 8.1 for a description of the intended use of cells from each biopsy procedure.

4. STUDY DESIGN

- A multi-centre, non-drug treatment study to compare differences in immune cells derived from iLN and peripheral blood of NOT1D subjects and HVs.
- After informed consent, each subject will attend a screening visit followed by a study visit for iLN biopsy and peripheral blood collection (Day 1). Follow up phone calls after the study visit will be carried out at approximately 2 to 4 days and 7 to 14 days after the study visit. The total duration of this study will be up to 10 weeks, including the screening period.
- A data look will be carried out after the recruitment of a cohort of up to 5 HVs to determine if the quality and quantity of cells derived from either fine needle aspirate or core biopsy of the inguinal lymph node and from peripheral blood are likely to be sufficient to continue the study to meet its primary objective. Full details on this feasibility analysis will be provided in the SRM and Reporting and Analysis Plan (RAP).
- Following the data look, for the remainder of the study the team will seek to match NOT1D subjects to HVs at a group level according to the number of subjects for each gender and the distribution of age within gender. Full details on group matching will be provided in the SRM.
- An interim analysis will be carried out after the recruitment of 5 evaluable HVs and 5 evaluable NOT1D subjects. The primary purpose of this interim analysis will be to facilitate decision making and study design for a potential follow-up interventional study.

4.1. Overall Design



4.2. Type and Number of Subjects

Up to 15 evaluable NOT1D subjects and up to 15 evaluable HVs will be enrolled. If subjects prematurely discontinue the study, additional replacement subjects (up to 5 subjects) may be enrolled at the discretion of the sponsor in consultation with the investigator. Evaluable subjects are defined as subjects that provide both iLN biopsy (at least sufficient amounts of cells by one of the two biopsy methods employed to enable the conduct of assays related to at least the primary endpoint) AND at least sufficient amounts of peripheral blood to enable the conduct of assays related to at least the primary endpoint).

4.3. Design Justification

Immunophenotyping of cells obtained by iLN biopsies with a similar study design was described by van Baarsen [van Baarsen, 2013]. The authors of that report examined the cellular composition of inguinal lymph nodes in HVs and subjects with early inflammatory arthritis or individuals at risk of developing the disease. A similar study design is applied in this study.

4.4. Benefit:Risk Assessment

The following section outlines the risk assessment and mitigation strategy for this protocol:

4.4.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Short and/or persistent pain following the lymph node biopsy procedure	Short period of pain in the groin region has been reported in up to 7% of subjects and persistent pain for up to 2 months has been reported in up to 0.9% of subjects after the lymph node biopsy procedure previously [de Hair, 2012].	• Subjects will be followed up as described in the Time & Events Table (see Section 6.1) and asked to promptly report any pain. Subjects will be asked to contact the investigator for an examination if they report any symptoms related to persistent pain in the biopsy area.

Potential Risk of Clinical Significance	**Summary of Data/Rationale for Risk**	**Mitigation Strategy**
Bruising / Bleeding at biopsy site	Core biopsy and fine needle aspirate have the risk of moderate bleeding, especially if a large blood vessel is punctured. A haematoma incidence of up to 80 % has been reported in the literature and post procedure haematoma may cause further discomfort for the subject [de Hair, 2012].	• Biopsy procedure will be performed under ultrasound guidance by an experienced radiologist. Adequate manual pressure haemostasis will be performed and the puncture site will be reviewed by a physician prior to completion of the study visit. • Subjects with bleeding disorders or those who take anticoagulant / antiplatelet agents will be excluded from the study as described in Section 5.2. • Subjects in whom the procedure may be technically difficult will be excluded on a case by case basis subject to investigator's discretion. • The number of core biopsies taken in this study will be limited to a maximum of five core biopsies in addition to a maximum of two fine needle aspirate passages in each subject.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Infection risk	All biopsy procedures carry a risk of infection. This risk may be higher in subjects with (uncontrolled) Diabetes Mellitus.	• Subjects in whom the procedure may place them at increased risk of infection (e.g., large pannus, poor glycemic control) will be excluded on a case by case basis subject to investigator's discretion. • Subjects with groin infection will be excluded. • Strict aseptic technique during the procedure will be followed. • Subjects will be followed up as described in the Time and Events Table (see Section 6.1) and will be asked to contact the investigator for an examination if they report any signs or symptoms of infection.
Inadvertent trauma to femoral nerve	The biopsy procedure carries a hypothetical risk of trauma to the femoral nerve which may cause persisting paraesthesia (hypothetical because the site of biopsy is distant to the femoral nerve).	• Ultrasound guided biopsy procedure will avert the risk of inadvertent damage to large nerves.
Post-procedure lymphoedema	Damage to the lymphatic system carries a hypothetical risk of lymphoedema in the	• The number of core biopsies taken in this study will be limited to a maximum of five core biopsies in

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	ipsilateral leg.	addition to a maximum of two fine needle aspirate passages in each subject. Subjects will be followed up as described in the Time and Events Table (see Section 6.1) and will be asked to contact the investigator for an examination if they report any signs or symptoms of lymphoedema.
Other		
Toxicity from Local Anaesthetic agents	Administration of these agents may cause systemic e.g., hypersensitivity, cardiac or neurological toxicity in susceptible individuals.	• Subjects with allergy to local anaesthetic agents will be excluded. • The administration and use will be in keeping with the relevant SmPC.

4.4.2. Benefit Assessment

This is an exploratory study to identify peripheral immune system biomarkers associated with T1D activity and there is no anticipated potential for direct benefit to subjects. The results from this study may contribute to understanding of disease pathogenesis, and identifying potential new biomarkers for future early phase clinical studies, in which the pharmacology of new treatments of NOT1D subjects will be tested.

4.4.3. Overall Benefit:Risk Conclusion

No medical benefit is anticipated by NOT1D subject's participation in this study. Taking into account the measures taken to minimise risk to subjects, the potential study procedure-associated risks are justified by the anticipated general benefits in the future that may be afforded to subjects affected by T1D or other diseases.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information that may impact subject eligibility is provided in the SRM.

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

Subjects may be re-screened for the study, provided that re-screening can be accomplished to meet the timeframe of the inclusion criteria of a subject entering the study, i.e. with an interval of up to 8 weeks between the initial diagnosis and day 1 of the study (see Section 5.1).

5.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

AGE (all subjects)
1. Between 18 and 40 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
Healthy volunteers 2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination and laboratory tests. 3. Subject values for the following parameters: fasted glucose, HbA1c, INR, APTT,

platelet count, red blood cells and total lymphocyte count within the normal range at screening.

NOT1D subjects

4. Documented diagnosis of diabetes mellitus according to ADA and WHO criteria and consistent with Type 1a (autoimmune) Diabetes Mellitus, with an interval of up to 8 weeks between the initial diagnosis and day 1 of the study (day 1 = "iLN biopsy" day, see Section 6.1, Time and Events Table).
5. Currently requires insulin treatment for T1D and has received insulin therapy for at least 7 days prior to screening.
6. Positive, at screening, for at least one autoantibody associated with T1D: anti-GAD, anti-IA 2, anti-ICA, anti-IAA, anti-ZnT8.
7. Evidence, at screening, of residual functioning β cells as measured by fasted C-peptide levels ≥0.15 nmol/L.
8. Subject values for the following parameters: INR, APTT, platelet count, red blood cells and total lymphocyte count within the normal range at screening.

SEX (all subjects)

9. Both, male or female subjects are eligible to participate in this study.

A female subject is only eligible to participate if she is not pregnant (as confirmed by a negative urine) human chorionic gonadotrophin (hCG) test), not lactating at screening AND study visit(s) or has documented evidence to not be of child bearing potential.

INFORMED CONSENT (all subjects)

10. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the consent form and in this protocol.

OTHER (all subjects)

11. A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the investigator, in consultation with the Medical Monitor if required, agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.

5.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

HEALTHY VOLUNTEERS
1. Family history of T1D (i.e. 1st degree relative has been diagnosed with T1D) 2. Presence of one or more of serum autoantibodies, such as anti-GAD, anti-IA2, anti-ICA, anti-IAA, anti-ZnT8, anti-thyroid peroxidase antibodies, anti-tissue transglutaminase antibodies, anti-nuclear antibodies.

NOT1D SUBJECTS
3. History of autoimmune disease other than T1D 4. Presence of one or more of serum autoantibodies of the following: anti-thyroid peroxidase antibodies, anti-tissue transglutaminase antibodies or anti-nuclear antibodies

ALL SUBJECTS: CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION
5. Allergy or intolerance to local anaesthetic agents 6. Any localised groin condition which would contraindicate biopsy procedure including but not limited to: Active infection/inflammation at the intended puncture site, previous surgery/scarring or any other anatomical abnormality as deemed relevant to the procedure by the investigator, in consultation with the Medical Monitor if required. 7. History of bleeding disorders, current or anticipated continuous use of anticoagulant (including but not limited to warfarin, rivaroxaban) and antiplatelet agents (including but not limited to NSAIDS, clopidogrel, etc.) 8. Active or unresolved bacterial infection, viral infection, fungal infection within 4 weeks prior to day 1. 9. Known febrile episode over 38 degrees Celsius within 4 weeks prior to day 1. 10. Active organ dysfunction or previous organ allograft. 11. History of malignancy (with the exception of resected basal carcinoma of the skin or cervical carcinoma in situ).

12. Has undergone any major surgical procedure within 30 days before screening, and/or is planning to undergo any such surgery during the period of the study (i.e. from screening until the last follow-up telephone call, see Section 6.1).

CONCOMITANT MEDICATIONS
13. Present or previous treatment with any cell depleting therapies or immune-modulating or suppressive agents (e.g., oral steroids), including investigational agents such as the following but not limited to e.g., IL-2, CAMPATH, anti-CD4, anti-CD5, anti-CD3, anti-CD19, anti-CD-20.
14. Vaccination ≤ 28 days before day 1 of the study or planned during the study period.
15. Current participation in an interventional clinical trial. Subjects, who participated in an interventional clinical trial previously, must wait for 3 months after completing the previous interventional clinical trial before participating in this study.

CONTRAINDICATIONS
16. Any medical history or clinically relevant abnormality that is deemed by the investigator and/or medical monitor to make the subject ineligible for inclusion because of a safety concern or any situation that, in the investigator's judgment, is likely to cause the subject to be unable or unwilling to participate in study procedures or to complete all scheduled assessments.
17. A positive pre-study drug/alcohol screen (unless positive due to prescription medication). A minimum list of drugs that will be screened for include amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines.
18. Inability to access the groin area to perform the biopsy procedure as judged by the investigator.

5.3. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but did not undergo the study procedures (iLN biopsy and peripheral blood collection). In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 6.7.1.5).

5.4. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required visit or does not complete telephone calls:

- The site must attempt to contact the subject and re-schedule the missed visit/telephone call as soon as possible.
- The site must counsel the subject on the importance of maintaining the assigned visit/telephone calls schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- In cases where the subject is deemed 'lost to follow up', the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and if necessary a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up".

A subject may withdraw from the study at any time at his/her own request, or may be withdrawn at the discretion of the investigator at any time if the subject undergoes a change, which would render her/him unsuitable to take part in the study according to the stated inclusion / exclusion criteria. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

Replacement of withdrawn subjects for inclusion in the protocol will be at the discretion of the PI in consultation with the medical monitor.

5.5. Subject and Study Completion

A completed subject is one who has completed all phases of the study including the follow-up call(s).The end of the study is defined as the last subject's last telephone call.

5.6. Treatment after the End of the Study

Subjects will not receive any treatment from GSK after completion of the study because no medicinal products are being investigated in this study, the indication being studied is not life threatening or seriously debilitating and other treatment options are available.

The investigator is responsible for ensuring that consideration has been given to the post-study care of the subject's medical condition.

5.7. Lifestyle and/or Dietary Restrictions

5.7.1. Caffeine, Alcohol, and Tobacco

- Subjects should maintain their usual caffeine- or xanthine-containing products consumption levels (e.g., coffee, tea, cola drinks and chocolate).
- During each study session, subjects will abstain from alcohol for 24 hours prior to the screening visit, each assessment day and whilst present in the hospital / clinical unit. No more than 21 units (for men) and 14 units (for women) of alcohol per week should be consumed from the time of screening until the end of the clinical phase of the study.

5.7.2. Activity

Subjects must abstain from strenuous exercise for 24 hours post biopsy, including weight lifting, repetitive leg movements, riding a non-motorized bicycle or sexual intercourse.

Subjects must refrain from operating any motorised vehicle for at least 24h following the procedure.

5.8. Concomitant Medications and Non-Drug Therapies

5.8.1. Permitted Medications and Non-Drug Therapies

All concomitant medications for co-morbid conditions taken during the study will be recorded in the CRF. The minimum requirement is that drug name, total daily dose and the frequency of administration are to be recorded. See Section 5.8.2 for subjects' reporting obligations.

All participants

- Medication use by eligible subjects may be considered on a case by case basis by the Investigator (in consultation with the Medical Monitor if required).
- Eligible subjects who have well controlled concurrent medical conditions must be stable on their current treatment prior to study enrolment, and for the duration of the study, but may be excluded from the study at the investigators discretion.

NOT1D subjects

- Regular medication can be continued throughout the study but see Section 5.8.2 for reporting obligations.

5.8.2. Prohibited Medications and Non-Drug Therapies

Subjects must report the use of any prescription and non-prescription medications during the study period to the investigator.

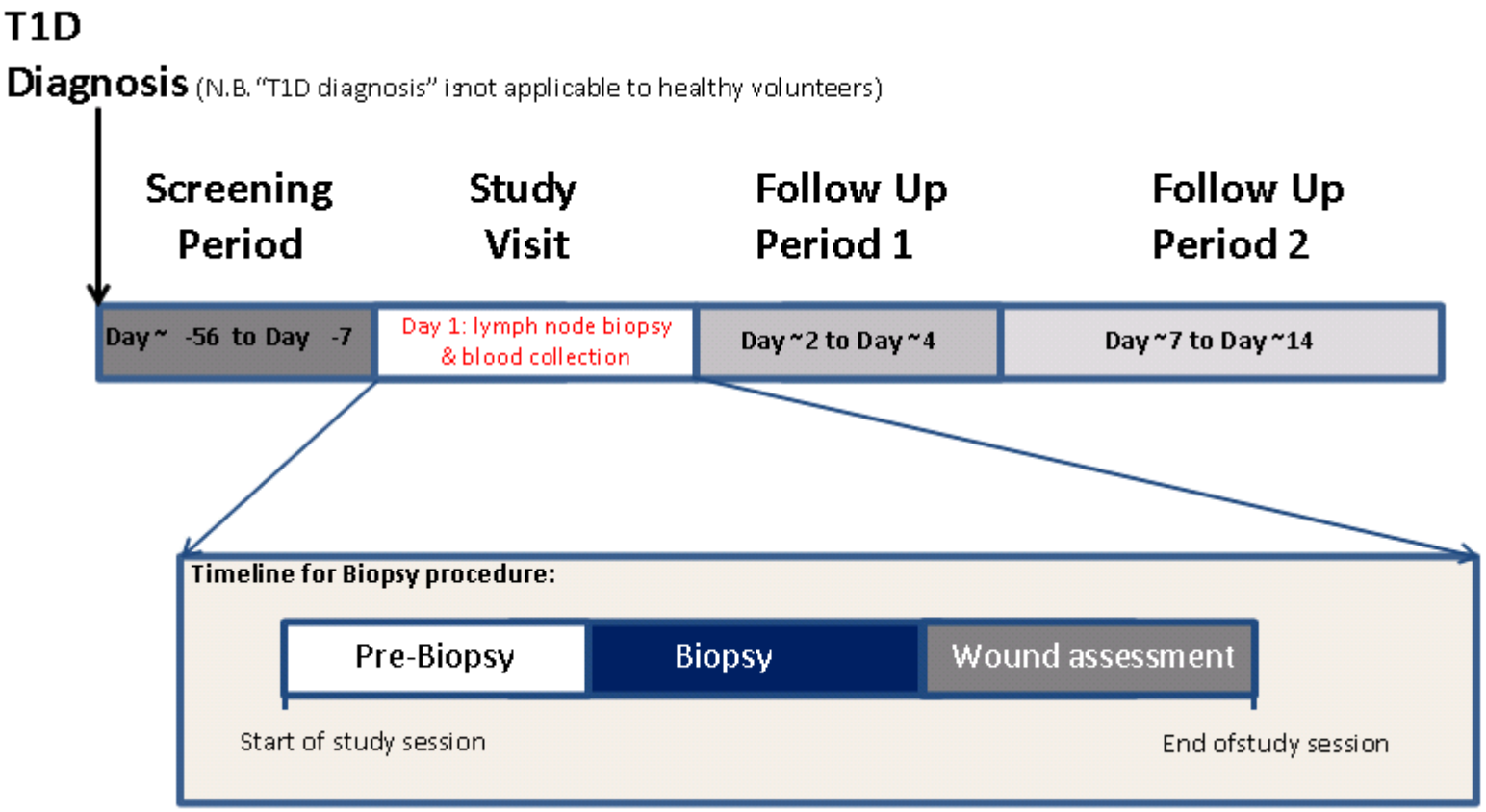
6. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment. The timing of each assessment is listed in the Time and Events Table Section 6.1

- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.
- No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

6.1. Time and Events Table



Screening procedures in addition to those listed below are (outpatient visit): Informed consent; Inclusion / Exclusion criteria; demography.

Procedure	**Screening (between -56 to 7 days prior to study session)**	**Study Session Day 1**			**Telephone follow-up Day ~2 to ~ Day 4**	**Telephone follow-up Day ~7 to Day ~14**	**Notes**
		Pre biopsy	**Biopsy**	**End of study session visit**			
Full physical exam	X						To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)
Medical history / Past and current medical conditions	X	X					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)

Procedure	**Screening (between -56 to 7 days prior to study session)**	**Study Session Day 1**			**Telephone follow-up Day ~2 to ~ Day 4**	**Telephone follow-up Day ~7 to Day ~14**	**Notes**
		Pre biopsy	**Biopsy**	**End of study session visit**			
Laboratory assessments (see Section 6.7.5)	X[1]	X[2]					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator) **1.** Blood testing in fasted condition at screening **2.** Haematology and clinical chemistry only on Day 1 in a non fasted condition
Concomitant medication review	X	X			X	X	
Alcohol Urine Test and Test for Drugs of Abuse	X	X					
Urine Pregnancy test (in women of child bearing potential)	X	X					

Procedure	**Screening (between -56 to 7 days prior to study session)**	**Study Session Day 1**			**Telephone follow-up Day ~2 to ~ Day 4**	**Telephone follow-up Day ~7 to Day ~14**	**Notes**
		Pre biopsy	**Biopsy**	**End of study session visit**			
Vital signs	X	X		X			To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)
Proposed biopsy site review		X					
Peripheral blood collection for cell isolation		X					
Peripheral blood collection for genetics		X					Optional in consenting subjects
Inguinal lymph node biopsy (fine needle aspirate & core biopsy)			X				
Wound assessment				X	X (telephone)	X (telephone)	To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)

Procedure	**Screening (between -56 to 7 days prior to study session)**	**Study Session Day 1**			**Telephone follow-up Day ~2 to ~ Day 4**	**Telephone follow-up Day ~7 to Day ~14**	**Notes**
		Pre biopsy	**Biopsy**	**End of study session visit**			
Pre- & post-biopsy questionnaire to assess subject experience of lymph node biopsy		X			X (fill in questionnaire on the day after the procedure, communicate answers via telephone a few days after)		
SAE review	←----------					----------→	
AE review		←----------			----------	----------→	

6.2. Screening and Critical Baseline Assessments

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

Procedures conducted as part of the subject's routine clinical management, for example but not limited to laboratory assessment, and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure(s) meet(s) the protocol-defined criteria and has (have) been performed within the timeframe of the study.

6.3. Pre-& post-biopsy Questionnaire

All subjects will be asked to complete a questionnaire about their expectations/experiences of undergoing the procedure of fine needle aspirate biopsy followed by core needle biopsy. The questionnaire focuses on the motivation of taking part in this study and aspects associated with experience before and after the procedure. Please see T&E table to study timings (see Section 6.1) and SRM for the questionnaire.

6.4. T1D auto-antibodies and other auto-antibodies

Tests for one or more antibodies will be performed at screening. These can include anti-GAD, anti-IA 2, anti-ICA, anti-IAA, anti-ZnT8, anti-nuclear antibodies, anti-thyroid peroxidise antibodies, anti-tissue glutaminase antibodies.

6.5. Fine needle aspirate & core biopsy of the inguinal lymph node

All study participants will undergo needle biopsies in an outpatient clinical setting by a radiologist. Both, iLN fine needle aspirate and iLN core biopsy will be taken after obtaining local anaesthesia and by using ultrasonography. First a fine-needle aspirate of a lymph node will be obtained and following that procedure up to five core biopsies of a lymph node will be acquired.

Using a standard clean technique, and with the ultrasound probe covered, local anaesthetic will be administered into the skin and subcutaneous tissues adjacent to the identified lymph node(s). The iLN will be localised using the assistance of ultrasonography and marked by the radiologist. For the fine-needle aspirate, an iLN will be localised by ultrasonography and sampled using a 21-gauge needle and a 5-ml syringe, as described by Tatovic et al 2015 [Tatovic, 2015]. Briefly, the LN cortex will be sampled using small to and fro needle movements while applying 1 ml suction with the syringe. One to two passages will be obtained per subject using this method.

Following the fine-needle aspirate, an approximately 0.5 cm long incision will be made as described by De Hair et al 2012 [de Hair, 2012]. From the same or a separate inguinal lymph node to the one sampled by fine-needle aspirate, up to five core biopsies (14 or 16

or 18 gauge) will be obtained using the assistance of ultrasonography , according to local standard operating procedures. All samples will be processed and stored immediately according to standard procedures for the various analyses (see SRM).

Following the procedures, manual pressure will be applied to the area for approximately 5 minutes to ensure haemostasis and to reduce the risk of haematoma formation. A sterile dressing will be applied to the small wound. Written information about after care will be provided to the subject, which will include advice about wound care.

6.6. Wound assessment

A visual assessment of the LN biopsy site will be conducted by the investigator before the end of the study visit to ensure adequate haemostasis and wound management. A sterile adhesive dressing will be applied to the wound. Subjects will be asked to contact the investigator for an examination if they report any signs or symptoms related to persistent pain in the biopsy area or signs or symptoms indicating infection.

6.7. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 6.1). Additional time points for safety tests (such as vital signs, physical exams and laboratory safety tests) may be added during the course of the study based on newly available data to ensure appropriate safety monitoring, but this will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme.

6.7.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The definitions of an AE or SAE can be found in Appendix 3.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

6.7.1.1. Time period and Frequency for collecting AE and SAE information

- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests) will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.
- AEs will be collected from: the start of study procedures until the follow-up contact (see Section 6.7.1.3), at the time points specified in the Time and Events Table (see Section 6.1).
- Medical occurrences that begin prior to the start of study procedure but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Appendix 3.

- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study procedures or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Appendix 3.

6.7.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?"

6.7.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Section 11.3) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up (as defined in Section 5.4). Further information on follow-up procedures is given in Section 11.3.

6.7.1.4. Death Events

The Death sections of the CRF will be required to be completed for all deaths.

The Death CRF is provided immediately after the occurrence or outcome of death is reported. Initial and follow-up reports regarding death must be completed within one week of when the death is reported.

6.7.1.5. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK or designee of SAEs related to study procedures (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects (and the safety of a product under clinical investigation are met).

GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it and will notify the IRB/IEC, if appropriate according to local requirements.

6.7.2. Pregnancy

- Details of all pregnancies in female subjects will be collected after the start of the study until completion of the study.
- If a pregnancy is reported then the investigator should inform GSK within 2 weeks of learning of the pregnancy and should follow the procedures outlined in Section 11.4.

6.7.3. Physical Exams

- A complete physical examination will include, at a minimum, assessment of the Cardiovascular, Respiratory, Gastrointestinal and Neurological systems, as well as palpation of lymph nodes and proposed puncture sites. Height and weight will also be measured and recorded.
- A proposed biopsy site review will involve palpation of lymph nodes and proposed puncture sites as well as an assessment of the skin around the area of the proposed puncture site.
- Investigators should pay special attention to clinical signs related to previous serious illnesses

6.7.4. Vital Signs

Vital signs will be measured in semi-supine position after approximately 5 minutes rest and will include temperature, systolic and diastolic blood pressure and pulse rate. These will be recorded in the CRF.

6.7.5. Clinical Safety & Other Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 1, must be conducted in accordance with the SRM, and Protocol Time and Events Schedule (see Section 6.1). Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit date. Details for the preparation and shipment of samples will be provided by the laboratory and will be detailed in the SRM. Reference ranges for all safety &other parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE) the results must be recorded in the CRF.

Information for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws will be described in the SRM.

All study-required laboratory assessments will be performed by a central laboratory, apart from:

- Haematology, Clinical Chemistry, Routine Urinalysis and "Other Screening Tests" as described in the appropriate row of Table 1 below, which will be conducted locally at each site (with the exception of auto-antibody screening which may be conducted by a central laboratory)

NOTE: Local laboratory results are only required in the event that the central laboratory results are not available in time for either a treatment and/or response evaluation to be performed. If a local sample is required it is important that the sample for central analysis is obtained at the same time. Additionally if the local laboratory results are used to make either a treatment or response evaluation, the results must be entered into the CRF.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 1.

Table 1 Protocol Required Safety & Other Laboratory Assessments

Laboratory Assessments	Parameters			
Haematology	Platelet Count	*RBC Indices*:	*WBC count with Differential:*	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit		Monocytes	
	INR (derived from Prothrombin time) and APTT		Eosinophils	
			Basophils	
Clinical Chemistry	Urea	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose[1]	Calcium	Alkaline phosphatise	Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol and drug screen • Urine hCG Pregnancy test (as needed for women of child bearing potential)[2] • Fasted C-peptide • HbA1c • anti-GAD, anti-IA2, anti-ICA, anti-IAA, anti-ZnT8, anti-thyroid peroxidase, anti-tissue transglutaminase and anti-nuclear antibody			

NOTES :

1. Fasted glucose at screening, non fasted glucose on Day 1.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

6.8. Biomarker(s)

With the subject's consent, iLN and peripheral blood samples will be collected during this study and used for the purposes of measuring biomarkers to aid the understanding of the pathogenesis of NOT1D and/or medically related conditions. If relevant, this approach will be extended to include the identification of biomarkers associated with adverse events. A peripheral blood sample may also be used for genotyping. Further details of experimental procedures will be provided in the SRM.

Blood samples (volume up to 30 ml) and up to two fine needle aspirate passages and up to five lymph node core biopsy samples will be collected at the time point indicated in Section 6.1.

Primary endpoint: Candidate biomarkers (= absolute numbers and/or proportion of leukocyte subsets) associated with either location of cells (i.e. iLNs derived leukocytes versus peripheral blood derived cells) and/or disease-status (i.e. HV versus NOT1D subjects) may be identified by application of flow cytometry.

- Objective: To assess the frequency and phenotype of leukocyte subsets in iLN and peripheral blood in HVs and T1D subjects
    - Leukocyte subset phenotyping will be carried out on peripheral blood and iLN-derived cells by assessing expression of but not restricted to the following antigens; CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD24, CD25, CD38, CD45RA, CD56, HLA-DR and FOXP3.

Secondary endpoint: Candidate biomarkers (=absolute numbers and/or proportion of leukocyte subsets) associated with type of biopsy (core biopsy versus fine needle aspirate) may be identified by application of flow cytometry.

- Objective: To assess the frequency and phenotype of leukocyte subsets from iLN core biopsy versus iLN fine needle aspirate in HVs and T1D subjects:
    - Leukocyte subset phenotyping will be carried out on iLN-derived cells by assessing expression of but not restricted to the following antigens; CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD25, CD24, CD38, CD45RA, CD56, HLA-DR and FOXP3

Exploratory endpoints: Novel candidate biomarkers and functional analysis of iLN-derived cells and cells from peripheral blood will be assessed depending on the material available.

- Objective: To assess suppression activity of T regulatory lymphocytes from the iLN and peripheral blood in HVs and NOT1D subjects
    - T regulatory suppression analysis may be carried out by a micro suppression assay or alternative equivalent.
- Objective: To assess the frequency of cytokine-producing lymphocytes in HVs and NOT1D subjects.
    - Percentages of lymphocytes (and subsets) producing pro- and or anti-inflammatory cytokines may be identified by flow cytometry (for example, but not restricted to, Th1 and Th17 cells).
- Objective: To assess clonal expansion of T and B lymphocyte populations in HVs and NOT1D subjects
    - T and B lymphocyte clones may be assessed by TCR and BCR deep sequencing.
- Objective: To assess the gene expression fingerprint of auto-antigen specific lymphocytes in HVs and NOT1D subjects

    - For analysis of autoreactive T lymphocyte function, iLN-derived lymphocytes and PBMCs may be stimulated with antigens such as GAD65 or proinsulin. Gene expression of autoreactive T lymphocytes may be analysed by RNA (Ribonucleic Acid) sequencing (and/or equivalent methodologies) of sorted T cell populations. The RNAs assayed/identified may be those involved with the pathogenesis of T1D.
- Objective: To assess the phenotype and transcriptomic fingerprint of stromal cells from iLN core biopsy samples in HVs and NOT1D subjects
    - Phenotyping of stromal cells, including but not limited to fibroblastic reticular cells, from iLN core biopsy samples may be carried out by assessing expression of, but not limited to, the following antigens: CD45, CD31, gp38, MHC II.
    - Gene expression of stromal cells, including but not limited to fibroblastic reticular cells, may be analysed by RNA sequencing (and/or equivalent methodologies)

All samples will be retained for a maximum of 15 years after the last subject completes the trial.

6.9. Genetics

Information regarding genetic research is included in Appendix 2.

7. DATA MANAGEMENT

- For this study, subject data will be entered into GSK defined CRFs, transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials or other Personally Identifiable Information (PII) will not be collected or transmitted to GSK according to GSK policy.
- Screen failure information will be entered in to the clinical database (see also Section 5.3)

8. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

This is a non-randomized study, without an investigational drug.

All data required for the analysis of primary, secondary and exploratory objectives will be captured in the clinical database and included in the clinical report. Further details will be provided in the RAP.

8.1. Hypotheses

This study is designed to explore the phenotype of immune cells in the iLN and peripheral blood (such as, but not restricted to PBMCs) of NOT1D subjects compared to HVs.

There are no formal hypotheses being tested due to the exploratory nature of the study. Primary comparisons of NOT1D subjects to HVs will be made using an estimation approach, providing point estimates and confidence intervals.

Secondary comparisons of biopsy methods will be made within NOT1D subjects and HVs using an estimation approach:

- iLN biopsies will be carried out by two related methods, fine-needle aspirate and core biopsy sampling:
- If sufficient numbers of cells to meet the cell number requirements of the primary endpoint are obtained by both biopsy methods, the primary endpoint analysis will be carried out independently for each biopsy method, otherwise only the biopsy method for which sufficient numbers of cells were obtained will be used for primary analysis.
- If no meaningful differences between biopsy methods are observed, material from both biopsy methods may be pooled to meet exploratory objectives 1, 2, 3 and 4. Material from fine-needle aspirate and core biopsies will **not** be pooled unless supported by the comparison described above.
- If meaningful differences in the proportion of leukocyte subsets between cells derived from each biopsy method **are** observed, then :
    - If sufficient number of core biopsy derived cells are available, these will be analysed for exploratory objectives 1, 2 and 5.
    - Fine needle aspirate derived cells will be used to meet exploratory objectives 3 and 4. If insufficient number of core biopsy derived cells are available to meet exploratory objectives 1 and 2, then fine needle aspirate derived cells may be used to meet either or both of those exploratory objectives

8.2. Sample Size Considerations

This is an exploratory study and there are no formal sample size calculations. The upper limit for sample size (n=15 per group) has been based on feasibility.

8.2.1. Sample Size Assumptions

For PBMCs, literature data was available for lymphocytes CD4+, CD8+ and regulatory T-cells in HVs using a similar gating strategy to that planned here. Computer simulation were performed to assess the expected precision of estimation of odds ratios and relative risks for a cross sectional comparison of PBMC regulatory T-cells for a range of differences between NOT1D subjects and HVs. The average precision for both PBMC estimates is expected to be within 1% of the point estimate using n=15 per group

8.2.2. Sample Size Sensitivity

No formal sample size sensitivity analysis has been performed.

The precision for iLN estimates is expected to be lower than the precision for PBMC estimates, since it is anticipated that fewer cells will be available from iLN biopsies for flow cytometry analysis. No published data was found for the phenotypes of interest in iLN biopsies.

8.2.3. Sample Size Re-estimation or Adjustment

Data for primary endpoints may be reviewed and/or analyzed on an ongoing basis. Based on this information the study team may make a recommendation to the investigator that sufficient data has been obtained prior to the maximum of n=15 subjects per group, such that further recruitment of subjects is not needed.

8.3. Data Analysis Considerations

8.3.1. Analysis Populations

Population	Definition / Criteria
Safety	• Comprised of all subjects who complete any study assessment.
Core Biopsy	• Subjects in the 'Safety' population for whom at least one lymph node core biopsy was taken
Fine needle aspirate Biopsy	• Subjects in the 'Safety' population for whom at least one lymph node fine needle aspirate biopsy was taken
Biopsy	• Subjects in both 'Core Biopsy' and 'fine needle aspirate biopsy' populations
Blood	• Subjects in the 'Safety' population for whom one blood sample was taken for analysis of cells derived from peripheral blood.

8.3.2. Interim Analysis

A data look will be carried after the recruitment of a cohort of up to 5 HVs to determine if the quality and quantity of cells derived from either fine needle aspirate or core biopsy of the inguinal lymph node and from peripheral blood are likely to be sufficient to continue the study to meet its primary objective.

An interim analysis will be carried out after the recruitment of 5 evaluable HVs and 5 evaluable NOT1D subjects. The primary purpose of this interim analysis will be to facilitate decision making and study design for a potential follow-up interventional study. In addition the interim analysis results would also help to enable prioritization of the exploratory assays and to facilitate the final RAP. Due to uncertainty around variability and precision, the primary analyses described in Section 8.4.1 will be performed in order to re-assess the precision of estimates for the comparisons of interest.

8.4. Key Elements of Analysis Plan

8.4.1. Primary Analyses

Descriptive statistics will be calculated for each of the primary flow cytometry cell types by group and sample type (blood / core biopsy / fine needle aspirate biopsy). The flow cytometry data for each cell type will be displayed graphically using scatter plots of cell count and % cell count versus group and/or sample type.

Generalized linear models with appropriate link functions will be used to analyze data separately for each flow cytometry cell type. Models will include terms for group, sample type, and the interaction of group with sample type. Subject will be included as a random effect term.

- For absolute cell counts, these models will estimate the mean cell count for each sample type in each group, comparing means as outlined in the primary hypothesis section.
- For proportional cell counts, these models will estimate the average odds (or relative risk) of having a particular cell type for each sample type in each group, comparing these odds (or relative risks) by estimating the ratios for the primary comparisons of interest.

All estimates will be presented with 95% confidence intervals.

Model assumptions will be checked, and modifications to the model or alternative approaches may be considered in the event of significant deviations from model assumptions.

Further details will be provided in the Reporting and Analysis Plan (RAP).

8.4.2. Secondary Analyses

Generalized linear models as described in Primary Analyses will be used to compare absolute and proportion cell counts between core biopsies and fine needle aspirate biopsies.

Details of this and further secondary analyses will be provided in the RAP.

8.4.3. Other Analyses

Details of other analyses will be provided in the RAP.

9. STUDY GOVERNANCE CONSIDERATIONS

9.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrolment of subjects begins.

9.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favourable opinion/approval of the study protocol and amendments as applicable
- Obtaining signed informed consent
- Investigator reporting requirements (e.g., reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.

- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

9.3. Quality Control (Study Monitoring)

- In accordance with applicable regulations including GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements.
- When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the "CRF" will serve as the source document.

GSK will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents

9.4. Quality Assurance

- To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.
- In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

9.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.

- If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.
- If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities (where applicable) of the suspension or premature discontinuation of the study and the reason(s) for the action.
- If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

9.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.
- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.
- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

9.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis, if applicable.

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

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11. APPENDICES

11.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
ADA	American Diabetes Association
CD	Cluster of Differentiation (glycoprotein)
CRF	Case Report Form
DNA	Deoxyribonucleic acid
GAD	Glutamic acid decarboxylase
GSK	GlaxoSmithKline
HLA	Human Leukocyte Antigen
hCG	Human chorionic gonadotrophin
HV	Healthy Volunteers
IAA	Insulin autoantibodies
IA-2	Protein tyrosine phosphatase-related islet antigen-2
ICA	Islet-cell antigen
IGRP	Islet-specific glucose-6-phosphatase catalytic subunit-related protein
iLN	inguinal lymph node
IRB/IEC	Institutional Review Board / Institutional Ethics Committee
LN	Lymph node
MHC	Major Histocompatibility Complex
mRNA	messenger RNA
NMR	Nuclear Magnetic Resonance spectroscopy
NOD	Non-Obese Diabetic
NOT1D	New Onset Type 1 Diabetes Mellitus
PBMC	Peripheral Blood Mononuclear Cells
RA	Rheumatoid Arthritis
RAP	Reporting and Analysis Plan
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SRM	Study Reference Manual
T1D	Type 1 Diabetes
TCR / BCR	T-cell Receptor / B-cell Receptor
WHO	World Health Organisation
ZnT8	Zinc transporter 8

Trademark Information

Trademarks owned by the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
None

11.2. Appendix 2: Genetic Research

Genetics – Background

Naturally occurring genetic variation may contribute to inter-individual variability in response to medicines, as well as an individual's risk of developing specific diseases. Genetic factors associated with disease characteristics may also be associated with response to therapy, and could help to explain some clinical study outcomes. For example, genetic variants associated with age-related macular degeneration (AMD) are reported to account for much of the risk for the condition [Gorin, 2012] with certain variants reported to influence treatment response [Chen, 2012]. Thus, knowledge of the genetic aetiology of disease may better inform understanding of disease and the development of medicines. Additionally, genetic variability may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), or pharmacodynamics (relationship between concentration and pharmacologic effects or the time course of pharmacologic effects) of a specific medicine and/or clinical outcomes (efficacy and/or safety) observed in a clinical study.

Genetic Research Objectives and Analyses

The objectives of the genetic research are to investigate the relationship between genetic variants and:

- antigen-specific T-cells in T1D and T1D susceptibility, severity, and progression

Genetic data may be generated while the study is underway or following completion of the study. Genetic evaluations may include focused candidate gene approaches and/or examination of a large number of genetic variants throughout the genome (whole genome analyses). Genetic analyses will utilize data collected in the study and will be limited to understanding the objectives highlighted above. Analyses may be performed using data from multiple clinical studies to investigate these research objectives.

Appropriate descriptive and/or statistical analysis methods will be used. A detailed description of any planned analyses will be documented in a Reporting and Analysis Plan (RAP) prior to initiation of the analysis. Planned analyses and results of genetic investigations will be reported either as part of the clinical RAP and study report, or in a separate genetics RAP and report, as appropriate.

Study Population

Any subject who is enrolled in the study can participate in genetic research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the genetic research.

Study Assessments and Procedures

A key component of successful genetic research is the collection of samples during clinical studies. Collection of samples, even when no *a priori* hypothesis has been

identified, may enable future genetic analyses to be conducted to help understand variability in disease and medicine response.

- A 6 ml blood sample will be taken for Deoxyribonucleic acid (DNA) extraction. A blood sample is collected at the study visit (Day 1), after the subject has been randomized and provided informed consent for genetic research. Instructions for collection and shipping of the genetic sample are described in the laboratory manual. The DNA from the [blood/saliva] sample may undergo quality control analyses to confirm the integrity of the sample. If there are concerns regarding the quality of the sample, then the sample may be destroyed. The blood sample is taken on a single occasion unless a duplicate sample is required due to an inability to utilize the original sample.

The genetic sample is labelled (or "coded") with the same study specific number used to label other samples and data in the study. This number can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number).

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study, or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will only use samples collected from the study for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

Subjects can request their sample to be destroyed at any time.

Informed Consent

Subjects who do not wish to participate in the genetic research may still participate in the study. Genetic informed consent must be obtained prior to any blood being taken.

Subject Withdrawal from Study

If a subject who has consented to participate in genetic research withdraws from the clinical study for any reason other than being lost to follow-up, the subject will be given a choice of one of the following options concerning the genetic sample, if already collected:

- Continue to participate in the genetic research in which case the genetic DNA sample is retained
- Discontinue participation in the genetic research and destroy the genetic DNA sample

If a subject withdraws consent for genetic research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records.

Genotype data may be generated during the study or after completion of the study and may be analyzed during the study or stored for future analysis.

- If a subject withdraws consent for genetic research and genotype data has not been analyzed, it will not be analyzed or used for future research.
- Genetic data that has been analyzed at the time of withdrawn consent will continue to be stored and used, as appropriate.

Screen and Baseline Failures

If a sample for genetic research has been collected and it is determined that the subject does not meet the entry criteria for participation in the study, then the investigator should instruct the subject that their genetic sample will be destroyed. No forms are required to complete this process as it will be completed as part of the consent and sample reconciliation process. In this instance a sample destruction form will not be available to include in the site files.

Provision of Study Results and Confidentiality of Subject's Genetic Data

GSK may summarize the genetic research results in the clinical study report, or separately and may publish the results in scientific journals.

GSK may share genetic research data with other scientists to further scientific understanding in alignment with the informed consent. GSK does not inform the subject, family members, insurers, or employers of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from genetic studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined. Further, data generated in a research laboratory may not meet regulatory requirements for inclusion in clinical care.

11.3. Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

11.3.1. Definition of Adverse Events

Adverse Event Definition:
• In this study an AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with study procedures • NOTE: An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the study procedures.

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study procedure even though it may have been present prior to the start of the study.

Events <u>NOT</u> meeting definition of an AE include:
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE. • Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

11.3.2. Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease, etc).

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject 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.
c. Requires hospitalization or prolongation of existing hospitalization NOTE: • In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in disability/incapacity NOTE: • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the

other outcomes listed in the above definition. These should also be considered serious.

- Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

11.3.3. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.
- Subject-completed questionnaires and the collection of AE data are independent components of the study.
- Responses to each question in the questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer.

11.3.4. Evaluating AEs and SAEs

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and will assign it to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event that prevents normal everyday activities. - an AE that is assessed

as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

- An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study procedure and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study procedure will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information (where applicable), for marketed products, in the determination of his/her assessment.
- For each AE/SAE the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.
- The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings,

including histopathology.

- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

11.3.5. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool

- Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool
- If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor.
- Site will enter the serious adverse event data into the electronic system as soon as it becomes available.
- The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.
- After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the [Medical Monitor or the SAE coordinator] by telephone.
- Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

11.4. Appendix 4: Collection of Pregnancy Information

The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of a subject's pregnancy. The subject will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported as such.

Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the study treatment/procedure by the investigator, will be reported to GSK as described in Appendix 3. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

11.5. Appendix 5: Country Specific Requirements

No country-specific requirements exist.

11.6. Appendix 6: Protocol changes

This amendment applies to all sites.

11.6.1. Amendement No. 3

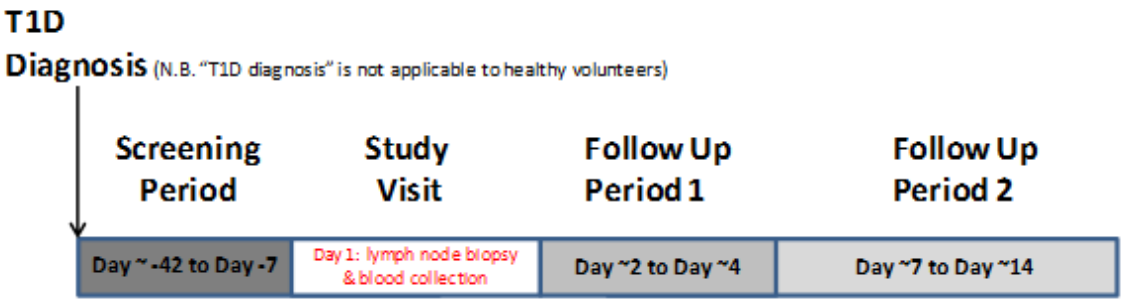
General summary of changes:

- Extended the interval between the initial diagnosis and Day 1, from 6 weeks to 8 weeks following recruitment challenges. 8 weeks is the upper limit for the NOTID population and does not affect the scientific rationale for the study. The 8 week change was updated throughout the protocol.
- Clarified exclusion criteria, vaccination ≤ 28 days before day 1.
- Interim Analysis: Added additional information for the Interim Analysis after the recruitment of 5 evaluable HVs and 5 evaluable NOTID subjects. This will facilitate the decision making of the study and prioritization of the exploratory assays.
- Clarified fasting at screening in the Time and Event table and added footnote to Table 1 to clarify fasted glucose at screening and non fasted glucose on Day 1

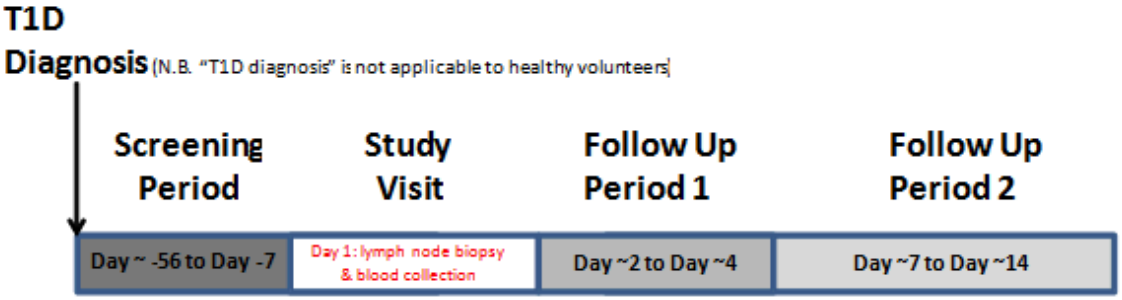
List of specific changes:

Section 1: Overall Design. Updated schematic to reflect extended screening window from Day~ -42 to Day~ -56. The change was also applied to Section 4: Study Design and Section 6.1: Time and Events Table.

Original:



Amended:



Section 1: Overall Design. Last sentence in second bullet point, changed the total duration of the study from 8 weeks to 10 weeks. This change was also applied to Section 4. Study Design.

Original:

- After informed consent, each subject will attend a screening visit followed by a study visit for iLN biopsy and peripheral blood collection (Day 1). Follow up phone calls after the study visit will be carried out at approximately Day 2-4 and Day 7 to 14. The total duration of this study will be up to 8 weeks, including the screening period.

Amended:

- After informed consent, each subject will attend a screening visit followed by a study visit for iLN biopsy and peripheral blood collection (Day 1). Follow up phone calls after the study visit will be carried out at approximately Day 2-4 and Day 7 to 14. The total duration of this study will be up to ~~8~~ **10** weeks, including the screening period.

Section 1: Overall Design, added Interim Analysis (4th bullet point). This was also added to Section 4 (5th bullet point).

Added text:

- An interim analysis will be carried out after the recruitment of 5 evaluable HVs and 5 evaluable NOT1D subjects. The primary purpose of this interim analysis will be to facilitate decision making and study design for a potential follow-up interventional study.

Section 5: Selection of study population and withdrawal criteria. Changed timeframe of the inclusion criteria to 8 weeks. This change also applies to section 5.1 NOTID subjects bullet point number 4.

Original:

Subjects may be re-screened for the study, provided that re-screening can be accomplished to meet the timeframe of the inclusion criteria of a subject entering the study, i.e. with an interval of up to 6 weeks between the initial diagnosis and day 1 of the study (see Section 5.1).

Amended:

Subjects may be re-screened for the study, provided that re-screening can be accomplished to meet the timeframe of the inclusion criteria of a subject entering the study, i.e. with an interval of up to 8~~6~~ weeks between the initial diagnosis and day 1 of the study (see Section 5.1).

Section 5.1.

Original:

4. Documented diagnosis of diabetes mellitus according to ADA and WHO criteria and consistent with Type 1a (autoimmune) Diabetes Mellitus, with an interval of up to 6 weeks between the initial diagnosis and day 1 of the study (day 1 = "iLN biopsy" day, see Section 6.1, Time and Events Table).

Amended:

4. Documented diagnosis of diabetes mellitus according to ADA and WHO criteria and consistent with Type 1a (autoimmune) Diabetes Mellitus, with an interval of up to **8** ~~6~~ weeks between the initial diagnosis and day 1 of the study (day 1 = "iLN biopsy" day, see Section 6.1, Time and Events Table).

Section 5.2. Exclusion Criteria: Concomitant medications: Bullet point number 14. Deleted i.e. from screening visit to last telephone call to make clearer exclusion period applied to before Day 1.

Original:

14. Vaccination ≤ 28 days before day 1 of the study or planned during the study period (i.e. from screening visit to last telephone call)

Amended:

14. Vaccination ≤ 28 days before day 1 of the study or planned during the study period. ~~(i.e. from screening visit to last telephone call)~~

Section 6.1. Time and Events Table. Screening days updated in header and clarified fasting at screening and non fasting on Day 1 in the notes associated to laboratory assessments.

Original:

Procedure	Screening (between -42 to 7 days prior to study session)	Study Session Day 1			Telephone follow-up Day ~2 to ~ Day 4	Telephone follow-up Day ~7 to Day ~14	Notes
		Pre biopsy	Biopsy	End of study session visit			
Laboratory assessments (see Section 6.7.5)	X	X[1]					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator) 1.Haematology and clinical chemistry only on Day 1

Amended:

Procedure	Screening (between -4256 to 7 days prior to study session)	Study Session Day 1			Telephone follow-up Day ~2 to ~ Day 4	Telephone follow-up Day ~7 to Day ~14	Notes
		Pre biopsy	Biopsy	End of study session visit			
Laboratory assessments (see Section 6.7.5)	X[1]	X[2]					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator) 1. Blood testing in fasted condition at screening 2. Haematology and clinical chemistry only on Day 1 in a non fasted condition

Section 6. Table 1: Clinical Chemistry added footnote to clarify fasted glucose at screening, non fasted glucose on Day 1.

Original:

Clinical Chemistry	Urea	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium	Alkaline phosphatise	Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol and drug screen • Urine hCG Pregnancy test (as needed for women of child bearing potential)[1] • Fasted C-peptide levels • anti-GAD, anti-IA2, anti-ICA, anti-IAA, anti-ZnT8, anti-thyroid peroxidase, anti-tissue transglutaminase and anti-nuclear antibody			
NOTES : 1. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.				

Amended:

Clinical Chemistry	Urea	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose[1]	Calcium	Alkaline phosphatise	Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol and drug screen • Urine hCG Pregnancy test (as needed for women of child bearing potential)[2] • Fasted C-peptide • HbA1c • anti-GAD, anti-IA2, anti-ICA, anti-IAA, anti-ZnT8, anti-thyroid peroxidase, anti-tissue transglutaminase and anti-nuclear antibody			
NOTES : 1. **Fasted glucose at screening, non fasted glucose on Day 1.** 2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.				

Section 8.3.2. Interim Analysis. Added additional text for interim analysis.

Original:

Due to uncertainty around variability and precision, the primary analyses described in Section 8.4.1 may be performed as data looks on one or more occasions in order to re-assess the precision of estimates for the comparisons of interest. See also Section 8.2.3

Amended:

~~Due to uncertainty around variability and precision, the primary analyses described in Section 8.4.1 may be performed as data looks on one or more occasions in order to re-assess the precision of estimates for the comparisons of interest. See also Section 8.2.3~~
An interim analysis will be carried out after the recruitment of 5 evaluable HVs and 5 evaluable NOT1D subjects. The primary purpose of this interim analysis will be to facilitate decision making and study design for a potential follow-up interventional study. In addition the interim analysis results would also help to enable prioritization of the exploratory assays and to facilitate the final RAP. Due to uncertainty around variability and precision, the primary analyses described in Section 8.4.1 will be performed in order to re-assess the precision of estimates for the comparisons of interest.

11.6.2. Amendement No. 2

General summary of changes:

- Time and Event table: Duplicate assesment removed and updated (medical history/past current medical conditions and laboratory assessments). Clarified when telephone call for Follow Up Period 1 would be collected. Clarified genetics testing was optional in consenting subjects. Added SAE/AE review.

- Added HbA1c to Clinical Chemistry in Table 1.

List of specific changes:

Section 6.1. Time and Events table

Original:

Procedure	Screening (between -42 to 7 days prior to study session)	Study Session Day 1			Telephone follow-up Day ~2 to ~ Day 4	Telephone follow-up Day ~7 to Day ~14	Notes
		Pre biopsy	Biopsy	End of study session visit			
Medical history / Past and current medical conditions	X						To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)
Laboratory assessments (see Section 6.7.5)	X						To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)
Medical history / Past and current medical conditions		X					
Peripheral blood collection for haematology, clinical chemistry only, (see Section 6.7.5)		X					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)

Amended:

Procedure	Screening (between -42 to 7 days prior to study session)	Study Session Day 1			Telephone follow-up Day ~2 to ~ Day 4	Telephone follow-up Day ~7 to Day ~14	Notes
		Pre biopsy	Biopsy	End of study session visit			
Medical history / Past and current medical conditions	X	X					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator)
Laboratory assessments (see Section 6.7.5)	X	X[1]					To be completed if a post study visit is required. Attendance to the unit post study sessions only if necessary (at discretion of investigator) 1.Haematology and clinical chemistry only on Day 1

Orignal:

Pre- & post-biopsy questionnaire to assess subject experience of lymph node biopsy		X			X (via telephone: questionnaire to be filled in on the day after the procedure)		

Amended:

Pre- & post-biopsy questionnaire to assess subject experience of lymph node biopsy		X			X (fill in questionnaire on the day after the procedure, communicate answers via telephone a few days after)		

Original:

Peripheral blood collection for genetics		X					

Amended:

Peripheral blood collection for genetics		X					Optional in consenting subjects

Section 6.7.5, Table 1:

Added HbA1c test to other screening test to support inclusion criteria and changed Fasted C-peptide levels to Fasted C-peptide.

11.6.3. Amendment No. 1

General summary of changes:

- Objective "To assess the biochemical fingerprint derived from metabonomic analyses of peripheral blood in HVs and NOT1D subjects" & associated endpoint removed in Section 3. References to this objective and endpoint in Section 6.1; and Section 6.8 were removed accordingly. This objective and associated endpoint were removed because they should be addressed in separate studies in the future.
- New exploratory objective ("To assess the phenotype and transcriptomic fingerprint of stromal cells from iLN core biopsy samples in HVs and NOT1D subjects") and associated endpoint added in Section 3, Section 6.8 and Section 8.1 were updated accordingly. This objective and associated endpoint were added to provide additional insights into the exploration of the peripheral immune system in subjects with New Onset T1 Diabetes, which were not envisaged during the finalisation of the original protocol.
- The Questionnaire in Appendix 1 was modified to avoid any duplication with Informed Consent procedures and duplication of data collection in eCRF. The entire questionnaire has been moved from the Study protocol to the Study Reference Manual (SRM) and is therefore not present in the amended protocol anymore.
- All appendix numberings and in-text references to appendices have been updated to reflect that the Lymph Node Questionnaire is no longer located in Appendix 1 of the Study Protocol.
- Clarifying wording was added and typos were corrected in Section 6.2; Section 6.7.5 and Section 6.8.
- Appendix 1 - Abbreviations and Trademarks lists were updated.

List of specific changes:

<table>
<tr><th>**Original:**</th><th>**<u>Amended</u> & *Rationale for amendment***</th></tr>
<tr><td>Section 3, Objectives & Endpoint:

<table><tr><td>5) To assess the biochemical fingerprint derived from metabonomic analyses of peripheral blood in HVs and NOT1D subjects</td><td>• List of biochemical markers / biomarkers (immune & non-immune function related) derived from analysis of peripheral blood</td></tr></table>
Section 6.1 Time and Events Table, Procedure 10:

• Peripheral blood collection for cell isolation and metabonomics analyses</td><td>

<table><tr><td><u>5) To assess the phenotype and transcriptomic fingerprint of stromal cells from iLN core biopsy samples in HVs and NOT1D subjects</u></td><td>• <u>Absolute number and/or proportion of stromal cell subsets and gene expression levels of stromal cell subsets from iLN core biopsy samples</u></td></tr></table>
Section 6.1 Time and Events Table, Procedure 10:

• Peripheral blood collection for cell isolation ~~and metabonomics analyses~~

The original exploratory objective & endpoint were removed because they should be addressed in separate studies in the future.

A new exploratory objective & endpoint were added to provide additional insights into the exploration of the peripheral immune system in subjects with New Onset T1 Diabetes, which were not envisaged during the finalisation of the original protocol.</td></tr>
<tr><td>Section 11.1, Appendix 1: Lymph Node Questionnaire: Core-needle Biopsy and Fine Needle Aspirate of inguinal lymph</td><td></td></tr>
</table>

nodes Part 1: • Please check the appropriate box to identify which study group you belong to: ☐ Newly Diagnosed Diabetes Mellitus subject ☐ Healthy volunteer 1. Gender: ☐ Male ☐ Female 2. Age:.....…… years 3. Did you ever undergo a procedure under local anaesthetics? ☐ No → Go to question ~~5~~ • 6. Please circle the appropriate box below to indicate how well you are prepared for the lymph node biopsy procedure, with respect to: a. understanding the aim of the procedure and the background information b. what to expect during the procedure c. after-care d. possible complications Part 2: 1. With the benefit of hindsight, what aspects do you feel could have been better explained about the procedure: ☐ None, I am content with the given information and how it was discussed with me Or: More detailed	~~• Please check the appropriate box to identify which study group you belong to:~~ ~~☐ Newly Diagnosed Diabetes Mellitus subject~~ ~~☐ Healthy volunteer~~ ~~1. Gender:~~ ~~☐ Male~~ ~~☐ Female~~ ~~2. Age:.....…… years~~ 3. Did you ever undergo a procedure under local anaesthetics? ☐ No → Go to question 3 ~~• 6. Please circle the appropriate box below to indicate how well you are prepared for the lymph node biopsy procedure, with respect to:~~ ~~a. understanding the aim of the procedure and the background information~~ ~~b. what to expect during the procedure~~ ~~c. after-care~~ ~~d. possible complications~~ 1. With the benefit of hindsight, what aspects do you feel could have been better explained about the procedure: ☐ None, I am content with the given information and how it was discussed with me Or: More detailed

explanations about: □ The procedure itself □ Anaesthetic procedure □ After-care □ Complications to be expected	explanations about: □ The procedure itself □ Anaesthetic procedure ~~□ After-care~~ □ Complications to be expected *Part 1 study group and age information, Part 1 question 6 and Part 2, Question 1 ("After care" option) were deleted to avoid any duplication of data collection in eCRF and duplication with Informed Consent procedures respectively. A cross-reference in Part 1 question 3 was updated to reflect updated numbering of questions. The entire questionnaire has been removed from the protocol and added to the SRM.*
Section 6.2, Screening and Critical Baseline Assessments; Paragraph 3, sentence 1: • Procedures conducted as part of the subject's routine clinical management, for example but not limited to blood count, and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure(s) meet(s) the protocol-defined criteria and has (have) been performed within the timeframe of the study	• Procedures conducted as part of the subject's routine clinical management, for example but not limited to ~~blood count~~ laboratory assessment, and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure(s) meet(s) the protocol-defined criteria and has (have) been performed within the timeframe of the study. *Clarified wording by using a more general example of subject's routine "clinical management".*
Section 6.7.5, Clinical Safety Laboratory Assessments Paragraph 1, sentence 1: • All protocol required laboratory assessments, as defined in Table 1, must be conducted in accordance with the Laboratory	Section 6.7.5, Clinical Safety & Other Laboratory Assessments *Clarified wording by adding "other" to both Section Title; in paragraph 1, sentence 4 and Table 1 Title.* • All protocol required laboratory assessments, as defined in Table 1, must be conducted in accordance with the ~~Laboratory Manual (and/or~~ SRM~~)~~

Manual (and/or SRM), and Protocol Time and Events Schedule (see Section 6.1).	and Protocol Time and Events Schedule (see Section 6.1). *Clarified wording to reflect that the Laboratory manual will be included in the SRM.*
Section 6.8, Biomarkers Paragraphs 4 and 6: • [...]by assessing expression of but not restricted to the following antigens; CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD24, CD25, CD24, CD38, CD45RA, CD56, HLA-DR and FOXP3	• [...]by assessing expression of but not restricted to the following antigens; CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD24, CD25, ~~CD24,~~ CD38, CD45RA, CD56, HLA-DR and FOXP3 *Deleted duplication of antigen "CD24" from list of antigens.*