FULL TITLE OF THE STUDY:

Understanding beta-cell destruction through the study of EXtremely Early-onset Type 1 diabetes
(A Musketeers' Memorandum Study)

SHORT STUDY TITLE / ACRONYM: EXE-T1D

RESEARCH REFERENCE NUMBERS AND DATES IRAS REC SPONSOR R&D CO-SPONSOR R&D	 **228082** **17/EM/0255** **1617/023** **1706443**
TRIAL REGISTRY NUMBER ClinicalTrials.gov	**NCT03369821**
PROTOCOL VERSION NUMBER AND DATE	**7.0** 24 November 2025
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OTHER RESEARCH REFERENCE NUMBERS NETWORK/PORTFOLIO CRF	 **35336** **CRF 228**

This protocol has regard for the HRA guidance

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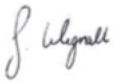
SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

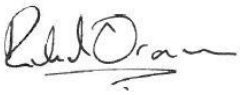
Signature: 

Date: 25/11/2025

Name (please print): **Suzy Wignall**

Position: Senior Clinical Research Governance Manager

Chief Investigator:

Signature: 

Date: 24/11/2025

Name: (please print): **Professor Richard Oram**

Position: Professor in Diabetes and Nephrology

STUDY SUMMARY/SYNOPSIS

Study Title	**Understanding beta-cell destruction through the study of Extremely Early-onset Type 1 Diabetes (EET1D)**
Short Title and Internal ref. no.	EXE-T1D CRF 228
Study Design	Retrospective observational and prospective observational cohorts
Lead Study Centre	Royal Devon University Healthcare NHS Foundation Trust
Study Summary	A study to assess clinical phenotype, beta cell function, genetics, and autoantibodies in EET1D and/or autoimmunity to better understand the aetiology and progression of beta cell destruction in T1D occurring in the first 2 years of life.
Study Participants	**Study 1:** Cross sectional study of existing and new cases of EET1D, diagnosed with T1D in the first year of life. Participants will be identified retrospectively and prospectively from referrals to the Exeter Molecular Genetic Laboratory or Professor Oram. **Study 2**: Newly diagnosed cases of EET1D, again identified by referral to Exeter Molecular Genetics Laboratory or Professor Oram. **Age-matched cohort without diabetes:** Children undergoing elective surgery/clinic will be recruited to collect samples for comparison of immunological development.
Planned Sample Size	**Study 1**: 100-130 EET1D and 100 controls **Study 2**: 40-60 EET1D and 10-40 controls **Age-matched non-diabetic control cohort:** 60-80
Treatment duration	**Study 1**: Single visit for sample collection (with possible 2nd visit to confirm abnormal findings) **Study 2**: One/Two visits close to diagnosis, and approx 2 years later for some children (see Follow up duration) **Age-matched non-diabetic control cohort**: recruitment and sample collection during elective surgical procedure
Follow up duration	2 years (+/- 18 months) for some UK Study 2 participants recruited in Phase 1, or if required to confirm abnormal findings.
Planned Study Period	Phase 1: 01/09/2017 – 30/11/2021 Phase 2: 01/12/2021 – 30/11/2025 Phase 3: 01/12/2025 – 30/11/2028
Main Inclusion Criteria	**Study 1:** • Aged 0 to 70 years • Clinical diagnosis of diabetes or autoimmunity <24 months **Study 2:** • Aged 0 to 2 years at recruitment • Clinical diagnosis of diabetes or autoimmunity <24 months **Age-matched non-diabetic control cohort:** • Aged 0 to 6 years at recruitment (priority for those aged <2 y) • Undergoing elective surgery or attending outpatients for routine blood draw • Do not have diabetes.

Objectives & Outcome Measures	**Objectives**	**Outcome Measures**
Primary	Measure β-cell function in EET1D compared to T1D and NDM.	C-peptide and GAD, IA2, ZnT8 autoantibody measurement
Secondary	Immune phenotyping in EET1D compared to T1D and NDM.	Presence/quantity of autoreactive CD8 and Treg; T cells; RNAseq; HLA alleles

FUNDING AND SUPPORT IN KIND:

FUNDER(S)	**FINANCIAL AND NON FINANCIAL SUPPORT GIVEN**
Diabetes UK Harry Keen Fellowship 16/0005529 University of Exeter 50793 DUK Diabetes UK Central Office Wells Lawrence House, 126 Back Church Lane, London E1 1FH	£ 799,275 01/01/2017-31/08/2024
The Leona M. and Harry B. Helmsley Charitable Trust • Type 1 Diabetes Program Grant #2018PG-T1D049 01/12/2018-30/11/2021	$ 749,303 (with King's College London) 01/12/2018-30/11/2021
• Grant #2103-05059 01/12/2021-30/11/2024	$1,632,721 (with King's College London) 01/12/2021-30/11/2025
• Grant #2607-09203 01/12/2025-30/11/2028	$1,499,984 (with King's College London) 01/12/2025-30/11/2028
Research & Development Directorate, Royal Devon University Healthcare NHS Foundation Trust	Study Co-Sponsorship and Research Governance Support
NIHR Exeter Clinical Research Facility and NIHR Clinical Research Network	Support Infrastructure
University of Exeter	Lead Sponsorship and Financial Management

ROLES AND RESPONSIBILITIES:

Sponsor

The sponsor has no role in the study design or data analysis, interpretation or manuscript writing.
The sponsor will review and approve the study protocol and supporting documents.
The sponsor takes overall responsibility for the study site monitoring that is carried out by the CI's team.

STUDY MANAGEMENT:

Day to day management of the study will be undertaken by the CI and Project Manager with the support of the NIHR Exeter Clinical Research Facility.

Study Management Group (SMG)

The SMG, to include the CI, Co-investigators, and Project Manager, will meet/communicate regularly to ensure all practical details of the trial are progressing and working well and that everyone within the study understands them correctly.
The SMG will meet/teleconference at 1, 2, 3, 6, then annually, and more frequently if necessary.

Table of Contents

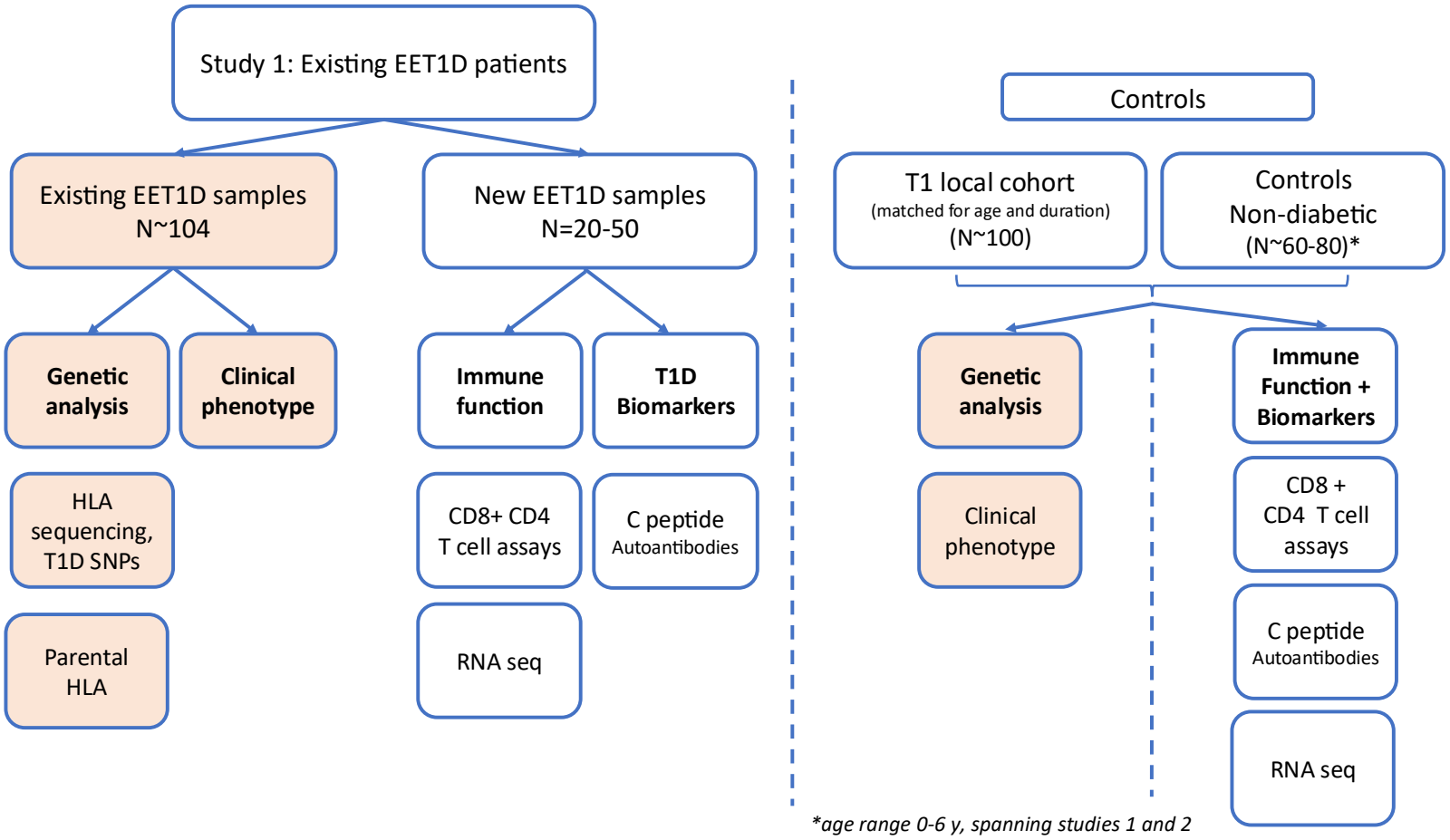
Glossary of Terms and Abbreviations

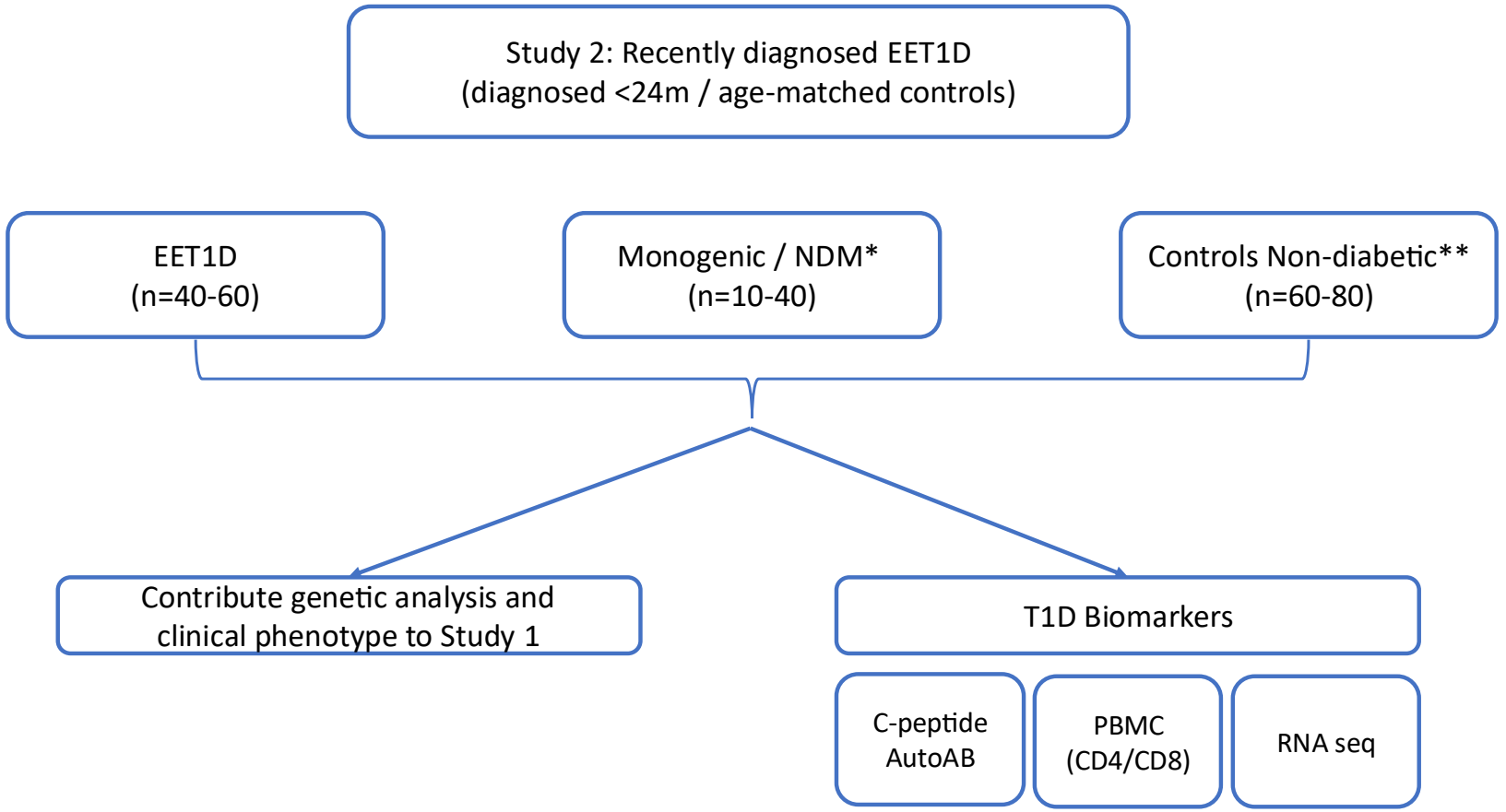
AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form (Data Collection Form)
CRN	Clinical Research Network
CRO	Contract Research Organisation
DAC	"DNA, Autoantibodies and C-peptide"
DCF	Data Collection Form (also known as Case Report Form)
EET1D	Extremely Early-onset polygenic Type 1 Diabetes or Autoimmunity
GCP	Good Clinical Practice
ICF	Informed Consent Form
IRB	Institutional Review Board
ISF	Investigator Site File
MT1D	Monogenic Type 1 Diabetes
NDM	Neonatal Diabetes Mellitus
NHS R&D	National Health Service Research & Development
Non-CTIMP	Non-Clinical Trial of Investigational Medicinal Product
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
Participant	An individual who takes part in a clinical trial
R&D	Research and Development Office
REC	Research Ethics Committee
RN	Research Nurse
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SIV	Site Initiation Visit
SDV	Source Document Verification
SMG	Study Management Group
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1D	Type 1 Diabetes
T1D-GRS	Type 1 Diabetes Genetic Risk Score
TMF	Trial Master File

KEY WORDS:

type 1 diabetes	monogenic diabetes	autoimmune diabetes
early-onset autoimmune diabetes	beta cell (β-cell) destruction	type 1 diabetes genetic risk
extremely early-onset Type 1 diabetes	neonatal diabetes	

STUDY SUMMARY FLOW CHARTS:





** Monogenic/NDM any age ** Non-diabetic age range 0-6 y, spanning studies 1 & 2*

Study Timeframe:

It is anticipated the project objectives will be met over a period of 4 years, as outlined below

Milestones	*Yr1* *Mar17 – Feb18*		*Yrs 2-10* *Mar18 – Feb 28*	*Yr 11* *Mar28 – Nov28*	
	0-5 m	6-12 m			
Ethical/Governance approvals					
Recruitment Study 1					
Recruitment Study 2					
Data collection					
Immuno analysis					
Data analysis					
Writing up/future funding applications					

1. Introduction

1.1 Background

Importance and potential benefit

Type 1 diabetes (T1D) is a common autoimmune disease that causes destruction of pancreatic, insulin producing beta cells, leading to high blood glucose. T1D is regarded as a childhood disease with an average age of diagnosis of 13 years, but the age presentation is very variable from young infants until late adulthood.

We have identified a group of rare children who have developed T1D in the very early life (1) and we have described them as Extremely Early-onset Type 1 Diabetes (EET1D). This study aims to study infants with EET1D or related forms of autoimmunity and ask why they have presented so early. This is important because they are presenting with autoimmunity right at the beginning of life when the immune system is not yet fully developed and at a time when pancreatic autoimmunity first emerges (2) and so study of these rare patients may give novel insights into the cause of T1D.

Exeter is a world referral centre for Neonatal Diabetes (NDM). Most cases of diabetes diagnosed under 6 months do not have EET1D but have genetic mutations in beta cell genes that lead to impaired insulin production (neonatal diabetes, NDM)(3, 4). The Exeter Molecular Genetics Laboratory is referred cases at diagnosis from around the world with neonatal diabetes (www.diabetesgenes.org). Exeter is in a unique position to be able to identify the remaining <20% without a mutation in a beta cell gene who actually have EET1D. We used a novel measure of T1D risk genes, called the T1D Genetic Risk Score (T1D GRS), to show that a proportion of the remaining patients had very high T1D risk and therefore had EET1D(1). This raises questions about how, why and when the immune system started to go wrong in these children. We hope that studying these very rare children with EET1D may help to improve our understanding of the whole spectrum of different ages of diagnoses. Understanding the mechanism for very early presentation could be highly important as immune strategies to intervene before or after people get T1D may differ by age of onset.

We will use the Exeter Neonatal Diabetes cohort to study people diagnosed with EET1D (<2 year ~ 1st centile), both those with specific monogenic autoimmunity (MT1D) and those with very early-onset polygenic T1D (EET1D). As there are <100 EET1D cases in the UK, this study falls under the *Musketeers' Memorandum* criteria, an NIHR UK Rare Genetic Disease Research Consortium Agreement which allows a single lead UK genetics unit to rapidly set-up a rare disease non-CTIMP (Clinical Trial of an Investigational Medicinal Product) project. This enables national recruitment without the need for individual contracts, material transfer agreements and increased administrative burden). The study will establish an infrastructure and protocols to study EET1D nationally and internationally. We will generate data from UK-based and international patient referrals. We will compare the immune system of infants diagnosed with EET1D to the immune system of control infants who either have a non-autoimmune form of monogenic diabetes, or who do not have diabetes. Relatively little is known about the immune system in very early life, so it is important to compare immune abnormalities in EET1D individuals, to children without diabetes, to confirm that any changes we see are related to autoimmunity and not due to young age.

How will the research impact on people living with diabetes?
This research will directly impact those diagnosed under 2 years of age who we are studying as it will define the subtype of diabetes that they have. The Exeter Molecular Genetics Laboratory is increasingly good at identifying the genetic mutations that cause most neonatal diabetes but a negative genetic test can be disappointing and often families and patients still want an explanation of why they have developed diabetes. This study will also try to address the question of why these children presented with diabetes so young.

We may better understand rapid/early-onset T1D. The study of extremely early-onset T1D may benefit the wider community of patients with other autoimmune disease as it is possible that the research will elucidate key influences in early life on the development of autoimmunity. As the mechanisms for monogenic T1D and other monogenic autoimmunity often overlap, we are looking at both of these groups in this study. The study will also aid the classification of very early-onset diabetes. The use of the genetic risk score in addition to other T1D biomarkers will help to confirm the diagnosis of T1D in those affected. As there are very few studies of the early life immune system, we may also learn about the natural changes that occur in the immune system in the first few years of life.

We may identify important pathways that could be future targets for intervention in T1D.
The long term impacts of this research will be in the identification of factors that contribute to very early-onset T1D. This may highlight key or novel pathways that are most important in the development of early-onset autoimmunity. The results may focus the research community on events that occur before birth. These results will be available by the end of this study. At this point the results may then inform new efforts to prevent or intervene in the underlying destruction of beta cells in T1D.

1.2 Lay Summary

Type 1 diabetes (T1D) results from destruction of insulin-producing beta cells in the pancreas by the body's own immune system (autoimmunity). We do not fully understand what causes this type of diabetes and why there is variation in age of onset and severity between people who develop the disease. The aim of this work is to study very unusual people who develop T1D and/or related autoimmunity extremely young, as babies under 2 years of age. We think that, for the condition to have developed that early, they must have an unusual or extreme form of autoimmunity.

Studying people with very early-onset diabetes or autoimmunity will enable us to look at exactly what goes wrong with the immune system because they have one of the most extreme forms of the disease. We may be able to learn a lot about the disease from a small number of rare individuals. We aim to confirm that they have autoimmune type 1 diabetes and then try to understand how it is possible that they have developed diabetes so young by studying their immune system genes, the

function of their immune system, and environmental factors (such as maternal genetics) that may play a role in their development of the disease.

People with diabetes diagnosed under 24 months are very rare and they live all over the world. We will take advantage of the fact that they are usually referred to Exeter for genetic testing. We will also study eligible patients that are referred directly to Professor Oram. As part of their wider clinical team, we will contact them via their clinician to ask for more information about their diabetes and their family history. We will collect samples to study whether they still make any of their own insulin and whether they make specific antibodies against their beta cells in the pancreas. Separately, we will study their immune system in depth using immune cells isolated from a blood sample. We will then study these cells using cutting edge techniques by Professor Tim Tree at King's College London, by Professor Bart Roep at Leiden University Medical Center, Netherlands, and Dr Cate Speake, Benaroya Research Institute, Seattle (USA). Some of these tests have never been used in people of young ages around the world, so an aim of this project will be to develop methods that can be used to study people even if they live far away.

We have secured additional funding to extend the study for a further 4 years (Phase 2) to include recruitment of infants without diabetes, aged 0-6 years, as controls to enable assessment of how the abnormalities found in autoimmune and non-autoimmune diabetes compare to normal early life development of the immune system. Our results so far highlight a successful strategy of identifying and studying extreme T1D presentations (19), by leveraging Exeter's role as an international referral centre for neonatal diabetes (2) and KCL's world leading role as an immune phenotyping core facility. The second phase of this study seeks to move from observation to mechanism, with a goal of proving that defined abnormalities have a causal role in diabetes development, thereby opening the way for trial interventions. We are continuing to recruit participants to studies 1 and 2, plus healthy controls without diabetes.

We observed differences in key immune features by age in cases and controls. We are now performing analyses that adjust for these age-associated features. Having recruited to a non-diabetes control arm of the study, we are expanding the number of these controls to 60-80 to allow for best comparison of all ages of case children. We are proposing to increase the numbers particularly in the 0-4y age range to allow for more robust analysis of immune phenotypes in cases at these ages.

EXE-T1D website: https://www.diabetesgenes.org/current-research/exe-t1d/

2. Rational for Study Design

Rationale

Studying the pathophysiology of EET1D and immune function in patients diagnosed very early will give insights into the development of autoimmunity and predictors of rapid beta cell destruction.

As EET1D is extremely rare (<100 cases identified by genetic testing in the UK), this study falls under the *Musketeers' Memorandum* criteria, an NIHR UK Rare Genetic Disease Research Consortium Agreement (as described above). This allows provision for coordination and management of the study at a single NHS site, as Exeter forms part of each patient's NHS team (although routine care will normally be delivered by their local clinician at a remote NHS site). We will identify people with EET1D or related autoimmunity who are referred to Exeter for genetic testing(3), and then aim to collect further samples and clinical information. Existing patients (and therefore of varying ages) will be recruited to study 1 - a cross-sectional study of T1D biomarkers, genetics and clinical information. Newly diagnosed patients who are able to give samples closer to diagnosis will be recruited to study 2, which allows assessment of the immune system and T1D biomarkers optimised for the small blood volumes possible in young infants. Newly diagnosed patients will also contribute their samples and clinical information to the cross-sectional study 1. Initial interim analysis found several immunological abnormalities in infants with EET1D. Our analyses also highlighted that little is understood about the normal development of the immune system in early life. Recruitment of controls without diabetes will enable assessment of how the abnormalities found in autoimmune and non-autoimmune diabetes compare to normal early life

development of the immune system. As we have sufficient follow-up data from phase 1, we are also able to simplify our study protocol and keep recruitment to study 2 as a single visit without the need for a follow up visit.

3. Study Objectives and Design

3.1 Hypotheses:

i) Extreme early-onset T1D (EET1D) is associated with classic biomarkers of T1D, such as islet specific autoantibodies, autoreactive islet specific CD8 T cells, and loss of beta cell function, whereas children with monogenic neonatal diabetes or without diabetes will not show abnormalities in these markers.

ii) EET1D will be associated with more rapid beta cell loss than T1D presenting at older ages.

iii) The mechanisms for EET1D will be due to rare changes in immune genes or due to a particularly potent, early response of the immune system to beta cells, as measured by autoreactive T cells or immune gene expression when compared to older onset T1D.

3.2 Study Aim

The EXE-T1D study will take people with T1D diagnosed before the age of 24 months and compare them to people with T1D diagnosed at more typical ages (1-20 years), and people diagnosed with non-autoimmune diabetes at a similar very young age (children with neonatal diabetes [NDM]), and infants without diabetes matched for age.

EXE-T1D is an observational study organised into two sub-studies:

Phase 1 aims:
Study 1: Cross-sectional study of existing patients with EET1D:
Aim: To compare clinical features, beta cell loss (measured by serum/urine C-peptide), islet-specific autoantibodies, T1D risk genes and autoreactive CD8 T cells in people already known to have EET1D and already referred to Exeter for genetic testing or directly to Professor Oram, to those people with T1D diagnosed at older ages (age 1-20 years).

Study 2: Study of newly referred patients:
Aim: To assess immune function (by measuring autoantibodies, autoreactive CD8 T cells and RNAseq of immune genes) at diagnosis and longitudinally in people with newly diagnosed EET1D compared to newly diagnosed monogenic neonatal diabetes.

Phase 1 recruitment:
Total recruited = 163: 137 to study 1, including 18 with fingerprick samples, and 24 to study 2. Numbers were affected by the COVID-19 pandemic and follow-up of UK study 2 patients is ongoing.

Phase 2 recruitment:
Total recruited = 267. To reflect the most valuable samples to date, we recruited 23 more patients with EET1D (reflecting incidence and referral rate), 1 monogenic / NDM control, 60 non-diabetic controls (age 0-6 years) and 20 newly diagnosed T1D controls. We prioritised recruitment to Study 2 (age <2 years). These patients are extremely rare and numbers are dependent on incidence and referrals so the above numbers reflect referral rates.
It is important to overlap recruitment of cases and controls to reduce risk of batch effects in immune and RNA analysis.

Phase 3 extension aims:
We will prioritise recruitment to Study 2 (age <2 years) plus age-matched non-diabetic controls and monogenic / NDM controls when identified. These patients are extremely rare and numbers are dependent on incidence and referrals so achieving the proposed targets depends on referral rates.

Primary Objective
The primary objective of the study is to compare beta cell function and islet autoantibodies in EET1D to T1D, NDM and non-diabetic controls.

Secondary Objectives
The secondary objectives of the study are:

i) To compare genetic and functional markers of autoimmunity in EET1D to T1D and NDM.

ii) To develop a robust pathway to identify, recruit and study early-onset diabetes for a prospective protocol for future study and assessment of patients close to diagnosis of diabetes.

Primary Endpoint/Outcome
Serum/urine C-peptide and autoantibodies (IAA, GAD, IA-2, ZnT8) in EET1D compared to T1D and NDM.

Secondary Endpoints/Outcomes

i) Prevalence and frequency of islet specific CD8 T cells in EET1D compared to T1D, NDM(5-7) and controls.
ii) Frequency and phenotype of pro-inflammatory and regulatory T cells in EET1D compared to T1D, NDM and controls.
iii) Difference in immune gene expression, as measured by RNAseq in newly diagnosed EET1D v NDM and v controls(8).
iv) Association of maternal and paternal non-inherited HLA alleles with EET1D v older onset T1D and NDM(9).

3.3 Study Design

Please refer to the Study Summary Flow Charts (pages 10-11).

Study 1 will recruit patients already identified and referred to Exeter irrespective of how close to diagnosis they are. We will assess whether EET1D patients have features of islet-specific autoimmune diabetes that causes progression to severe insulin deficiency (and whether this process differs from T1D at a later age of diagnosis). We will assess islet autoantibodies, islet T cell autoimmunity, C-peptide, RNAseq, genetics and clinical features of EET1D compared to T1D in selected patients *already known* to the Exeter genetics team who are of varying ages and durations of diabetes and have been referred over the last 15 years. This will involve re-contacting clinical teams and their patients to gain additional clinical information and, according to preference of the patient and clinical team, we will arrange the collection of a blood sample and optional urine sample either in Exeter, or at the patients' local centres. Where possible, the research visit will be combined with a routine review to minimise burden, but it may need to be arranged separately.

Through promotion of the study, we may be approached by patients' GPs and by patients themselves, particularly because EET1D is rare. Recruitment to the study in this setting will be by our team, including our Paediatric Diabetes Specialist Nurse, who will provide information about the study and feedback inclusion of the participant in the study to the GP and diabetes clinician as appropriate.

We plan to repeat sampling in a small subset of participants with monogenic autoimmunity to confirm any striking abnormalities.

Study 2 will recruit newly diagnosed patients with EET1D who are referred to Exeter for diagnostic testing, or directly to Professor Oram. This will allow assessment of immune phenotype in patients close to diagnosis(5-7). We will assess immune function longitudinally by collecting a blood sample for serum and peripheral lymphocytes shortly after referral, and some children may have a second sample approximately 2 years after Visit 1 (+/- 18 months). We will assess islet autoantibodies, C-peptide, and RNAseq at the same time as peripheral lymphocyte (PBMC) sampling. Blood sampling will be timed to fit with routine outpatient appointments where possible.

Non-diabetic controls. We will recruit children between the age of 0 and 6 who are attending hospital for surgery and will therefore require venous cannulation. After administration of a general anaesthetic, we will collect blood samples to assess the same metabolic, immune and genetic markers being assessed in infants with diabetes. Most children under the age of 2 years undergo induction of anaesthesia using inhalational techniques, to avoid venepuncture in an awake and uncooperative child. Inhalational anaesthesia (eg sevoflurane) is known to have a potential impact on immune phenotype (20-23).
A first cohort of 10 children aged 4 to 6 years was recruited to evaluate the effects of sevoflurane on the immune phenotype. Children were cannulated after application of topical prilocaine cream (minimum 15min) and use of cryogenic spray to facilitate a pain-free insertion. Pre-induction bloods were drawn as a baseline. General anaesthesia were induced using up to 8% of inhaled sevoflurane on FiO2=1 administered via a facemask. Immediately following the induction similar blood tests were taken to document any immediate effect of volatile anaesthetic agents on our measures of immune phenotype. We collected samples to assess the same metabolic, immune and genetic markers being assessed in infants with diabetes.
We will then proceed to recruit >60 children aged 0-6 years, with a focus on recruitment of children under the age of 2 years.

3.4 Study Setting

To minimise risk of COVID-19 infection to participants, the research/clinical teams will follow local Trust policy and procedures based on the current UK government guidance.

The Exeter neonatal genetics testing service is part of the clinical team responsible for diagnosing NDM, EET1D and monogenic autoimmunity in the UK and internationally. We will identify people with EET1D, and NDM controls, for this research by the clinical diagnostic tests performed as part of this service.

Patients in the UK will be given information about the EET1D study by the Exeter/local clinical team liaising with them (this will give patients the chance to accept or decline contact about the research study). If willing to be contacted, a trained member of the EET1D/local study team will contact patients initially by phone, and arrange a visit in person at their local care provider (timed with a clinical visit where possible to minimise patient burden). All study information is available to clinicians and patients on our study website (https://www.diabetesgenes.org/current-research/exe-t1d/).

UK participants will be recruited under UK wide ethics. As an international referral centre, Exeter will recruit patients from international centres in collaboration with local clinicians that have specific Institutional Review Board (IRB) approval.

We have developed the protocol for recruitment of paediatric non-diabetic controls with collaboration from the Children's Surgical Services Directorate and R&D Department at Guy's and St Thomas' NHS Foundation Trust and affiliate of King's College London.

The *Exeter Clinical Laboratories* encompass the Exeter Blood Sciences Laboratory and Exeter Molecular Genetics Laboratory at the Royal Devon University Healthcare NHS Foundation Trust and will perform C-peptide, islet autoantibody and genetic tests.

Peripheral lymphocyte (PBMC) analysis for autoreactive CD4 and CD8 T cells will be performed by Bart Roep (Leiden University Medical Center, Netherlands) and Tim Tree (King's College London).

RNAseq will be performed by the Exeter team and by Cate Speake at the Benaroya Research Institute, Seattle (USA).

Sites outside the UK will need to confirm the following capabilities in order to deliver the study:

- IRB approval (Study CI and Project Manager can offer support with the application).
- Sufficient staff time to undertake the recruitment, consent, sample and data collection, documentation and arrangements for sample transfer to Exeter, data entry, and all follow-up procedures, as detailed in the study design, for the full duration of their participants' involvement in the study.
- Ability to despatch blood samples on day of collection (or following day latest) to the Exeter Clinical Laboratories at the lead site in Exeter, UK.

NOTE:

- Samples transferred to the Royal Devon and Exeter Hospital Central Laboratories for analysis will be labelled with participant identifiable data (name, date of birth & NHS//CHI/hospital number) in accordance with the requirements for clinical sample analysis. Once initial analysis is complete, remaining samples will be anonymised and assigned an individual study code prior to storage that links to the participant's study ID. Consent to store samples will be sought; samples without consent to store will be destroyed.
- Consent status, data collected and local test results will be entered on the online database within 7 working days of the study visit.
- Full contact details for both participants and clinicians will be included in the information transferred to the lead site, to facilitate feedback of visit results. C-Peptide and antibody results will be provided to the Site PI within 10 days of sample receipt at Exeter. T1D-GRS and genetic results will be advised to the Site PI within approximately 8 weeks of sample receipt if patients meet criteria for genetic testing (ie diagnosed before 9 months). T1D GRS results will be reported following batched analysis.

4. Subject Selection

4.1 Number of Subjects and Subject Selection

Please refer to the Study Summary Flow Charts (pages 10-11).

Study 1: Study of existing patients with EET1D (n=100-130 v 100)
Study 2: Study of patients newly diagnosed with diabetes <24 mths (n=40-60 EET1D v 10-40 Monogenic / NDM controls)
Non-diabetic controls: As comparators, healthy non-diabetic patients, aged 0-6 y, undergoing elective surgery (n=60-80)

The Exeter Molecular Genetics Laboratory has been referred >3500 patients diagnosed <12 months from 84 countries and we currently receive ~200 referrals per year. This is the largest cohort in the world of babies diagnosed with diabetes in the first year of life.

40% of patients already identified are based in the UK. The remainder are located at international centres (n=30 centres) that have referred patients as part of clinical care. We will initially recruit within the UK and target people who have been diagnosed with diabetes and referred to the Exeter Molecular Genetics Laboratory already. We have already identified EET1D patients and their clinicians who are willing to take part in the study and are establishing the infrastructure to recruit

and study these people. The majority of patients already identified are diagnosed under the age of 6 months, but we will recruit patients with an age of diagnosis up to 24 months. We will also recruit eligible patients referred directly to Professor Oram or who contact us upon learning of the study and will arrange for genetic testing where this has not been performed. A cohort of non-diabetic children will be recruited as controls at specified participating hospitals when attending elective surgery (eg hernia repair), with samples being collected after anaesthetic induction to minimise patient discomfort and burden.

During the first year of the study, recruitment from UK referrals was slower than anticipated and subsequently the COVID-19 pandemic has affected recruitment rates, so we will allow alternative referral methods and recruitment approaches:

i) attempt to recruit patients from distant sites with multiple cases.
ii) We have now thoroughly reviewed patients already referred to the Exeter team and anticipate fewer newly recruited patients from this cohort than originally predicted (e.g. due to death or out of date contact details). We have been approached by eligible patients directly and, with new referrals from clinicians, these may make up for this shortfall, so alternative referral methods will be permitted. Additionally, the rarity of this condition means predictions of recruitment are very difficult, as are power calculations for the proposed analyses. We plan to recruit as per this updated protocol (and the new recruitment routes) and then may propose revised recruitment targets in 12 months based on these altered recruitment strategies.
iii) attempt to recruit healthy non-diabetic children as controls for Studies 1 and 2. Option 1 is to recruit healthy children when attending elective surgery at specified participating sites, as described above. As a second option, if recruitment proves slower than anticipated, we would recruit children with congenital non-immune thyroid disease when they attend paediatric clinic for blood draw (~5 times in the first year of their life), giving an opportunity for a sample to be collected for functional immune analysis. The recruitment priority will be children aged under 2 years but eligibility will be 0 to age 6 years.

Patients for study and controls (Studies 1 and 2)
Defining patients as EET1D:
Patients will be selected on the basis of:

i) diagnosis of diabetes <24 months (with continued primary focus on recruitment of those diagnosed <12months)
ii) exclusion of mutation in all 23 monogenic non-autoimmune neonatal diabetes genes using targeted capture Next Generation Sequencing(NGS) if diagnosed <12 months (3)
iii) T1D GRS within distribution of T1D reference population(10).
iv) Diagnosis of monogenic type 1 diabetes defined as a mutation in a gene known to cause type 1 diabetes (e.g. *FOXP3* and *STAT3*) or a Down's syndrome (added with additional funding for recruitment and analysis from The Leona M. and Harry B. Helmsley Charitable Trust).

Study 1: existing cases of any age meeting above criteria.
Study 2: new cases referred to Exeter Genetics Service or Professor Oram within 12 months of diagnosis, meeting above criteria, and aged <24 months.

Comparison groups:
Study 1: Older age onset T1D. To allow comparison with later onset T1D, we will select duration-matched patients from our T1D research cohort of patients diagnosed at 1-20 years of age. They will be defined as T1D by clinical diagnosis, age of diagnosis, and treatment with insulin from diagnosis.

Studies 1 & 2:
Age- and duration-matched controls with monogenic / NDM (neonatal diabetes). We will identify, from referrals to Exeter, age- and duration-matched controls with proven monogenic (non-autoimmune) neonatal diabetes. Eligible patients will include people with neonatal diabetes caused by mutations in the Kir6.2 and SUR1 genes(11).
Non-diabetic controls. We will identify healthy children <6 years of age undergoing elective surgery.

4.2 Inclusion Criteria

Study 1:

EET1D

- Aged 0 to 70 years
- Clinical diagnosis of diabetes or autoimmunity <24 months (+ evidence of WHO diabetes criteria)

T1D Controls

- Age 0-70 years (matched to above)
- Clinical diagnosis of T1D (diagnosed age 2-20 years)
- Insulin treated from diagnosis.

Monogenic / NDM controls

- Diagnosis of diabetes <12 months
- Diagnosis of monogenic / NDM (confirmed by Exeter Molecular Genetics Laboratory).

Study 2:

EET1D

- Aged 0 to 24 months at recruitment
- Clinical diagnosis of diabetes <24 months (+ evidence of WHO diabetes criteria); recruited within 12 months of diagnosis.
- Negative genetic test for mutations causing non-autoimmune neonatal diabetes
- Type 1 diabetes genetic risk score >50th centile of T1D reference group, or monogenic cause of T1D (e.g. *STAT3* or *FOXP3* mutation)

NDM controls

- Diagnosis of diabetes <12 months
- Diagnosis of NDM (confirmed by Exeter Molecular Genetics Laboratory).

Non-diabetic controls for Studies 1 and 2:

- Aged 0-6 years (The recruitment priority will be children aged under 2 years but to include children aged 2-6 years)
- Attending specified participating hospital sites for elective surgery, including but not limited to: inguinal hernia repair, umbilical/midline hernia repair, orchidopexy, gastrostomy insertion/change, hypospadias repair, cleft palate repair, excision of accessory digit, laryngoscopy, adenoidectomy, tonsillectomy, MRI under general anaesthesia, eye surgery.

Should recruitment be slower than anticipated, we would recruit children with congenital non-immune thyroid disease when they attend paediatric clinic for blood draw.

4.3 Exclusion Criteria

Study 1:

- Aged >70 years
- No diagnosis of diabetes
- MODY (e.g. caused by *HNF1A/HNF4A/HNF1B/GCK* mutations), type 2 diabetes or diabetes related to pancreatic insufficiency or syndromic diabetes
- Intercurrent illness at time of sampling for PBMCs (see below).

Study 2:

- Aged >24 months
- Clinical diagnosis of diabetes >24 months
- Intercurrent illness at time of sampling for PBMCs or RNA (see below).

Non-diabetic controls for Studies 1 and 2:

- Aged >6 years
- Diagnosis of diabetes or other autoimmune condition
- Known immunological disorder
- On immunosuppressive medication
- Ongoing infections/sepsis
- Major congenital abnormality or significant systemic illness that may affect the immune system, e.g. metabolic disease, 22q deletion syndrome
- Recent (within two weeks) febrile illness
- Renal failure.

For PBMC and RNA sampling: Exclusion for factors that may alter T cell function and RNAseq

Review the following exclusion criteria carefully at time of appointment as some details may have changed since initial contact:

- *Recreational drug use* (excluding cannabis use more than 1 week prior to blood sampling) - drug abuse may alter T cell function
- *Alcohol related illness* (excessive alcohol consumption may alter T cell function)
- *Renal failure:* Creatinine >200 (as may alter T cell function)
- Any other medical condition which, in the opinion of the investigator, would affect the safety of the subject's participation.

Factors that if temporary would lead to rearrangement of study visit but if long duration, may lead to exclusion subject to the CI's discretion:

- *Pregnant or lactating* (as this may limit blood sampling and affect T cell function)
- *Any infectious illness within the last 2 weeks if it was a febrile illness, or within 2-3 days if it was non-febrile* (as this may activate T cells non-specifically)
- *Taking steroids or other immunosuppressive medications* (as these may alter T cell function)
- *Received any immunoglobulin treatments or blood products in the last 3 months* (as these may alter T cell function).

5. Study Procedures

Please refer to the flow charts on pages 10 and 11 for a summary of the study procedures.

The study procedures described below will be conducted in accordance with detailed SOPs provided by the CI.

5.1 Informed Consent Procedures

All participants (or their legal guardian) recruited to the study will be required to give written informed consent. It is the responsibility of the PI, or an appropriately trained and delegated individual, to obtain written informed consent from each participant and/or their parent/guardian (if aged <16 years in the UK). Informed consent will only be taken following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study.

All participants will be informed of their right to withdraw from the study at any time without prejudice or jeopardy to any future clinical care. Prior to consent, all potential participants will be provided with detailed written information about the study and an opportunity to discuss it with one of the research team. As stipulated by GCP, participants will be provided with adequate time to consider giving consent.

Appropriately trained members of the research team may take informed consent for this study. If consent is taken by a member of the research team and the participant wishes to speak to a clinician who is present or contactable via telephone, further information can be given to the participant and questions can be answered quickly. Where this is not possible, informed consent should not be taken until the participant is content that all questions have been adequately answered.

A scanned copy of the consent form will be sent to Exeter from remote sites, to allow storage of samples and monitoring.

The Exeter team, with the participant's local clinical team, will review local laboratory and genetic results and confirm eligibility to the study. Following screening, a trained member of the study team will discuss the study in detail with the potential participant and/or their parent/guardian. A unique study ID will be allocated to the participant to link all participant information and samples. The participant's clinical characteristics and local sample results will be recorded from the hospital records.

5.2 Visit sample collection and processing

Study 1 and Study 2

Clinical data collection

The local clinical team (diabetes specialists and specialist nurses) of patients referred to the Exeter Molecular Genetics Laboratory or directly to Professor Oram will be contacted by the Exeter study team to complete any missing clinical and biochemical data identified.

Genetic analysis

Participant and parental DNA samples are already stored in the Exeter Genetics Beta Cell Research Bank at the time of referral for genetic testing and we will perform genetic analysis as per appendix 3 with these samples. The genetic testing will not include any non-diabetes related analysis.

Sample for HbA1c, C-peptide, Autoantibody, RNAseq and PBMC analysis in UK

A patient identified and screened as being suitable for this study will have a blood sample and optional urine sample collected by the clinical team at a time and location suitable for the patient, clinical and study teams. Healthy children without diabetes, identified and recruited when attending hospital for elective surgery, will have their samples collected after anaesthetic induction to minimise patient discomfort and burden (except for the initial 10 patients aged 4-6 recruited to evaluate the direct effect of sevoflurane on markers of interest who will have pre- and post-induction samplings, using specific pain relieve technics to minimise any discomfort, as described above).

In addition to a clinical blood sample analysed locally, the following samples will be collected and sent (by courier) to the Exeter Clinical Laboratories: one serum tube for autoantibody analysis (13),

one to three EDTA tubes for DNA, flow cytometry, HbA1c and C-peptide analyses(12) and, dependent on age and weight (http://www.who.int/bulletin/volumes/89/1/10-080010/en/ - see example below), one Tempus tube with 0.5 ml minimum blood sample for RNAseq, and one to five 5 ml Sodium Heparin tubes for PBMC extraction and cryopreservation. An optional urine sample, if collected, will be provided in a 20 ml boric acid tube.

We will use a home sampling pack that allows collection of a blood sample from a fingerprick (for example, when the participant is testing their blood glucose from a fingerprick anyway) and post it (using prepaid postage packaging provided) to the Exeter Clinical Laboratories for analysis of HbA1c, C-peptide, autoantibody analysis and extraction of DNA. We will offer this option to individuals that meet Study 1 criteria but are of longer duration or cannot provide a standard EXE-T1D sample. Referred patients interested in the study will be sent the pack with study information sheet(s), consent form(s) and prepaid packaging. A telephone call will be arranged with the study research nurse and patient/parent/guardian to discuss the study, answer any questions, obtain informed consent and talk through the process of sample collection and posting to Exeter.

It is possible that, especially with young children, venepuncture during a study visit will not be successful (for example because insufficient blood draw is possible, or the infant is unwilling to have the blood draw). If this happens, we will offer the study participants the choice of another visit, the option to post a fingerprick blood sample, or to withdraw or not participate further in the study.

Example of blood volume used for research samples in infants:
WHO guidelines specify 1-5% of blood volume in one day. Children tend to have a blood volume of 75 ml/kg, so the limit would be 0.75 – 3.25 ml/kg. Therefore, for a child weighing 10 kg, the safe maximum, as advised by WHO, would be 7.5 – 32.5 ml.

Samples will be sent with 3 forms of identifiable information (name, DOB, NHS/CHI/hospital number) using Exeter-specific analyses request forms. The samples for C-peptide and autoantibodies will be tested in the Exeter Clinical Laboratory as clinical samples and results will be returned to the local clinical teams. Study results will be anonymised and stored by study ID on the study database. PBMC samples will be anonymised and cryopreserved.

Any travel costs related to the study will be reimbursed to study participants.

Study 2
In addition to the first visit, as detailed above, a repeat visit may be conducted two years (+/- 18 months) after Visit 1. We will not routinely perform a second study visit for participants recruited in phase 2 of the study, but, if we find major abnormalities, we may arrange a second sample to confirm findings.

Second Sample for PBMC, C-peptide and Autoantibody analysis in UK
For Study 2 children recruited in the UK during Phase 1, a second sample for HbA1c, C-peptide, Autoantibody, RNAseq and PBMC analyses may be collected in an identical manner to the first sample, approximately 2 years (+/-18 months and timed with a clinic visit where possible) after the initial sample at Visit 1, using the methods outlined above.

Non-UK Centres
Samples will be collected as detailed above at collaborating international centres with their own IRB approval. The difference will be that the local team will spin and freeze the EDTA plasma sample and store it on site while the PBMCs are extracted as per Exeter's SOP. All tubes will then be couriered to Exeter. If no local team is available to extract PBMCs, we will arrange immediate couriering of all tubes back to Exeter or King's College London (if more practical) for analysis.

The samples will be processed as per the study SOP.

5.3 Laboratory Assessments and Results Reporting

- Local sample results and data will be inputted into the study database within 7 working days of the visit.
- Results of tests analysed in Exeter will be uploaded directly to the database within 10 working days of availability. The Exeter Clinical Laboratories will provide the Site PI (and clinician) with the results of HbA1c, C-peptide, and Autoantibodies within 10 working days of receipt of the sample. Results for the non-diabetic control cohort will only be reported if results might inform clinical care.
- T cell, T1D-GRS, RNA analysis and HLA typing will not be individually reported back to patients and clinicians but the results of the final analyses will be made available to clinicians and participants involved in the study. These analyses will be performed in a batched manner at the completion of study procedures to reduce impact of batch effects on analysis.

5.4 End of Study Definition

The parameter marking the end of the study is: last participant's final study visit plus 6 months to enable follow-up data capture.

5.5 Subject Withdrawal

Subjects will be informed that they are free to withdraw from the study at any time up to and including data and sample analysis, without prejudice or jeopardy to any future clinical care. If a patient permanently withdraws from the study, or is lost to follow-up, the reason will be recorded.

Criteria for premature withdrawal from the study:

- Participant withdrawal of consent
- Investigator's discretion that it is in the best interest of the participant to withdraw
- Termination of the study by the study sponsor.

5.6 Data Collection and Follow-up for Withdrawn Subjects

Where a participant withdraws, data will be collected up to the point of withdrawal in line with the protocol and study SOPs. If appropriate, the PI or delegated member of the research team will follow-up with the participant to ensure wellbeing and that ongoing clinical care is unaffected.

Where a participant has prematurely withdrawn but not revoked consent, data and samples will remain within the study and be included in any analysis. Where consent is withdrawn, study samples and data will be kept/destroyed as per the following options, in line with data SOPs and local guidelines:

- samples and data remain
- samples withdrawn, data remains
- samples remain, data withdrawn
- both samples and data withdrawn.

Withdrawal will be documented and recorded on the study database, including the participant's choice of the above options. A withdrawn participant is not obliged to give a reason for withdrawing their consent but where they are willing to do so this reason will be documented. Details of all withdrawn participants will be flagged to the central coordinating centre via monthly accruals and feedback to enable any study-wide trends relating to study procedures to be ascertained.

6. Samples

6.1 Collection/Labelling/Logging

UK samples will be collected by a suitably qualified and trained member of the research/clinical team, whose role is documented on the study delegation log. Where the low volume fingerprick home sampling method is used, the samples will be collected by the participant or parent/guardian and posted to the Exeter Clinical Laboratories using the packaging provided with prepaid postage.

Samples coming from international centres will operate under local guidelines and IRB approval. A detailed SOP will be provided detailing the clinical procedure for collecting samples and the logistics of sample labelling, logging and management.

6.2 Sample Receipt/Chain of Custody/Accountability

The Exeter Clinical Laboratories have an established pipeline for receiving and processing all research samples, including documentation of chain of custody. Upon receipt, the specimens will be assessed for content and integrity. The samples will be logged immediately and all samples will be tracked using the laboratory information management system (LIMS).

6.3 Sample Analysis Procedures

Analyses that are routine biochemistry tests available in the NHS test repertoire use assays that are CE marked, fully validated and accredited by CPA (Clinical Pathology Accreditation).

6.4 Sample Storage Procedures (if applicable)

Surplus samples for storage should be processed, logged and frozen at -80°C within 24 hours of receipt at the Exeter Clinical Laboratories.

The consent status and visit data will be indicated on the study database and will therefore be available to the Exeter Clinical Laboratories if required. Consent to store samples will be checked; samples without consent to store will be destroyed. After initial processing, samples will be registered into the study database and the Study ID and barcode set will be linked providing a robust link-anonymised system for all samples.

All study samples (with the exception of DNA extraction which is logged on the Exeter Molecular Genetics Laboratory database) must be logged on the study database. The coordinating centre will monitor sample collection via the database. Where samples are unable to be collected, this should be documented under the participant Study ID with the reason for non-collection provided.

Procedures for sample collection, processing, and courier or postal packaging will be provided via the sample handling SOP. Relevant consumables and request forms will be provided by the coordinating centre.

Samples collected for local analysis should be labelled in line with local clinical guidelines, including all required identifiable information and sent to local laboratories with relevant paperwork.

Research sites will arrange for study samples to be couriered (using the study recommended courier) or posted to the Exeter Clinical Laboratories on the day of collection (or within 24 hours latest), as detailed in the study-specific sample transfer SOP. Research sites will be asked to complete transfer documentation to ensure all samples are accounted for and that the integrity of samples has not been compromised up to the point of transfer.

Upon receipt at the Exeter CRF/ Exeter Clinical Laboratories, samples will be stored at -80°C +/- 10°C.

6.5 Long-term Sample Analysis and Storage

Consent to store samples will be checked by the co-ordinating centre; samples without consent to store will be destroyed. All saved plasma, serum and urine samples will be stored under the study ID, with the file linking the study code to personal identifiable information held securely by the Principal Investigator and accessible only to personnel with training in data protection who require this information to perform their duties. Those with access to personal identifiable data will be documented on the delegation log.

All samples will be appropriately labelled in accordance with the 2018 Data Protection Act. Biological samples collected from participants as part of this study will be transported, stored,

accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

Participants will have the opportunity to consent to gift samples at the end of the study for future research. Enduring consent will be sought to store the samples in an ethically-approved research tissue bank (Peninsula Research Bank [PRB]) and all future analysis of samples and/or data will be conducted with the specific agreement of the PRB steering committee.

7. Data Recording/Reporting

Each participant will be allocated a unique study identity number under which all study data and samples will be link-anonymised and stored, using a secure password-protected study database. Personal identifiable data will only be accessible to personnel with training in data protection who require this information to perform their study role. Personal data to be collected will include, name, date of birth, NHS/CHI/hospital number, gender, contact details and preferences. Identifiable information will be stored in a separate but linked database to enable local research teams to undertake the study. Only those members of the research team whose role requires access to personal identifiers will have access.

All paper copies of study data will be stored under ID number and kept in locked offices within the research facilities; research data will be held separately to identifiable information. No identifiable data will be included in research publications or progress reports.

Results of analyses undertaken by the Exeter Clinical Laboratories will be electronically uploaded directly to the study database and linked to Study IDs.

8. Safety, Definitions and Reporting

8.1 Risks

Blood samples will be collected by staff trained in venepuncture, usually at a clinic visit. Any potential discomfort or side-effects will be equivalent to that experienced in routine clinical care.

8.2 Benefits

The C-peptide and autoantibody results may help to confirm a diagnosis of T1D for the clinical team and so will be reported back to clinicians responsible for the patient's diabetes care. Decisions about ongoing clinical care and treatment will be made externally to the research study but treatment will be recorded.

8.3 Definitions and reporting of adverse effects

It is not anticipated that participants involved in this study will be subject to adverse effects, as we are not administering any medicinal products. Should any unforeseen adverse events arise that are possibly, probably, or definitely related to a study procedure, they will be reported to the Sponsor and CI/central coordinating team within 24 hours of the CI or PI or co-investigators becoming aware of the event, as per standard NHS R&D protocols. The CI will assess causality and advise accordingly.

Serious Adverse Event (SAE)

An SAE fulfils at least one of the following criteria:

- Is fatal – results in death (NOTE: death is an outcome, not an event)
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect.

All reportable SAEs will be recorded in the subject's notes, the subject's study record, the Sponsor SAE form and reported to the Sponsor and CI/central coordinating team, within 24 hours of the CI or PI or co-investigators becoming aware of the event.

All non-reportable SAEs will be recorded in the subject's notes and the subject's study record, but the Sponsor SAE form will not be filled out or reported to the Sponsor.
The following SAEs will be considered recordable but **not reportable** to the Sponsor:

- Hospitalisation for elective treatment
- Hospitalisation for treatment of a pre-existing condition
- Hospitalisation due to road traffic accident.

9. Data Handling and Record Keeping

9.1 Confidentiality

The PI is responsible for ensuring that participant anonymity is protected and maintained, they must also ensure that their identities are protected from any unauthorised parties. The CI is the 'Custodian' of the data. All information related to study participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

Participant data will be held in a pseudo-anonymised format, with personal identifiable data only accessible to personnel with training in data protection who require this information to perform their study role. Personal data to be collected will include, name, date of birth, NHS/CHI/hospital number, gender, contact details and preferences. Identifiable information will be stored in a separate but linked database to enable local research teams to undertake the study. Only those members of the research team whose role requires access to personal identifiers will have access.

A unique Study ID will be allocated, under which all study data and samples will be pseudo-anonymised and stored on a secure password-protected study database.

All paper copies of study data will be stored under ID number and kept in locked offices within the research facilities; research data will be held separately to identifiable information. Researchers involved in data and sample analysis will not have access to personal identifiable data, only the anonymised research data. No identifiable data will be included in research publications or progress reports.

Any participant information required to be sent to a third party will adhere to these pseudo-anonymised parameters, this includes the patient initials, date of birth, and gender, as well as unique study ID.

9.2 Case Report Form (Data Collection Form)

The visit CRF will capture all the relevant information to ensure that all the documented statistical information thus dictated in the protocol is captured and documented at each visit. This also serves to monitor patient eligibility and safety at Sponsor level.

10. Study Documents

- Signed protocol and any subsequent amendments
- Current/Superseded Patient Information Sheets (as applicable)
- Current/Superseded Consent Forms (as applicable)
- Indemnity documentation from Sponsor
- Conditional/Final R&D Approval
- Signed site agreement

- Ethics/HRA submissions/approvals/correspondence
- CVs of CI and site staff
- UK regulations (GCP) course certificate for each of study team
- Delegation log
- Recruitment log
- Monitoring visit log and subsequent reports
- Protocol training log
- Correspondence relating to the study
- Communication Plan between the CI/PI and members of the study team
- Case Report Forms (Data Collection Forms) for each visit.

11. Record Retention and Archiving

During the course of research, all records are the responsibility of the Chief Investigator / Principal Investigator and must be kept in secure conditions. When the research study is complete, it is a requirement of the Research Governance Framework and Sponsor Trust Policy that the records are kept for a further *15 years*.

Local investigator site files must be archived at the external site according to local R&D requirements. They will not be stored at the coordinating centre's archiving facility.

The R&D Standard Operating Procedure ARCHIVINGS03 can be consulted for further details – the coordinating centre will make this available to sites on request. However, local SOPs and policy (where present) will be considered the key basis for archiving.

12. Statistical Considerations

12.1 Sample Size

Total recruitment target is at least 260). These are rare patients and the aim is to recruit:
Study 1: 100-130 with EET1D plus 50-100 controls (N=~200).
Study 2: 20-40 to study plus 10-40 controls (N=30-80).
Non-diabetic controls (aged 0-6y) for Studies 1 & 2 (N=60-80).

12.2 Feasibility

This is a study to establish infrastructure to study rare patients with EET1D and MT1D. The sample size has been selected to assess feasibility rather than on the basis of statistical power. In reality with these extremely rare but potentially very interesting patients, every single patient recruited could contribute on their own to a novel discovery. Whilst accepting that we do not know what immune, beta cell or autoantibody differences we are going to find, a group of 20 v 20 gives an 80% power (alpha 0.05) to detect a difference of 10% v 50% in a proportion between the two groups and a power of 85% (alpha 0.05) to detect a 1 SD difference in a continuous variable. Each case of EET1D included in the study increases the statistical power to find new immune and genetic associations. Sometimes individual cases have specific mutations which we are now funded to test for with whole genome sequencing. Additionally, to reduce the chances of batch effects in immune and other -omics analysis, we will continue to keep case recruitment open in parallel to recruitment to the non-diabetes control cohort.

12.3 Statistical analysis

The EET1D as described are unique and findings in the various studies are difficult to predict given the novel nature of this study. The study using 100 EET1D v 100 controls gives a 90% power (alpha 0.05) to detect a difference in proportions of a binary variable of 50% v 30% and a 0.6SD difference in a continuous variable, and similarly a group of 20 v 20 gives an 80% power (alpha 0.05) to detect

a difference of 10% v 50% in the two groups and a power of 85% (alpha 0.05) to detect a 1 SD difference in a continuous variable.

13. Clinical Governance Issues

13.1 Compliance

The CI will ensure that the study is conducted in compliance with the principles of the Declaration of Helsinki (2013), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the UK Policy Framework for Health and Social Care Research (2020) and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006, 2008 and 2009 and any future relevant or replacement EU Regulations, Trust and R&D Office policies and procedures and any subsequent amendments.

13.2 Ethical Considerations

This protocol and any subsequent amendments, along with any accompanying material provided to the patient in addition to any advertising material, will be submitted by the Chief Investigator to an independent UK Research Ethics Committee (REC) and the Health Research Authority (HRA). Written REC and HRA approvals must be obtained and subsequently submitted to R&D to obtain Final Trust Approval.
All correspondence with the REC will be retained in the Trial Master File/Investigator Site File. Annual progress, safety reports and a final report at conclusion of the study will be submitted to the Sponsor and the REC. All protocol amendments will be submitted to the Sponsor, REC and Regulatory Authorities for approval. The CI will notify the REC of the end of the study and, should the study end prematurely, the CI will notify the REC, including the reasons for the premature termination.
Clinicians based worldwide will be involved in recruiting participants outside the UK and will operate under their own site's Institutional Review Board (IRB) approval.

14. Quality Control and Quality Assurance

14.1 Monitoring

Monitoring of this study will ensure compliance with Good Clinical Practice. The Investigators will permit monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (eg patients' case report forms, consent forms, etc). Periodic remote monitoring will be carried out by the CI's study team and the Sponsor. Consent will be obtained to permit access to data by external staff responsible for monitoring and auditing the study as a requirement of participating in the study.
Remote monitoring of recruitment activities at all sites will be carried out periodically by the study management team (on behalf of the CI & Sponsor) by exploring data, consent forms, delegation logs & recruitment logs copied to the CI in accordance with the study SOPs.

To facilitate this remote monitoring, and ensure timely and efficient transfer of data to the central study database, recruiting sites will be required to:

- Transfer scanned (PDF) copies of completed CRFs (data collection forms), consent forms and biochemistry results (via secure nhs.net email) to the CI site within 5 working days of the study visit.
- Monitoring reports will be stored in the study Trial Master File and copied to the Sponsor.

NHS Indemnity will apply to UK-based sites.

14.2 Audit and Inspection

A study may be identified for audit by any method listed below:

i) A project may be identified via the risk assessment process.
ii) An individual investigator or department may request an audit.

iii) A project may be identified via an allegation of research misconduct or fraud or a suspected breach of regulations.
iv) Projects may be selected at random. The UK Department of Health states that Trusts should be auditing a minimum of 10% of all research projects.
v) Projects may be randomly selected for audit by an external organisation (e.g. MHRA).

Internal audits will be conducted by a Sponsor's representative.

14.3 Amendments

Under the Medicines for Human Use (Clinical Trials) Regulations 2004, the Sponsor may make a non-substantial amendment at any time during a trial. If the Sponsor wishes to make a substantial amendment to the REC application or the supporting documents, the Sponsor will submit a valid notice of amendment to the REC for consideration. The REC will provide a response regarding the amendment within 35 days of receipt of the notice. It is the Sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the REC. Amendments will be notified to NHS R&D departments of participating sites to assess whether the amendment affects the NHS permission for that site. The amendment history will be tracked in the Trial Master File/Investigator Site File to identify the most recent protocol version.

14.4 Financial and other competing interests for the Chief Investigator, PIs at each site and committee members for the overall study management

No financial or other competing interests to disclose.

14.5 Indemnity

NHS Indemnity will apply to UK participants and public liability insurance is provided by the University of Exeter.

14.6 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol. Accidental protocol deviations can happen at any time and must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.
Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

14.7 Non-Compliance

Defined as a noted systematic lack of both the CI and the study staff adhering to SOPs/protocol/ ICH-GCP and UK regulations, which leads to prolonged collection of deviations, breaches or suspected fraud.

These non-compliances may be captured from a variety of different sources including monitoring visits, CRFs, communications and updates. The Sponsor will maintain a log of the non-compliances to ascertain if there are any trends developing which need to be escalated. The Sponsor will assess the non-compliances and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the R&D will agree an appropriate action, including an on-site audit.

15. Study Committees

The EXE-T1D Study Management Group (SMG) will be defined as the CI, Co-investigators, and Project Manager, who will meet/communicate regularly to ensure all practical details of the study are progressing and working well and that everyone within the study understands them correctly.

16. Access to the final study dataset

The Exeter study team will have access to the final dataset. Of the multiple analyses done during the study, relevant co-investigators for each analysis (e.g. RNAseq for Cate Speake) will have access to the datasets they have contributed to.

17. Public and Patient Involvement

This study was born out of the CI's direct contact with patients referred to the Exeter Molecular Genetics Laboratory from around the world with a diagnosis of diabetes in the first 12 months of life. They were suspected to have a possible genetic diagnosis for their diabetes but were shown to have likely Type 1 diabetes. The "absence" of a genetic diagnosis has been disappointing for some patients, and has prompted them to question why they have developed diabetes so early when the immune system is still developing. In addition, some patients and parents have asked why some people have developed T1D near birth whereas their affected siblings or parents have been diagnosed at much more typical ages. Both patients, relatives and clinicians have been in agreement that there would be significant benefit to knowing why and how T1D can present so young and whether understanding this could help with treatment or prevention.

A particular focus in this study design has been appreciation of the difficulty in collecting blood samples from young children. We have, therefore, specifically designed the study around a protocol that requires minimal blood samples that can usually be collected at the same time as routine clinical tests with no additional venepuncture, and have used a novel approach to collection, postage and analysis of fingerprick blood samples that again requires no additional fingerpricks to the patient's standard blood glucose monitoring.

This work is part-funded by a patient-focused charity, Diabetes UK that represents people with Diabetes in the UK, and The Leona M. and Harry B. Helmsley Charitable Trust; both charities consider this to be an important area of research.

The study team also has access to the user representative group of the NIHR Exeter Clinical Research Facility (ECRF). In keeping with the NHS Patient Carer and Public Involvement (PCPI) strategy, the Exeter CRF invites user representatives to contribute to the development of various projects within its portfolio. These individuals have agreed to maintain contact and regular meetings have been established at which researchers discuss the development of current projects within the Exeter CRF. Additional PPI has been conducted with parents of healthy young children, paediatricians and paediatric anaesthetists to agree an optimal strategy to recruit a cohort of healthy non-diabetic controls.

18. Publication Policy

On completion of the study, the data will be analysed and tabulated and a Final Study Report will be prepared and submitted to the Funder, Sponsor and REC. Results will be written up and submitted for publication in a peer-reviewed journal(s). Abstracts will be submitted to national and international conferences. Results will be presented to clinical colleagues at regular in-house meetings.

Written information in the form of a letter/newsletter outlining the key findings of the study will be posted on the study website.

- The University of Exeter owns the data arising from the study.
- There are no time limits or review requirements on the publications.
- The Funder will be acknowledged within publications but does not have review or publication rights.
- After the Final Study Report has been compiled, participants may specifically request results from their PI.
- The study protocol has been registered with ClinicalTrials.gov: NCT03369821.

19. References

RD&E NHS FT R&D Standard Operating Procedure ARCHIVING S03

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20. Appendices

20.1 Appendix 1 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
SA1	2.0	01 May 2018	Dr Richard Oram Michelle Hudson	1. Revised recruitment strategy to enable recruitment of eligible patients referred directly to Dr Oram, or who contact the study team. 2. Amended age of diagnosis to ≤24 months 3. Allow collection of RNAseq sample at visits for Study 1 and Study 2. Therefore, in Study 2, parents will not collect fingerprick samples for RNA analyses. 4. Where venepuncture is difficult, participants will be offered the option of another visit or collecting a fingerprick sample and posting it to Exeter (for analysis of HbA1c, C-peptide, autoantibodies and DNA), or to withdraw from the study.
SA3	3.0	01 November 2019	Dr Richard Oram Michelle Hudson	1. Allow additional visit for a small subset of Study 1 participants. 2. Updated information sheets and consent forms to provide greater clarity that some participants may not have developed diabetes yet, or may be invited for a second visit, or may be offered a home fingerprick sampling method instead of a face-to-face visit. NIHR branding has also been updated.
1617-23-SA-08	4.0	01 March 2022	Prof Richard Oram Michelle Hudson	1. Updated protocol for Phase 2, to include addition of non-diabetic controls cohort. 2. New activities added for Guy's & St Thomas' NHS FT and Royal Devon University Healthcare FT, to recruit non-diabetic controls cohort.

				3.New participant information sheet and consent form for parents of non-diabetic controls. 4.Minor edits to study participant information sheets and consent forms to make up-to-date for Phase 2.
1617-23-A12	5.0	24 November 2022	Prof Richard Oram Michelle Hudson	1.Protocol and information sheets (studies 1 & 2) updated to remove home visit as an option, and to indicate that the research visit may be arranged separately if it cannot be combined with review visit. 2.Consent and Assent forms (studies 1 & 2) updated to refer to new PIS version.
1617-23-SA4	6.0	17 July 2024	Prof Richard Oram Michelle Hudson	1.Protocol updated to indicate study extension to 30/11/2025 and increased recruitment total from 240 to at least 260, mainly by increasing the healthy control cohort as described.
1617-23-S-A20	7.0	24 November 2025	Prof Richard Oram Michelle Hudson	1.Protocol updated to indicate Phase 3 funding award and study extension to 30/11/2028, plus increased recruitment total to at least 300.

20.2 Appendix 2 – Study 1: Confirmation of EET1D as an autoimmune disease leading to beta-cell destruction

This study aims to assess autoimmunity and beta-cell function. Since most patients live outside Exeter, small blood volumes (as taken from very young subjects) and robust techniques will be required because samples will need to survive being sent to Exeter by courier.

Assessment of Autoimmunity

i) **Autoantibodies:** To establish whether EET1D is an islet specific B-cell autoimmune disease, we will test serum/plasma from >50 EET1D patients for islet autoantibodies (GAD, IA-2 and ZnT8) in Exeter (established assays, part of international QA scheme lead McDonald) and also duration-matched T1D controls.

Islet specific autoreactive T cells in EET1D (visiting Lab of Professor Bart Roep, Leiden University Medical Center, Netherlands). We will collect peripheral lymphocytes (PBMCs) for assessment of autoreactive CD8 T cells samples from >20 UK patients with EET1D, duration-matched T1D controls and age/duration-matched monogenic non-autoimmune diabetes. We may send the participants' PBMCs to Professor Bart Roep to investigate the presence and specificities of islet-specific CD8 T cells using the Diab-Q-kit developed by Professor Roep. The QDot analyses require only 2 million cryopreserved PBMCs and has separated T1D from non-T1D in numerous settings(5-7), making it an ideal assay to assess autoimmunity in EET1D. We will determine the frequencies of islet-specific CD8 T cells against insulin B10-18, PPI15-24, IA-2797-805, GAD65114-123, GRP265-273 and pplAPP5-13.using peptide-MHC multimers labelled with Qdots. We will compare the activation status of the islet-specific CD8 T cells by flow cytometry. Cells will be analysed for the expression of markers involved in effector activation and memory states (CCR7/CD45RA) and capacity to migrate to the inflamed pancreas (CXCR3)(14,15).With this analysis, we aim to elucidate whether islet-specific CD8 T cells from EET1D patients express an immune signature consistent with autoimmune T1D and how it varies from T1D with a normal onset. Through additional funding from The Leona M. and Harry B. Helmsley Charitable Trust, this study now includes additional analysis of CD4 T cells by Tim Tree (King's College London).

ii) **Assessment of loss of beta-cell function**: We will assess the loss of endogenous beta-cell function in >50 EET1D patients and controls by measuring C-peptide in parallel with the autoantibody measurement. C-peptide will be taken in EDTA plasma which allows it to be stable

for 24 hours in whole blood at room temperature(12). A non-fasting random post-meal sample has been shown to be a sensitive and specific measure of insulin deficiency without the need for omission of insulin or a formal stimulation test(16).

iii) **Outcome:** The focus of study 1 is to establish evidence that the EET1D patients have features of islet-specific autoimmune diabetes that through beta-cell destruction causes progression to severe insulin deficiency (and whether this process differs from T1D at a later age of diagnosis).

20.3 Appendix 3 – Study 2: Investigation of mechanisms of EET1D

In this second study, we will examine for mechanisms that can explain the very early onset of islet-specific autoimmunity in EET1D. This will involve looking for differences in genetics, maternal genetics, intra-uterine environment, and autoimmune function.

Genetic analysis

i) **HLA high resolution sequencing:** we aim to define HLA to 6-digit definition by sequencing the entire 3.8Mb HLA region using a combination of CATCH-seq targeted capture with Pacific Biosciences sequencing in 100 patients with EET1D and 100 T1D patients (diagnosed 1-10 years) matched for 2 digit HLA genotype (DR3/DR4 haplotype combinations). The Pacific Biosciences technology provides an opportunity to fully interrogate the HLA region in these patients using long-read sequencing that avoids the difficulties of haplotyping with shorter read approaches(17). The Exeter group has extensive experience of analysing PacBio data (having been recently awarded a £1m MRC clinical infrastructure grant for the PacBio sequencing machine and associated computing). We will use the PacBio SMRT portal for analyses including de-multiplexing, generation of haplotypes, read alignment and variant calling. We will look for differences in frequencies of rare or novel risk variants between EET1D and T1D. For example, we will have 80% power to detect a rare variant that is present in 0.1% of controls, but >15% of cases at a P<0.0005.

ii) **Maternal genetic factors: Non-inherited maternal allele (NIMA) and microchimerism analysis.**
We will assess whether non-inherited maternal alleles (NIMA) are important in EET1D. Non-inherited maternal HLA alleles that are associated with no/reduced risk for T1D can increase the likelihood of T1D in offspring(18). We have parental DNA from 100 EET1D cases so we can test if the NIMA of mothers of EET1D are more likely than by chance to be non-DR3 and non-DR4 (comparing to paternal HLA) and whether at non-HLA loci the NIMA are more likely to be protective alleles than expected by chance. Maternal DNA (microchimerism-presence of maternal cells in the offspring) is measurable in T1D at higher proportions than in controls and non-diabetic siblings in the peripheral blood and pancreas of patients with T1D. We will use assays developed by Kathleen Gillespie (University of Bristol)(9) to assess the presence of maternal microchimerism in fetal circulation in EET1D compared to NDM and T1D (diagnosed 1-10 years).

iii) **Longitudinal analysis of Immune Phenotype:** The initiation of autoimmunity in T1D has been very difficult to characterize due to uncertainty regarding when the process starts. The identification of EET1D patients so close to birth allows a unique opportunity to study T1D very close to the initiation of autoimmunity. We will study autoimmunity in newly referred patients with EET1D (n=20) and NDM (age-matched controls n=20) at diagnosis and after 2 years follow up and assess autoimmunity using 3 methods that can all be performed in early life due to the small samples required:
   - **a)** Islet autoantibody measurement
   - **b)** QDot analysis for CD8 T cells
   - **c)** T1D-specific transcriptomic analysis using RNA sequencing (8). This last analysis is in collaboration with Dr Cate Speake, (Benaroya Research Institute). The key outcome will be to assess which markers of autoimmunity are present at diagnosis and how they change at 2 years follow-up. It has never previously been possible to study T1D that has occurred in such a short space of time after birth and this offers the possibility to identify the first immune signature of the disease.

20.4 Appendix 4 – Details of immunophenotyping assays to be performed

Name	Technology	Parameter measured	Sample requirement	Priority
Global Immune Profiling	Multiparameter flow cytometry using modified FITMaN panels.	Frequency of subsets of major immune cell populations (T Treg, B, NK, DC, monocytes & granulocytes)	500µl whole blood	1
	Luminex	Soluble markers of immune activation and inflammation	100µl serum	1
CD8 Q-Dot	MHC-CI multimers combined with multiparameter FACS	Frequency and phenotype of islet specific CD8 T cells	3 x 10^6 cryopreserved PBMC (2 ml fresh blood*)	2
	CD4+ $FOXP3^+$ Treg suppressive function	Autologous co-culture suppression assay	1 ml whole blood	3
CD4 ELISpot	Frequency of islet specific CD4 T-helper cell subsets	IFN-γ, IL-10 (and IL-17) ELISpot/ FluoroSpot.	5-15❖ x 10^6 fresh PBMC (3.3-10 ml fresh blood*)	4
* Estimates for PBMC yields are based on lymphocyte counts for individuals aged 2-6 years of age from published sources and pilot data. Yields from individuals <2 years of age are expected to be significantly higher. ❖ Range indicates minimum and maximum PBMC required to study responses to 3-12 islet peptides.				