Trial Title: Low dose glibenclamide and dapagliflozin in Type 1 Diabetes Mellitus

Short title: Low dosE GlibENclamide and Dapagliflozin in type 1 Diabetes (LEGEND-D)

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There are no potential conflicts of interest to declare

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

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1. KEY TRIAL CONTACTS

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Committees	Two committees will be involved in the management and oversee of the trial: Trial management group (TMG) and Trial Steering Committee (TSC)

2. LAY SUMMARY

Type 1 diabetes (T1D) affects around 400,000 people in the UK and is caused by nearly complete loss of insulin-producing cells in the pancreas. It is often challenging to achieve good control of blood glucose throughout the day in T1D, in part because of fluctuations associated with giving insulin. Severe episodes of low blood sugar levels, commonly called “hypos”, are one of the most feared complications of managing diabetes with insulin.

The impaired release of other hormones, such as glucagon which raises blood sugar, during hypo episodes is also part of type 1 diabetes. A better understanding of this mechanism could lead to treatments aimed at reducing the risk of hypoglycaemia.

Glibenclamide is a type of anti-diabetic medication (sulfonylurea) which is commonly used to increase the amount of insulin released by the pancreatic beta-cells. Recent pre-clinical studies have shown that sulfonylureas can also improve glucagon levels when used in very small doses by working on pancreatic alpha-cells, which release glucagon. We have previously conducted a pilot study (LEGEND-A), which suggested that low doses of glibenclamide (0.3mg/day) could alter glucagon release in some people with type 2 diabetes without increasing the risk of hypoglycaemia. In addition, another type of anti-diabetic medication, called dapagliflozin, has also been shown to work on pancreatic alpha-cells.

Therefore, the aim of this follow-up study (LEGEND-D) is to find out whether similar doses of glibenclamide or a single dose of dapagliflozin could restore glucagon release in people with T1D. We hope that add-on therapies such as these may become a new way of helping people with T1D to prevent hypoglycaemia.

The trial will involve 2 groups of participants:

a) 20 people with T1D, who will be given a liquid form of glibenclamide for a maximum of 54 days (at 3 different doses, in 3 blocks of 14-18 days), followed by a single dose of dapagliflozin, and undergo five controlled hypoglycaemia challenges.

b) A control group of 10 people without diabetes, who will undergo one hypoglycaemia challenge without receiving any medication.

During these challenges, we will gradually drop the participants’ blood sugar from a normal level (around 6 mmol/L) to a lower level (around 2.5mmol/L) for 40 minutes. While this is a well-established procedure, this change in blood sugar can be stressful and participants without diabetes will probably not have experienced the symptoms before. We will screen all participants for high risk conditions, and monitor them closely during the entire procedure.

We will use a continuous glucose monitor during the study in participants with T1D. All participants will need to attend the OCDEM Clinical Research Unit at the Churchill Hospital, Oxford for an initial screening visit. This will be followed by 8 study visits over a period of 8-10 weeks (divided into around 2-week blocks) for the people with T1D, and the people without diabetes will have just 1 study visit.

This trial is funded by The Leona M. and Harry B. Helmsley Charitable Trust.

3. SYNOPSIS

Trial Title	**Low dose glibenclamide and dapagliflozin in Type 1 Diabetes Mellitus**		
Short title	Low dosE GlibENclamide and Dapagliflozin in type 1 Diabetes (LEGEND-D)		
Trial registration	**ISRCTN and ClincialTrials.gov**: TBD		
Sponsor	University of Oxford Research Governance, Ethics and Assurance Team, Joint Research Office , Boundary Brook House, Churchill Drive, Headington, Oxford OX3 7GB		
Funder	The Leona M. and Harry B. Helmsley Charitable Trust		
Clinical Phase	Phase II (Therapeutic exploratory trial)		
Trial Design	Randomised, pilot, open-label triple cross-over		
Trial Participants	People with type 1 diabetes (either on insulin pump or multiple daily injections) compared with non-diabetic individuals.		
Sample Size	20 participants with type 1 diabetes and 10 non-diabetic participants		
Planned Trial Period	Individual participant's involvement: T1D group = maximum 77 days, including 8 days washout non-DM group = 1 day Trial duration: 19 months Trial start date: 15 August 2023 Trial End Date: 30 May 2025		
Planned Recruitment period	Estimated recruitment dates: August'23 – 30 November 2024		
	Objectives	Outcome Measures	Timepoint(s)
Primary objective	To determine whether treating people with T1D for 14-18 days with glibenclamide at 0.3mg, 0.6mg or 3mg daily can increase the counter-regulatory glucagon response during induced hypoglycaemia compared to baseline (pre-treatment).	Concentration of plasma glucagon at 40min during the hypoglycaemic phase of the hyper-insulinaemic hypo-glycaemic clamp, at baseline and at each dose step of glibenclamide (T1D group only).	At 40min during the hypoglycaemic phase of the hyper-insulinaemic hypo-glycaemic clamp.
Secondary objectives (A)	1. To compare the glucagon response during induced hypoglycaemia in people with T1D prior	1. Concentration of plasma glucagon during the hypoglycaemic phase of the	1. Glucagon levels during the hypoglycaemic phase of the hyperinsulinaemic

	to glibenclamide treatment, and at each dose step, to that of participants without diabetes (non-DM group). 2. To compare the increase in glucagon from euglycaemia to hypoglycaemia in T1D participants prior to treatment, and at each dose step of glibenclamide, to that of participants without diabetes (non-DM group). 3. To compare the percentage of time spent in hypoglycaemia at each dose step of glibenclamdie, compared to baseline (T1D group only)	hyperinsulinaemic hypoglycaemic clamp (T1D and non-DM group) 2. Proportion of increase in plasma glucagon at each timepoint (i.e. glucagon concentration at 0min during the hypoglycaemic phase divided by the glucagon concentration at 0min during the euglycaemic phase) (T1D and non-DM group). 3. Percentage of time spent in hypoglycaemia (<4.0mmol/L) from Freestyle Libre 2 data (T1D group only)	hypoglycaemic clamp (0, 15, 30 and 40min). 2. Glucagon levels at each timepoint (0, 15, 30 and 40min) during the euglycaemic and hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp. 3. Freestyle libre data collected for the 7 days prior to each dose step until the start of the hyper-insulinaemic hypo-glycaemic clamp
Secondary objectives (B)	1. To compare the change in glucagon from euglycaemia to hypoglycaemia in T1D participants prior to and following administration of a single dose of dapagliflozin 10mg, to that of participants without diabetes (non-DM group). 2. To compare the change in plasma somatostatin from euglycaemia to hypoglycaemia in T1D participants prior to and following administration of a single dose of dapagliflozin 10mg, to that	1. Proportion of change in plasma glucagon at each timepoint (i.e. glucagon concentration at 0min during the hypoglycaemic phase divided by the glucagon concentration at 0min during the euglycaemic phase) (T1D and non-DM group). 2. Proportion of change in plasma somatostatin at each timepoint (i.e. somatostatin concentration at 0min during the hypoglycaemic phase divided by the somatostatin	1. Glucagon levels at each timepoint (0, 15, 30 and 40min) during the euglycaemic and hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp. 2. Somatostatin levels at each timepoint (0, 15, 30 and 40min) during the euglycaemic and hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp.

	of participants without diabetes (non-DM group). 3. To characterise the development of hypoglycaemic symptoms during induced hypoglycaemia at baseline, and during each dose of glibenclamide and dapagliflozin. 4. To measure any residual C-peptide participants with T1D only. 5. To measure the frequency of adverse events in participants treated with Glibenclamide and Dapagliflozin.	concentration at 0min during the euglycaemic phase) (T1D and non-DM group). 3. Self-reported hypoglycaemia symptoms during the hyperinsulinaemic hypoglycaemic clamp. 4. Fasting C-peptide prior to the baseline hyperinsulinaemic hypoglycaemic clamp (T1D group only) 5. Number of Adverse events reported	3. Blood glucose value at onset of self-reported hypoglycaemia symptoms during the hyperinsulinaemic hypoglycaemic clamp. 4. Plasma C-peptide level at the start the hyperinsulinaemic hypoglycaemic clamp 5. At each study visit following the receipt of informed consent
Intervention(s) • IMP(s)	Oral glibenclamide suspension (Amglidia) - Oral liquid suspension at strengths of 0.6 mg/ml. Dosing schedule will range from 0.3 – 3 mg daily, split into morning and evening (half dose in the morning, half dose in the evening). Dapagliflozin (Forxiga) 10mg oral tablets.		
Comparator	IMP: No active comparator. The baseline samples taken at visit 1 for all participants, both T1D & non-diabetes participants - who will not receive either of the study IMPs and will undergo one hypoglycaemia challenge only, will provide the comparator.		

4. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
AUC	Area under curve
α-cells	Alpha-cells
βhCG	beta human chorionic gonadotrophin
CGM	Continuous Glucose Monitor
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CRU	Clinical Research Unit
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DEPTH	Dapagliflozin during exercise for the prevention of hypos
DM	Diabetes mellitus
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
DTU	Diabetes Trials Unit
δ-cells	Delta-cells
EPR	Electronic Patient Record
eTMF	Electronic Trial Master File
FGM	Flash Glucose Monitoring
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
HTA	Human Tissue Authority
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IRB	Independent Review Board
KATP	ATP-sensitive potassium channel
LEGEND-A	Low-dose Glibenclamide in Diabetes – Part A

LEGEND-D	Low-dose Glibenclamide and Dapagliflozin in type 1 Diabetes
MHRA	Medicines and Healthcare products Regulatory Agency
mmol/mol	millimoles per mole
ng/ml	nanograms per millilitre
NHS	National Health Service
Non-DM	Without diabetes
RES	Research Ethics Service
RGEA	Research Governance, Ethics and Assurance
OCDEM	Oxford Centre for Diabetes, Endocrinology and Metabolism
OUH	Oxford University Hospitals
pmol	picomoles
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
POC	Point of care
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SGLT2i	Sodium-glucose co-transporter 2 inhibitor
SmPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
$t_{1/2}$	half-life
T1D	Type 1 diabetes
TMF	Trial Master File
TSG	Oxford University Hospitals NHS Foundation Trust / University of Oxford Trials Safety Group
USAN	United States adopted name

5. BACKGROUND AND RATIONALE

Type 1 diabetes mellitus (T1D) is a multi-hormonal disorder that results from near-complete loss of insulin secretion and dysregulation of glucagon secretion (1). It is often very challenging to achieve good blood glucose control throughout the day in type 1 diabetes, in part because of fluctuations in insulin levels associated with insulin administration. Severe hypoglycaemia ("hypos") are reported as one of the most feared complications of diabetes and its management with insulin (2). In addition, the frequency of hypos is often underestimated, as >80% of people with type 1 diabetes said they did not report non-severe hypoglycaemia to their healthcare team (3).

Glucagon is the body's principal hyperglycaemic hormone. It is normally released from the alpha-cells of the pancreatic islets in response to a fall in plasma glucose (4). Following its release, glucagon travels through the blood to the liver where it stimulates hepatic glucose production until normoglycemia is restored, a process referred to as counter-regulation (5).

In many patients with T1D, a fall in blood glucose fails to stimulate glucagon secretion (loss of the normal counter-regulatory response), thereby enhancing insulin's hypoglycaemic effect (6). This has also been demonstrated in our lab using islets from deceased donors with type 1 diabetes, in which hypoglycaemic conditions induced only 25% of the glucagon secretion seen in non-diabetic donors (7).

5.1. Glibenclamide component

Sulfonylureas are a class of medication which include tolbutamide and glibenclamide, among others. They act on the ATP-sensitive potassium channel (K_{ATP}) (8), and have been used for >60 years to manage type 2 diabetes by stimulating insulin secretion. However, their effects on glucagon secretion have not been extensively studied. Previous in vitro studies have shown that normal glucose regulation of glucagon secretion can be restored by low concentration of tolbutamide (<10% of the concentration used to stimulate insulin secretion) in islets obtained from diabetic mice and organ donors with type 2 diabetes (9,10). It is thought that low-dose sulfonylureas act by subtly altering the resting membrane potential of the alpha-cells (via their action on the K_{ATP} channels), thereby restoring physiological glucose-regulated glucagon secretion (i.e. suppression during hyperglycaemia, and stimulation during hypoglycaemia) (11). Whether low concentrations of sulfonylureas restore counter-regulatory glucagon secretion in T1D is not known. Our own experiments in islets from an insulinopenic mouse model of diabetes (in which insulin content was reduced by 98%) suggest this may offer therapeutic benefits (12).

We previously conducted a small pilot study using low doses of glibenclamide in patient with type 2 diabetes (LEGEND-A) (13). The results demonstrated that 0.3mg/day of glibenclamide (<10% the normal starting dose) was able to alter glucagon secretion in a sub-population of patients who had inappropriately high fasting glucagon levels. We will now test whether the principle of restoring appropriate glucose-mediated glucagon secretion could also apply in type 1 diabetes.

The main aims of the "Low dose glibenclamide and dapagliflozin in Type 1 Diabetes Mellitus" (LEGEND-D) trial are to investigate whether treatment with low-dose glibenclamide can improve glucagon counter-regulation and reduce time spent hypoglycaemic in patients with type 1 diabetes. Participants with T1D will undergo an induced hypoglycaemic challenge (hyperinsulinaemic hypoglycaemic clamp) before and

after treatment with low doses of glibenclamide (0.3mg, 0.6mg and 3mg daily), and their results will also be compared to a control group without diabetes who will not receive glibenclamide.

5.2. Dapagliflozin component

Finally, we will also investigate whether a single 10 mg dose of the medication dapagliflozin, a type of medication already used in type 2 diabetes therapy, could improve glucagon counter-regulation during an induced hypoglycaemic challenge.

Anecdotal reports from some people with T1D suggest SGLT-2 (sodium/glucose cotransporter-2) inhibitors (SGLT2i) such as dapagliflozin can prevent exercise-induced hypoglycaemia. SGLT2i promote glucose excretion without causing hypoglycaemia (14). They appear to also increase endogenous glucose production and stimulate glucagon secretion in the absence of hypoglycaemia (15,16). Studies in diabetic rats indicate that the physiological counter-regulatory response is suppressed in insulin-treated diabetes, a defect that can be corrected by somatostatin antagonists (17).

Better understanding of the interplay between pancreatic α- and δ-cells (which secrete glucagon and somatostatin, respectively), as well as of the impact medications have on their function is key to unravelling the pathophysiology of diabetes. We will explore glucagon and somatostatin secretion during the hypoglycaemic challenge before and after treatment with a single oral dose of dapagliflozin 10mg in participants with T1D, and compare this to the response of participants without diabetes (who will not be given the medication).

5.3. Justification of the starting doses

Glibenclamide, also known as glyburide by its United States adopted name (USAN), belongs to a class of medication called sulfonylureas, which (at the usual dose of 5mg used for the treatment of type 2 diabetes (18)) work by stimulating insulin secretion from pancreatic beta-cells. As such, there is a risk of causing hypoglycaemia in individuals with preserved beta-cell function – however this is not the case in people with T1D, as the majority of their beta-cells have been destroyed. Studies using dosing regimens different from what is normally prescribed are scarce. Nonetheless, our recent study showed that the administration of low doses of glibenclamide (0.3mg daily) could potentially restore more physiological glucagon secretion (13). The current study aims to identify whether similar doses can have a beneficial effect in people with T1D. As described above, in T1D the majority of beta-cells are destroyed and insulin secretion does not occur. We therefore do not anticipate that administering glibenclamide at 0.3mg/day, 0.6mg/day or 3mg/day in people with T1D will increase their risk of hypoglycaemia.

Dapagliflozin was previously licenced for use as an adjunct therapy in T1D at a dose of 5mg daily, however in December 2021 the licence in T1D was withdrawn. This was related to an increased risk of diabetic (or euglycaemic) ketoacidosis, which occurred more frequently in people with T1D. We do not anticipate an increased risk of ketoacidosis in participants with T1D receiving a single dose of 10mg dapagliflozin prior to the hyperinsulinaemic clamp. From a clinical perspective the insulin infusion would be expected to counteract ketone production, and capillary blood ketone levels will be checked at the end of the clamp, as per standard clinical care.

6. TRIAL DESIGN

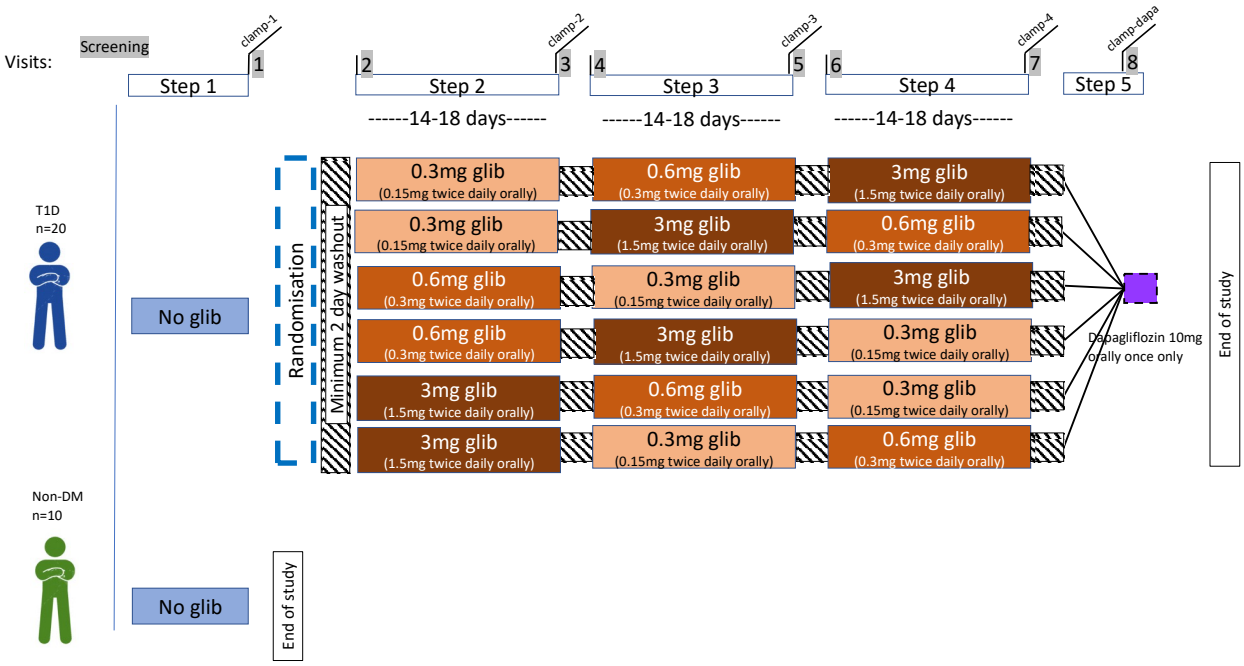

Figure 1 Trial flow chart. See APPENDIX A: Trial flow chart, for larger version

The LEGEND-D trial is a pilot, randomised cross-over, single-centre, non-blinded, clinical trial which aims to investigate the effect low doses of glibenclamide (0.3mg, 0.6mg and 3mg/day) and dapagliflozin (single dose of 10mg) on the glucagon response during induced hypoglycaemia in people with type 1 diabetes, compared to non-diabetic individuals (Figure 1). The trial will be conducted by the Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) Clinical Research Unit (CRU) at the Churchill Hospital.

A summary timeline of the trial can be found in **APPENDIX A: Trial flow chart**. For participants with T1D, the trial is divided into 5 steps, and involves 5 hyperinsulinaemic hypoglycaemic clamps (see section 8.9 for more details). Once step 1 (no medication) has been completed, the participants with T1D will be randomised to a sequence of glibenclamide doses for the subsequent steps, followed by a single dose of 10mg dapagliflozin. There will be a washout phase of at least 48 hours between each step (the half-life of the glibenclamide suspension is 8 hours; **Table 1**). Participants without diabetes will undergo a single hyperinsulinaemic hypoglycaemic clamp, as they will not receive any study medication (section 8.9).

	0.83ml GlibenTek 6mg/ml	**8.33ml GlibenTek 0.6mg/ml**	**5mg Daonil**
t_{max}	2.5h	2.5h	3h
C_{max}	201.71± 71.43 ng/ml	206.93± 67.33 ng/ml	148.34± 46.74 ng/ml
$AUC_{0-\infty}$	1120.9± 400.5 ng.h/ml	1172.3± 422.0 ng.h/ml	
Bioavailability	114.1% (relative to Daonil)	121.6% (relative to Daonil)	100%

$t_{1/2}$	8h	8h	10.45h

Table 1 Pharmacokinetic properties of glibenclamide suspension (GlibenTek, now Amglidia) at 2 difference strengths (6mg/ml and 0.6mg/ml), compared to crushed tablets of the licensed tablet Daonil (glibenclamide) [8].

The total daily dose as defined by the dosing schedule will be split into AM and PM as follows:

Total dose	Strength	AM	PM	Total volume over 14 days (min)	Total volume over 18 days (max)
0.3mg	0.6mg/ml	0.25ml	0.25ml	7ml	9ml
0.6mg	0.6mg/ml	0.5ml	0.5ml	14ml	18ml
3mg	0.6mg/ml	2.5ml	2.5ml	70ml	90ml
			Total:	**91ml**	**117ml**

Table 2 Oral glibenclamide suspension doses and volumes.

6.1. Dapagliflozin component

As mentioned above, for participants with T1D the final hyperinsulinaemic hypoglycaemic clamp will involve them taking a single dose of dapagliflozin 10mg orally at the start of the study visit.

The main pharmacologically relevant site of action of dapagliflozin (Forxiga, AstraZeneca), is the sodium-glucose cotransporter 2 in the proximal convoluted tubules of the kidneys. By inhibiting this transporter, the threshold for glucose reabsorption is reduced, leading to increased glucose excretion. The pharmacokinetic properties of dapagliflozin are summarised in **Table 3**.

Dapagliflozin also leads to increased glucagon secretion by acting directly on the pancreatic islet cells (16). Plasma glucagon concentrations are increased compared to baseline 2 hour after dapagliflozin administration, and reach a peak of 79 pg/ml (23.7pmol/L) after 4 hours (15).

	10mg Dapagliflozin (Forxiga)
t_{max}	2 hours
C_{max}	158 ng/ml
$AUC_{0-\infty}$	628 ng h/ml
Bioavailability	78%
$t_{1/2}$	12.9 hours

Table 3 Pharmacokinetic properties of dapagliflozin (Forxiga)

In total there will be 8 study visits for participants with T1D and 1 study visit for the non-DM participants.

7. PARTICIPANT IDENTIFICATION

7.1. Trial Participants

The anticipated recruitment period is 12 months; the recruitment target is 20 participants with T1D and 10 non-DM controls. The study will aim to include participants of different age groups.

7.2. Inclusion Criteria

Type 1 diabetes group:

1. T1D diagnosed ≥12 months prior to screening
2. Age 18-75 years
3. Either on insulin pump or multiple daily insulin injections
4. HbA1c < 10% (86mmol/mol) at screening
5. Prior training regarding insulin dose-adjustment and management of hypoglycaemia
6. Willing and able to give informed consent for participation in the trial
7. In the Investigator's opinion is able and willing to comply with all trial requirements

Non-DM control group:

1. Age 18-75 years
2. HbA1c ≤6.0% (42mmol/mol) at screening
3. Willing and able to give informed consent for participation in the trial
4. In the Investigator's opinion is able and willing to comply with all trial requirements

7.3. Exclusion Criteria

T1D group only:

1. An episode of diabetic ketoacidosis in the previous 1 month
2. Severe hypoglycaemia requiring third party intervention on more than 1 occasion in the preceding 12 months
3. Active diabetic retinopathy (including active proliferative diabetic retinopathy or vitreous haemorrhage in the past 6 months)

T1D and non-diabetic group:

4. Haemoglobin < 125 g/L
5. History of seizure or coma
6. Pregnancy, breast feeding or women of childbearing potential without adequate contraception

7. Renal impairment (eGFR ≤50 ml/min) at screening
8. ALT >2.5×upper limit of the assay normal range or known liver disease, specifically bilirubin >30 µmol/L that is associated with other evidence of liver failure.
9. Uncontrolled hypertension (>180 mmHg systolic or > 100 mmHg diastolic)
10. History of ischaemic heart disease (unless has had successful reperfusion), stroke/transient ischaemic attack (TIA), ventricular rhythm disturbances or thromboembolic disease
11. On beta-blocker medication
12. A history of heart failure (New York Heart Association, NYHA, Class 3 or 4)
13. Untreated Grave's disease
14. History of ECG or stress test findings indicating active ischaemia or a condition that would compromise the participant's safety
15. Known history of porphyria
16. Concomitant use of bosentan
17. Known or suspected allergy to the trial product or related products
18. Have received any investigational drug within 3 months prior to screening
19. Systemic (i.e. other than topical) corticosteroid treatment within 30 days prior to the start or at any time during the trial period
20. Major psychiatric disease including eating disorders, history of drug and alcohol abuse
21. Known malignancy or any other condition or circumstance which, in the opinion of the investigator, would affect the participant's ability to participate in the protocol

It is worth noting that although the SmPC of both Amiglida (glibenclamide) and Forxiga (Dapagliflozin) present an extensive list of medications which might interact with these drugs, given the low dosage administration, the limited number of times that participants are required to administer these medications, as well as the different study population investigated, these interactions are not considered clinically relevant and will not form part of the exclusion criteria. However, potential participants will be advised that concomitant use of Bosentan is an exclusion criterion.

8. TRIAL PROCEDURES

8.1. Recruitment

Individuals with T1D will be recruited to the trial mainly through publicity, including posters as well as social media (Twitter etc.). The poster will also be used to share information about the study in the Hospital and University's newsletter. Potential T1D participants who have already given consent to be contacted regarding medical research will also be identified through the Clinical Research Unit (OCDEM, Oxford).

As the chief investigator is also a Specialty Clinician in diabetes and endocrinology, potential T1D participants will also be identified through his clinics. It is common practice for clinicians to ask patients reviewed in diabetes clinics whether they consent to being contacted about research projects. This can also be done by patients themselves via the Electronic Patient Record (EPR) system and the patient portal.

Potential participants without diabetes (non-DM controls) will be recruited via publicity through local institutions (hospital and university), and via social media and poster advertising.

Interested potential participants will be sent a study invitation pack (paper copy or via secured email). This will be carried out by using well-established correspondence methods already in adoption within the CRU. The study invitation pack will include a covering letter asking them whether they would like to join the study, the Participant Information Leaflet and details about how to register an interest (including by contacting the research team via email or phone). In addition, a pre-paid stamped addressed envelope will be included in the pack to enable a safe return of the reply slip present in the invitation letter. Participants will also be able to indicate whether they would prefer a telephone, e-mail or face-to-face discussion to have the trial protocol explained to them. If no response is received in 1 month, then a follow-up letter/email will be sent once only to the potential T1D participants only.

Interested potential participants will be invited to a screening visit where the research team will assess their eligibility criteria and obtain informed consent. Some initial checks regarding certain exclusion criteria can be carried out over the phone. As a guide, they will be invited to a screening visit 1-2 weeks after the information leaflet has been sent to them. A visit reminder shall be sent in accordance with the standard CRU procedure.

8.2. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form (ICF) before any trial specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as they wish to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the trial. Written Informed Consent will then be obtained by means of participant dated signature and

dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the study site.

8.3. Screening visit

This will take 1h and the following will be performed:

1. Inclusion and exclusion criteria checked (protocol waivers are not permitted).
2. Informed consent will be obtained by a member of the clinical research team.
3. Medical history checked with participants (including Gold score for T1D group, see APPENDIX H: Assessment of hypoglycaemia symptoms.
4. Concomitant medication checked.
5. Demographics documented in CRF: date of birth, gender, weight and height.
6. Observations documented in CRF: resting heart rate and blood pressure.
7. Blood sample for HbA1c and safety blood tests (full blood count, sodium, potassium, urea, creatinine, and liver function tests: bilirubin, ALT, AST, albumin, ALP).
8. Pregnancy test in women who are not postmenopausal (see below).
9. T1D group only: Flash glucose monitor (Freestyle Libre) provided
10. Arrangements made for first study visit.
11. Adverse events recording

A pregnancy test (urinary βhCG) will be performed at the screening visit in all women who are not postmenopausal (defined as no menses for 12 months without an alternative medical cause). Acceptable forms of contraception include the followings: -

- Established use of oral, injected or implanted hormonal methods of contraception.
- Placement of an intrauterine device or intrauterine system.
- Male sterilisation if the vasectomised partner is the sole partner for the participant.
- True abstinence (as defined as refraining from any sexual relationships in which the participant may become pregnant), when this is in line with the preferred and usual lifestyle of the participant. Note periodic abstinence and withdrawal are not acceptable methods of contraception.

The pregnancy test will be repeated at the first study visit (day 1) if the interval between the screening visit and the start of the trial is more than 28 days, and at the beginning of each “Step" (visits 2, 4, 6 and 8). At the discretion of the medical investigator, if considered necessary, the pregnancy test might also be performed during the other visits of the trial.

Any participant who becomes pregnant during the trial will not partake in further study activities and, with their permission, will be followed up for safety reasons until pregnancy outcome. Further venepuncture and blood sampling will not be performed on such participants. No other study information will be collected from such participant. Any information collected will be strictly limited to ensuring participant’s safety.

Approximately 10 mL of blood sample will be collected for safety blood test which results will usually be available within 1 day of the screening visit, and the potential participant will be contacted to let them

know if they meet the inclusion / exclusion criteria. Participants will be considered enrolled once eligibility is confirmed and they have signed the ICF.

There is no maximum duration allowed between screening and starting the trial / randomisation. There will be no exceptions made regarding eligibility, i.e. each participant must satisfy all the approved inclusion and exclusion criteria of the protocol.

Rescreening will be permitted if the participants' circumstances change such that the inclusion and exclusion criteria can be met in the future (e.g. time-limited use of oral steroids), or if the original screening visit was >6 months from the first study visit.

8.4. Flash Glucose Monitoring (FGM)

Participants with T1D will receive a flash glucose monitoring (FGM) system (FreeStyle Libre 2, Abbott), which will be used throughout the trial. The Freestyle Libre 2 is a CE marked device, which will be used in the trial for its intended purpose, in accordance with the manufacturer's instructions. This system, which is designed for use by the patient themselves, utilises a small subcutaneous sensor which measures glucose in the interstitial fluid every 15 minutes and lasts for up to 14 days. The sensor is attached to the arm and transmits data to the Libre monitor device (or mobile phone with compatible app) every time it is scanned by bringing the two close to each other. This must be done at least every 8 hours, and this will be explained to the participants at the start of the study. FGM values <3.0 mmol/L will be verified by capillary blood glucose measurement by the participants. They will be instructed to treat any verified values < 4.0 mmol/L (as per normal standard care, see APPENDIX H: Assessment of hypoglycaemia symptoms), and to contact the research team if there are values < 3.0 mmol/L.

The sensor will be connected to either the Libre monitor or via mobile phone app (whichever the participant prefers). If there is a problem with the sensors (e.g. they fail or detach) then they will be replaced as required. Data will be uploaded to an account on Libreview which will include the Study ID only. The Libre monitor device (if used) will be returned to CRU at the end of the trial.

If the participants already use a different CGM system as part of the normal management, then they will be allowed to continue using it in addition to using the Freestyle Libre 2.

Training on the use of the Libre 2 device is provided by the manufacturer on their website, and will be supported as/when required by the clinical research team. Participants will receive the standard manufacturer's instructions (as part of the Libre 2 sensor package). Tutorial videos illustrating how to make the best use of the device is provided on the manufacturer's website (https://www.freestyle.abbott/uk-en/support/tutorialsanddownloads.html). However, it is important to emphasise that the correct usage of the device is illustrated in a very comprehensive manner by the manufacturer in a leaflet which is part of the Libre 2 sensor package. Participants will be directed to the above mentioned website if retained necessary.

The participant will be advised to stop using the system and contact the study team for further guidance if they experience any significant irritation and/or discomfort.

8.5. Definition of hypoglycaemia

Hypoglycaemia episodes will be reported as per the recommendations of the International Hypoglycemia Study Group (19):

Level 1	< 4.0 mmol/L (70mg/dL) = **glucose alert**
Level 2	< 3.0 mmol/L (54mg/dL) = **clinically important hypoglycaemia**
Level 3	<4.0 mmol/L with severe cognitive impairment requiring external assistance for recovery = **severe hypoglycaemia**

Table 4 Hypoglycaemia levels

For the purposes of this study, level 1 and 2 will form part of the secondary outcomes, while level 3 will be reported as an adverse event, unless the event is serious enough to be reported as an SAE as per the definition in section 10.1. Flash glucose monitoring values <3.0 mmol/L (level 2) will be verified by capillary blood glucose measurement by the participants. They will be instructed to treat any verified values < 4.0 mmol/L (as per normal standard care) and contact the clinical research team if there are values < 3.0 mmol/L.

8.6. Randomisation

The trial will involve a computer-based randomisation system (Sealed Envelope Ltd, London, UK).

The randomisation schedule will be generated using simple computer-generated random numbers. Participants with T1D will be randomised to a sequence of glibenclamide dose steps (i.e. 0.3mg/day, 0.6mg/day or 3mg/day) at visit 1, after a date for visit 2 has been agreed (see **APPENDIX A: Trial flow chart**).

Both the participants and the clinical research team will be aware of the dose-step order (i.e. there will be no blinding to allocation).

8.7. Blinding and code-breaking

The trial will be open-label, therefore participants will not be blinded to allocation.

8.8. Baseline Assessments

Baseline assessments will be performed and recorded during the screening visit (as described in section 8.3), after informed consent has been collected.

8.9. Study visits

See **APPENDIX A: Trial flow chart** for an illustration visit sequences.

In order to take account of weekends / bank holidays etc (when no visits / procedures will take place), the following adjustments will be made:

- Each “Step” will last between 14-18 days.
- The hypoglycaemic clamps (clamp) can take place between day 14 and day 18
- There will be a minimum of 2 days gap between the clamp and the next visit. Where possible this will be within 14 days but flexibility will be permitted to allow for participant’s pre-standing

commitments (holidays etc.). In case of combined visits (explained in subsequent paragraphs), there will be a minimum of 2 days' gap between the clamp and the commencement of IMP administration. To ensure this is implemented, the research team will instruct the participants on the specific day they should start to take the study medication.

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Prior to the visits involving hyperinsulinaemic hypoglycaemic clamp, participants will be required to fast overnight, and those with T1D will be asked not to take their quick acting (meal-time) insulin on the morning of the tests (but they can take their basal insulin as usual).

Visits 1 (4 hours; Clamp):

1. Observations documented in CRF: resting heart rate and blood pressure
2. Hypo clamp – see **APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp)**.
3. Recording of self-reported hypoglycaemia symptoms – see **APPENDIX H: Assessment of hypoglycaemia symptoms**
4. Date for visit 2 agreed
5. Randomisation
6. Adverse events recording

Unscheduled Visits

Participants may require unscheduled visits for the purpose of IMP resupply, following the detachment of FGM or for any event which in the opinion of the investigator requires participants to be seen by the research team. Future visits will occur as scheduled.

Non-DM group:

Telephone follow-up (15min) – visit 1 +4 to +7 days:

Document if any adverse events occurred following the hypo clamp.

End of study

T1D group:

Visits 2, 4 & 6 (0.5 hour):

1. Observations documented in CRF: resting heart rate and blood pressure
2. FGM sensor provided / attached
3. Check concomitant medications
4. Participants issued with 0.6mg/ml Amglidia (glibenclamide suspension), dosing syringe and schedule. Educated on self-administration and management of hypoglycaemia and given dosing record and the medication information for user leaflet (N.B. Participants will be advised that this leaflet is aimed at paediatric care and will be advised which sections are relevant to them).
5. Dates for hypo clamp agreed.
6. Urine pregnancy test (as required)
7. Adverse events recording

Visits 3, 5, 7 (4 hours; Clamp):

1. Observations documented in CRF: resting heart rate and blood pressure
2. Adverse event recording.
3. Check compliance
4. Hypo clamp – see **APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp)**.

5. Recording of self-reported hypoglycaemia symptoms – see **APPENDIX H: Assessment of hypoglycaemia symptoms**
6. Unused oral glibenclamide suspension returned & document drug accountability.
7. Adverse events recording

In an effort to minimise inconvenience and time-commitment for the participants in the T1D group, and once a date has been agreed for the next Clamp visit, the investigator (at their discretion and where feasible) may agree with the participant to perform study activities related to visits 2, 4 & 6 during or at the end of activities of visits 1, 3 & 5, respectively. This approach has been added following feedback from initial participants in the trial. In the case of combined visits, once issued with IMP, participant will be instructed by the research team on the exact date they should start IMP administration.

Visit 8 (4 hours; clamp-dapa):

1. Observations documented in CRF: resting heart rate and blood pressure
2. Urine pregnancy test (as required)
3. Administer dapagliflozin
4. Hypo clamp – see **APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp)**
   Recording of self-reported hypoglycaemia symptoms – see **APPENDIX H: Assessment of hypoglycaemia symptoms**
5. Blood ketone levels checked at the end of the hypo clamp (CRU capillary blood ketone meter)
6. Adverse event recording

Telephone follow-up (15min) – Day last visit +4 to +7 days:

Document if any adverse events occurred following the discontinuation of the trial medication.
End of study.

8.10. Description of trial intervention(s), comparators and trial procedures (clinical)

a. Attachment of the flash glucose monitoring sensor

- Number of procedures that are additional to standard care: 4
- Average time taken per procedure: 5 minutes
- Details of who will conduct the procedure and where it will take place: a delegated member of the clinical research team at the Churchill Hospital, Oxford, will apply the sensor and/or show the participant how to apply it themselves.

b. Hyperinsulinaemic hypoglycaemic clamp

- Number of procedures that are additional to standard care: 5
- Average time taken per procedure: 4 hours
- Details of who will conduct the procedure and where it will take place: a delegated member of the clinical research team will perform the hypoglycaemic clamp procedure at the Churchill Hospital, Oxford. A maximum of 5 hyperinsulinaemic hypoglycaemic clamps will be performed for participants with T1D, and 1 for participants without diabetes.

Details of the hyperinsulinaemic hypoglycaemic clamp procedure can be found in **APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp).**

c. Administration of the oral glibenclamide suspension

- Number of procedures that are additional to standard care: 84 – 108 (14 – 18 days)
- Average time taken per procedure: 2 minutes
- Details of who will conduct the procedure and where it will take place: participants with T1D will self-administer the oral glibenclamide suspension at home twice per day for a maximum of 54 days. Participants without diabetes will not receive glibenclamide.

d. Administration of dapagliflozin

- Number of procedures that are additional to standard care: 1
- Average time taken per procedure: 2 minutes
- Details of who will conduct the procedure and where it will take place: participants with T1D will be given a single 10mg dapagliflozin tablet at the start of the final hyperinsulinaemic hypoglycaemic clamp. Participants without diabetes will not receive dapagliflozin.

8.11. Sample handling for trial purposes

Blood samples and any required urine samples will be collected by a qualified member of the research team.

Blood samples will be processed and stored in OCDEM (Churchill Hospital, University of Oxford) in -80^{o}C freezers. They will be retained for one year after the end of the study, in order to verify any results. Once all analysis has taken place and the results verified, the samples will be destroyed in accordance with the Human Tissue Authority's (HTA) code of practise.

Screening blood tests (as described in section 8.3) will be performed at the OUH NHS Foundation Trust clinical laboratories. The same will apply for plasma glucose and c-peptide measurements.

Glucagon assays will be performed using the Glucagon ELISA by Mercodia® and Somatosatin will be assayed using the Euro Diagnostica RB306 radioimmunoassay. The glucagon and somatostatin assays will be performed in OCDEM.

All samples kept in the -80C freezers will be plasma, which will be rendered acellular, or serum only, i.e. they will not be classified as "Relevant Material" under the Human Tissue Act 2004. They will be accessible to appropriately delegated members of the research team.

Urine samples will be discarded after testing according to the local procedure.

For each participant, the maximum anticipated volume of blood taken during the trial will be 180ml per hypo clamp.

This will equate to:

- T1D group: 180 x 5 = 900ml (approximately 1 ½ pint of blood, or 2 units)
- Non-DM group: 180ml (approximately 10 tablespoons of blood)

8.12. Early Discontinuation/Withdrawal of Participants

Each participant has the right to withdraw from the trial (i.e. withdraw consent) at any time without having to give a reason for the withdrawal. In case of withdrawal, participants will be asked if they will be willing for their information collected up to that date (including blood samples) to still be used for the purposes of the trial or if they wish for them to be discarded. No further data or blood samples will be collected or any other research procedures carried out on or in relation to the participant, other than a follow-up telephone call to check for AEs if they have received the IMP. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures
- Withdrawal of Consent
- Loss to follow up
- If information relating to the trial medication or any other aspect of the trial arises which may cause harm to the participants.

The reason for withdrawal will be documented in the case report form (an entry of "participant choice" will be made if a participant wishes to withdraw without giving a reason). If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised. The withdrawn participant will be asked to return all unused IMP as per accountability checks.

A drop-out rate of 5% has been taken into account during the sample size calculation (see section 11.2). If there are additional drop-outs, then further participants will be recruited in order to avoid loss of statistical power.

8.13. Definition of End of Trial

The end of trial is the point at which all the data has been entered and queries resolved.

9. TRIAL INTERVENTIONS

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

9.1.1. Glibenclamide suspension

Participants will be issued with an oral suspension of glibenclamide (Amglidia) at a strength of 0.6mg/ml (see section 0). The glibenclamide suspension is being purchased from commercial stock by the OUH NHS Foundation Trust using the authorised product Amglidia, which is made according to good manufacturing process. The OUH NHS Foundation Trust Clinical Trials pharmacy Churchill Hospital will be responsible for dispensing the preparations. The trial medication will be dispensed by the trial pharmacist upon receipt of a duly authorised prescription.

Each participant with T1D will receive 5 vials, to account for the 91ml required (see

Table *2*). Therefore, a total of 100 vials will be required. The package will contain the standard patient information leaflet for Amglidia; while this preparation is used in neonates and children and therefore the instructions describe how to administer it to children, it contains useful information about drawing up the suspension and storage. Participants will be made aware of this and instructed on which sections of the medication leaflet is relevant to the trial. These concepts will be explained to the participants verbally on the first visit when they are issued with the medication and will be remarked on every visit involving the dispensing of the study drugs.

9.1.2. Dapagliflozin

Participants will be issued with dapagliflozin (Forxiga) 10mg tablets. These will be purchased via the Oxford University Hospitals NHS Foundation Trust Pharmacy, and will be dispensed by the trial pharmacist upon receipt of a duly authorised prescription.

9.1.3. Blinding of IMPs

The IMPs will not be blinded.

9.1.4. Storage of IMPs

The OUH NHS Foundation Trust Clinical Trials pharmacy will be responsible for the storage and dispensing of the trial medications. The oral glibenclamide suspension can be stored at room temperature and has a shelf life of 3 years (30 days self-life once opened). It is recommended to keep the bottle in the outer carton in order to protect it from the light. Dapagliflozin is stored at room temperature and has a shelf life of 3 years.

9.1.5. Compliance with Trial Treatment

Participants will return all partly used vials of glibenclamide suspension to the CRU at the end of their participation in the dosing steps of the trial (APPENDIX A: Trial flow chart). Participants will be asked to document when they have taken the trial medication and compliance will be checked prior to each hyperinsulinaemic hypoglycaemic clamp. Significant non-compliance is defined as missing more than three doses of the trial medication during each dose step. If a participant has missed more than three doses, a decision on whether the planned hyper-insulinaemic hypo-glycaemic clamp should proceed and whether the participant should remain in the study will be considered by the CI.

9.1.6. Accountability of Trial Treatment

The oral glibenclamide suspension will be supplied to the OUH NHS Trust pharmacy, and all retrieved (partially used) vials of medication will be returned at the end of the trial to the OUH NHS Trust pharmacy. All movements of trial medication between OUH NHS Trust pharmacy and OCDEM will be documented.

The management of both drugs will be done by the OUH NHS Foundation Trust pharmacy, who will over-label the Amglidia medication with the trial-specific label. The pharmacy will also be responsible for the maintenance of their own accountability logs (as detailed in the SoECAT). Dapagliflozin as a single dose will be dispensed by pharmacy on the day of the final hypoglycaemic challenge, and will be administered by trained CRU staff. No over labelling will be required for this particular medication.

The CI will use a pharmacy-approved trial prescription form.

9.2. Concomitant medications

Throughout the trial period the CI may prescribe any concomitant medications or treatments which is considered necessary to provide supportive care, except for those listed in the exclusion criteria. If medications are required which match the exclusion criteria, then the participant will be withdrawn from the trial. Any medication administered during the trial, other than the trial medication, will be recorded in the CRF.

9.3. Post-trial Treatment

No trial IMP will be provided after the end of the trial.

9.4. Other Treatments (non-IMPS)

There are no non-IMPs in the trial design.

9.5. Other Interventions

There is no additional intervention in the trial design.

10. SAFETY REPORTING

The period of safety reporting is from the receipt of informed consent until the telephone follow-up at the end of the trial for each participant. Telephone follow-up at the end of the trial will also be available for participants without diabetes. Serious Adverse Events (SAE) related to the trial interventions will be followed-up until event resolution.

10.1. Adverse Event Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

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

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

10.2. Assessment results outside of normal parameters as AEs and SAEs

Relevant trial assessments include the capillary blood ketone check at the end of the hypo clamp. A capillary blood ketone result >2.9mmol/L will be recorded as an AE. The clinical significance of an abnormal results will be determined on a case by case basis by the medically qualified investigator and categorised based on the on the following scale: 1 = mild, 2 = moderate, 3 = severe.

10.3. Assessment of Causality

The relationship of each adverse event to the trial medication must be determined by a medically qualified individual according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

10.4. Procedures for Reporting Adverse Events

All AEs occurring during the trial that are observed by the Investigator or reported by the participant, other than level 1 and 2 hypos (see section 8.5), will be recorded on the CRF, whether or not attributed to trial medication.

The following information will be recorded:

- description,
- date of onset and end date,
- severity,
- assessment of relatedness to trial medication,
- other suspect drug or device,
- action taken.

Follow-up information will be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of trial assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

10.5. Reporting Procedures for Serious Adverse Events

All SAEs from the date the IMP is taken until the follow-up phone call must be reported on the SAE reporting form to the central trial team within 24 hours of the medical research team becoming aware of the event (see **APPENDIX D: SAE REPORTING FLOW CHART**). The reporting form should be submitted using the following email address: **legend-d@dtu.ox.ac.uk**. Once the form is received, the central trial team will assess each case and proceed to inform all relevant authorities within the necessary deadline. These actions will be executed following the latest version of the standard operating procedure, SOP:TM002, established and already in use in the DTU. A flow chart illustrating the process of SAE reporting can be found in Appendix D.

10.5.1. Events exempt from immediate reporting as SAEs

Hospitalisation for a pre-existing condition, including elective procedures planned prior to trial entry, which has not worsened, does not constitute a serious adverse event. In addition, standard supportive care for type 1 diabetes does not constitute a SAE, and does not require SAE reporting.

10.5.2. Procedure for immediate reporting of Serious Adverse Events

All SAEs must be reported on the SAE Reporting Form to the DTU Coordinating Centre immediately or within 24 hours of Site Study Team becoming aware of the event being defined as serious.

Site Study Team will complete an SAE Reporting Form for all reportable SAEs.

The SAE report form will be scanned and emailed (email address: **legend-d@dtu.ox.ac.uk**) to the DTU Coordinating Centre immediately *i.e.,* within 24 hours of site study team becoming aware of the event. Site Study Team will provide additional, missing or follow up information in a timely fashion.

DTU will perform an initial check of the report, request any additional information, and ensure it is reviewed by the CI or delegate. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and emailed to the DTU Coordinating Centre. A hard copy of the SAE will be retained in the Investigator Site File at the CRU site while the DTU will retain only electronic copies of the report which will be filed in the electronic Trial Master File.

10.6. Expectedness

For SAEs that require reporting, expectedness of SARs will be determined according to the RSI, section 4.8 of each relevant SmPC. The RSI used (within the SmPC) will be the current Sponsor and MHRA approved version at the time of the event occurrence. For assessment of expectedness in the Development Safety Update Report, see section 10.8 below.

10.7. SUSAR Reporting

All SUSARs will be reported by the sponsor delegate to the relevant Competent Authority and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current trial.

10.8. Development Safety Update Reports

The CI will submit (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK), Ethics Committee, HRA (where required), Host NHS Trust and Sponsor. Separate DSURs will be submitted for each IMP and will include the description of significant actions related to any safety issues that have been taken during the reporting

period which have had an impact on the conduct on the trial. The report will also include any changes to information relating to exclusion criteria, contraindications of the IMP, warnings, serious adverse events, adverse events of special interest and any important findings arising after the data lock point while the DSUR is in preparation.

For assessment of SARs in the DSUR, the RSI that was approved at the start of the safety reporting period will be used. When there has been approved changes to the RSI by substantial amendment during the reporting period, the RSI used for the DSUR will differ to the RSI used to assess expectedness at the time of SAR occurrence for SARs which require expedited reporting.

The CTA approval date will be used as the anniversary date for the submission of the DSURs.

11. STATISTICS

11.1. Statistical Analysis Plan

Primary Objective

Objectives	Outcome Measures	Timepoint(s)
To determine whether treating people with T1D for 14 to 18 days with oral glibenclamide at 0.3mg, 0.6mg or 3mg daily can increase the counter-regulatory glucagon response during induced hypoglycaemia compared to baseline (pre-treatment).	Concentration of plasma glucagon at 40min during the hypoglycaemic phase of the hyper-insulinaemic hypo-glycaemic clamp, at baseline and at each dose step of glibenclamide (T1D group only).	At 40min during the hypoglycaemic phase of the hyper-insulinaemic hypo-glycaemic clamp.

T1D group only: 3 separate paired t-tests will be used to compare 0mg vs 0.3mg, 0mg vs 0.6mg and 0mg vs 3mg. We calculated the sample size (see below) based on this analysis.

Secondary Objectives (A):

These analyses are being conducted on an exploratory basis, and no formal sample size computation has been conducted.

Objectives	Outcome Measures	Timepoint(s)
1. To compare the glucagon response during induced hypoglycaemia in people with T1D prior to glibenclamide treatment, and at each dose step, to that of participants without diabetes (non-DM group).	Concentration of plasma glucagon during the hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp (T1D and non-DM group)	Glucagon levels during the hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp (0, 15, 30 and 40min).

A mixed-effects model (treatment x timepoint) will be used to compare glucagon values at each timepoint (0, 15, 30 and 40min) during the hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp at baseline (T1D and non-DM group) and at each dose step (T1D group only).

Post-hoc analysis will be performed which will allow pairwise comparisons between the glucagon values obtained at each timepoint (T1D and non-DM group, see **Table *5***).

Groups for comparison:
T1D 0 vs nonDM 0
T1D 0 vs T1D 0.3
T1D 0 vs T1D 0.6
T1D 0 vs T1D 3
T1D 0.3 vs nonDM 0
T1D 0.3 vs T1D 0.6
T1D 0.3 vs T1D 3
T1D 0.6 vs nonDM 0
T1D 0.6 vs T1D 3
T1D 3 vs nonDM 0

Table 5 Groups for comparison

Objectives	Outcome Measures	Timepoint(s)
2. To compare the increase in plasma glucagon from euglycaemia to hypoglycaemia in T1D participants prior to treatment, and at each dose step of glibenclamide, to that of participants without diabetes (non-DM group).	Proportion of increase in plasma glucagon at each timepoint (i.e. glucagon concentration at 0min during the hypoglycaemic phase divided by the glucagon concentration at 0min during the euglycaemic phase) (T1D and non-DM group).	Glucagon levels at each timepoint (0, 15, 30 and 40min) during the euglycaemic and hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp.

Plasma glucagon values at each timepoint (0, 15, 30 and 40min) during the hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp will be divided by the value at the corresponding timepoint during the euglycaemic phase.

A mixed-effects model (treatment x timepoint) will be used to compare the proportion increase in glucagon (i.e. glucagon during the hypoglycaemic phase vs glucagon during the euglycaemic phase) at each timepoint (0, 15, 30 and 40min) at baseline (T1D and non-DM group) and at each dose step (T1D group only).

Post-hoc analysis will be performed which will allow pairwise comparisons between the glucagon values obtained at each timepoint (see **Table *5***).

Objectives	Outcome Measures	Timepoint(s)
3. To compare the percentage of time spent in hypoglycaemia at each dose step of glibenclamdie, compared to baseline (T1D group only)	Percentage of time spent in hypoglycaemia (<4.0mmol/L) from Freestyle Libre 2 data (T1D group only)	Freestyle libre data collected for the 7 days prior to each dose step until the start of the hyper-insulinaemic hypo-glycaemic clamp

Three separate paired t-tests will be used to compare 0mg vs 0.3mg, 0mg vs 0.6mg and 0mg vs 3mg. If the assumptions of t-test are not satisfied, then the percentages will be treated as proportion and a 2 proportions test will be used.

Secondary Objectives (B):

Objectives	Outcome Measures	Timepoint(s)
1. To compare the change in glucagon from euglycaemia to hypoglycaemia in T1D participants prior to and following administration of a single 10 mg dose of oral dapagliflozin, to that of participants without diabetes (non-DM group).	Proportion of change in plasma glucagon at each timepoint (i.e. glucagon concentration at 0min during the hypoglycaemic phase divided by the glucagon concentration at 0min during the euglycaemic phase) (T1D and non-DM group).	Glucagon levels at each timepoint (0, 15, 30 and 40min) during the euglycaemic and hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp.

Glucagon values at each timepoint (0, 15, 30 and 40min) during the hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp will be divided by the value at the corresponding timepoint during the euglycaemic phase.

A mixed-effects model (treatment x timepoint) will be used to compare the proportion change in glucagon (i.e. glucagon during the hypoglycaemic phase vs glucagon during the euglycaemic phase) after taking a single dose of dapagliflozin (T1D group only) versus baseline/no treatment (T1D and non-DM group).

Post-hoc analysis will be performed which will allow pairwise comparisons between the glucagon values obtained at each timepoint.

Objectives	Outcome Measures	Timepoint(s)
2. To compare the change in plasma somatostatin from euglycaemia to hypoglycaemia in T1D participants prior to and following administration of a	Proportion of change in plasma somatostatin at each timepoint (i.e. somatostatin concentration at 0min during the hypoglycaemic phase divided by the somatostatin concentration at	Somatostatin levels at each timepoint (0, 15, 30 and 40min) during the euglycaemic and hypoglycaemic phase of the

single dose of dapagliflozin 10mg, to that of participants without diabetes (non-DM group).	0min during the euglycaemic phase) (T1D and non-DM group).	hyperinsulinaemic hypoglycaemic clamp.

Somatostatin values at each timepoint (0, 15, 30 and 40min) during the hypoglycaemic phase of the hyperinsulinaemic hypoglycaemic clamp will be divided by the value at the corresponding timepoint during the euglycaemic phase.

A mixed-effects model (treatment x timepoint) will be used to compare the proportion change in somatostatin (i.e. somatostatin during the hypoglycaemic phase vs somatostatin during the euglycaemic phase) after taking a single dose of dapagliflozin (T1D group only) versus baseline/no treatment (T1D and non-DM group).

Post-hoc analysis will be performed which will allow pairwise comparisons between the somatostatin values obtained at each timepoint.

Objectives	Outcome Measures	Timepoint(s)
3. To characterise the development of hypoglycaemic symptoms during induced hypoglycaemia at baseline, and during each dose of glibenclamide and dapagliflozin.	3. Self-reported hypoglycaemia symptoms during the hyperinsulinaemic hypoglycaemic clamp.	3. Blood glucose value at onset of self-reported hypoglycaemia symptoms during the hyperinsulinaemic hypoglycaemic clamp.

A multilevel modelling approach (treatment x group) will be used to compare the onset of self-reported hypoglycaemic symptoms (see **APPENDIX H: Assessment of hypoglycaemia symptoms**) during the hyperinsulinaemic hypoglycaemic clamp between the group with and without diabetes.

Objectives	Outcome Measures	Timepoint(s)
4. To measure any residual C-peptide participants with T1D only.	Fasting C-peptide prior to the baseline hyperinsulinaemic hypoglycaemic clamp (T1D group only)	Plasma C-peptide level at the start the hyperinsulinaemic hypoglycaemic clamp

Descriptive statistics only will be used.

Objectives	Outcome Measures	Timepoint(s)

5. To measure the frequency of adverse events in participants treated with Glibenclamide and Dapagliflozin.	Number of Adverse events reported	At each study visit following the receipt of informed consent

Descriptive statistics only will be used.

11.2. Sample Size Determination

There is currently no data on glucagon levels from human trials using low-dose sulfonylureas in patients with type 1 diabetes. The sample size calculations are based on the data generated from the LEGEND-A trial (13) (NCT02830048 - completed) and interim analysis from the DEPTH trial (NCT03537131 – suspended due to the COVID-19 pandemic).

The LEGEND-A trial suggested glibenclamide (at a dose of 0.3mg/day) could change glucagon levels in participants with type 2 diabetes by 30% (magnitude of effect), while the DEPTH trial suggested that participants with type 1 diabetes who experienced exercise-induced hypoglycaemia had mean plasma glucagon values of 5.54pmol/L (SD 2.14pmol/L) at the time of hypoglycaemia (defined as <3.3mmol/L).

For the non-diabetic control group, data from a clinical study in healthy volunteers (20), which used similar methodology and a glucagon assay comparable to the one we will use in our trial, demonstrated that the mean glucagon values during the hypoglycaemia plateau of 23.3 pmol/L (SD 5.7pmol/L).

These figures indicate that **20 participants with T1D, and 10 participants without diabetes** (including 5% dropout) would give the LEGEND-D trial 80% power to detect a 30% increase in glucagon levels (the primary endpoint) during induced hypoglycaemia (hyperinsulinaemic-hypoglycaemic clamp).

11.3. Analysis populations

Data from all randomised participants will be included in the analysis.

11.4. Decision points

There is no planned interim analysis

11.5. Stopping rules

The trial will be terminated if there are serious adverse events that occur in three or more (>10%) of participants.

11.6. The Level of Statistical Significance

All statistical significance will be assessed using an alpha value of 0.05 (95% confidence interval).

11.7. Procedure for Accounting for Missing, Unused, and Spurious Data.

Any missing data regarding blood results will be checked with the corresponding labs (Oxford University Hospitals NHS Foundation Trust or University of Oxford). The same will apply to spurious data. If two or more data points for the primary outcome (glucagon concentration) are missing from the same participant (excluding the baseline hypo clamp, which is essential), then the participant will be withdrawn from the trial.

11.8. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any deviation from the original statistical plan will be described and justified in the final report of the trial.

11.9. Health Economics Analysis

No health economics analysis has been undertaken.

12. DATA MANAGEMENT

The plan for the data management of the trial are outlined below. There will also be a separate Data Management document in use for the trial.

12.1. Source Data

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

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study ID number, not by name.

Source data and the documents or electronic records in which they can be found, will be detailed in the Source Data Location list, which will be held in a server located in an access-restricted area at OCDEM, the Oxford University Hospitals.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the trial to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

All trial data will be entered onto a secure web-based data capture platform (OpenClinica). The clinical trial database will be built and managed by the DTU in line with DTU SOPs, in a validated clinical data management system, OpenClinica, that is compliant with ICH GCP and FDA 21 CFR Part 11. It is hosted on a secure, access-restricted server within the UK, within a virtual private cloud.

An electronic Trial Master File (eTMF) will also be adopted by the DTU for data keeping and filing. The eTMF is a web-based software system developed and approved in 2007 for the management of Trials and sharing of documents internally within DTU. The server is located in an access-restricted area in Oxford, UK and it is adopted to manage essential documents as defined in ICH GCP E6.

If paper copies of the data collection worksheets are used during any procedures (such as hypo clamp) prior to transfer to the OpenClinica system, then the paper copies will be considered the source data. The participants will be identified by a unique study ID in any database or worksheet. The name and any other identifying details will not be included in the trial data electronic file.

The pseudo-anonymised data (i.e. with study ID only) generated from this trial will be deposited in the Oxford Research Archive (http://ora.ox.ac.uk/). At the end of the retention period (currently 5 years), the data will be deleted from the archive.

All laboratory results will be reviewed and the reports signed by a qualified member of the clinical research team, who will record in the CRF whether it is normal, abnormal but not clinically significant, or abnormal and clinically significant. In the latter case, the eligibility of the participant will be reviewed by the study physician and they will be referred to their GP if required.

The Freestyle Libre data will be collected, stored and retrieved in accordance with the privacy policy of Freestyle Libre web site controlled by Abbott Laboratories. The cloud-based diabetes management system has been assessed and approved by the University of Oxford Information Security Team.

13. QUALITY ASSURANCE PROCEDURES

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

13.1. Risk assessment

A risk assessment and monitoring plan will be prepared before the trial opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

13.2. Monitoring

Regular monitoring will be performed according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13.3. Trial committees

Trial Management Group (TMG)

The TMG will meet approximately monthly during the trial to oversee day-to-day operations. A TMG charter will detail the committee's responsibilities and membership which will include the CI (or

delegate) the DTU Trial Manger, Senior Trial Manager, and representatives of all areas of the trial being managed by the DTU.

Trial Steering Committee (TSC)
Trial Steering Committee (TSC) will consist of independent academic members along with the Study Statistician, Chief Investigator, co-applicant and collaborator. The LEGEND-D Trial Manager and representatives of all areas of the trial being managed by the DTU may also attend these meetings but will have no voting rights. A TSC charter will detail the committee's responsibilities and membership.

Due to the low-risk nature and short timeframe of this trial a Data Monitoring Committee (DMC) is not required for this trial.

14. PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

15. SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

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

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

16.5. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration. Participants who wish to know the outcome of the trial will be addressed to the website of the study or directed to the journal paper where the results are published.

16.6. Participant Confidentiality

The trial will comply with the UK General Data Protection Regulation (UK-GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of identifiable data of participants will be minimised by making use of a unique participant study number only on all trial documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The trial staff will safeguard the privacy of participants' personal data.

The blood samples taken at the screening visit for baseline biochemical data will be analysed by the Oxford University Hospitals NHS Trust central clinical laboratory. In line with the laboratory's sample processing procedures, the samples will be identified by the participant's name, date of birth and NHS number. This information will only be seen by the CRU and laboratory personnel. The results of these samples will be accessible to the participant's GP as per normal practice, nevertheless, any significantly abnormal results will be reported to their GP. Participant names or other identifying details will NOT be included on the study CRFs. Blood samples analysed within the University of Oxford will be labelled with the Study ID only.

16.7. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

Participants with T1D will be reimbursed at the level of £625 upon completion of the trial, to account for loss of earnings and inconvenience. Participants who do not fully complete the trial will be reimbursed pro rata based on the number of completed hypo clamps. The participants without diabetes will therefore be reimbursed at a level of £125 upon completion of the trial.

17. FINANCE AND INSURANCE

17.1. Funding

This trial is being funded by The Leona M. and Harry B. Helmsley Charitable Trust.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

17.3. Contractual arrangements

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

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the trial. Authors will acknowledge that the trial was funded by The Leona M. and Harry B. Helmsley Charitable Trust. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

20. ARCHIVING

The data generated from this study will be deposited in the Oxford Research Archive (http://ora.ox.ac.uk/). At the end of the retention period (currently 5 years), the data will be deleted from the archive.

All research documents with participants' personal information, such as consent forms, will be stored at the research site and will be archived in line with the DTU SOPs.

The eTMF and other electronic files will be archived by the DTU per current regulatory requirements in accordance with DTU SOPs and for a maximum of 25 years. The anonymised trial database will be retained indefinitely. Investigator Site Files and source documents will be retained and archived by the site

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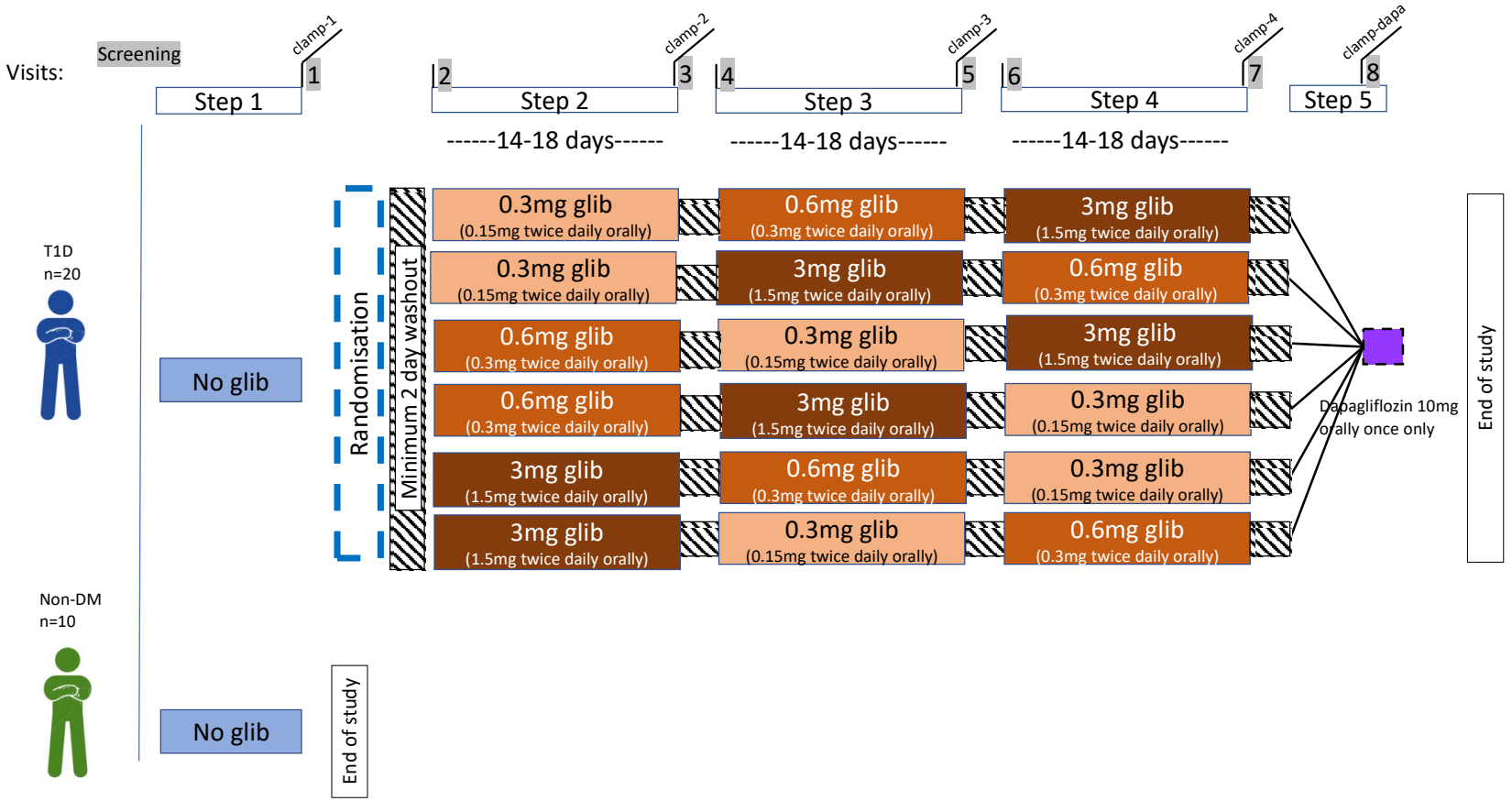
22. APPENDIX A: Trial flow chart



After an initial screening visit (around 1 hour), all participants will undergo a hypo challenge ("clamp") visit which will last around 4 hours. No extra medications will be used during this first Step. Participants with T1D only will then be randomised to a sequence of 0.3mg, 0.6mg and 3mg glibenclamide (glib), which will be provided at the start of each subsequent Step (visits 2, 4 and 6 – each will last around 30min). After taking the medication for 14-18 days, they will undergo another hypo challenge (visits 3, 5 and 7 – again around 4 hours). The final hypo challenge will occur on visit 8, when participants will be given a single tablet of 10mg dapagliflozin.

It is of note that some study visits, where feasible, can be combined at the discretion of the investigator and in agreement with the participant (see section 8.9).

23. APPENDIX B: Schedule of Procedures (T1D group)

Procedures		**Study visits**								
	Screening visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Follow-up**
	Running total of days using glibenclamide (showing maximum days, actual days may differ).									
		0	**0**	**18**	**18**	**36**	**36**	**54**		
	Running total of days using dapagliflozin									
									1	
	Screening									
Informed consent	x									
Demographics	x									
Observations: height & weight (screening only), blood pressure, heart rate	x	x	x	x	x	x	x	x	x	
Medical history	x									
Concomitant medications	x		x		x		x		x	
Laboratory tests:										
Blood: HbA1c, FBC*, U&E, LFTs.	x									
Urine pregnancy test (if applicable)	x	x**	x		x		x		x	
Hypo clamp (see 28APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp) for blood tests)		x		x		x		x	x	
Eligibility assessment	x									
Dispensing of study medication (Glibenclamide)			x		x		x			
Dispensing of study medication (Dapagliflozin)									x	
Ketone check at end of hypo clamp (CRU capillary blood ketone meter)									x	
Compliance check				x		x		x		
FGM provided	x		x		x		x			
Adverse event assessments				x		x		x	x	

Suspension returned & drug accountability				x		x		x		
Telephone follow-up										x

**Haemoglobin, white cell count, haematocrit and platelets*

***Required only if >28 days since initial screening visit*

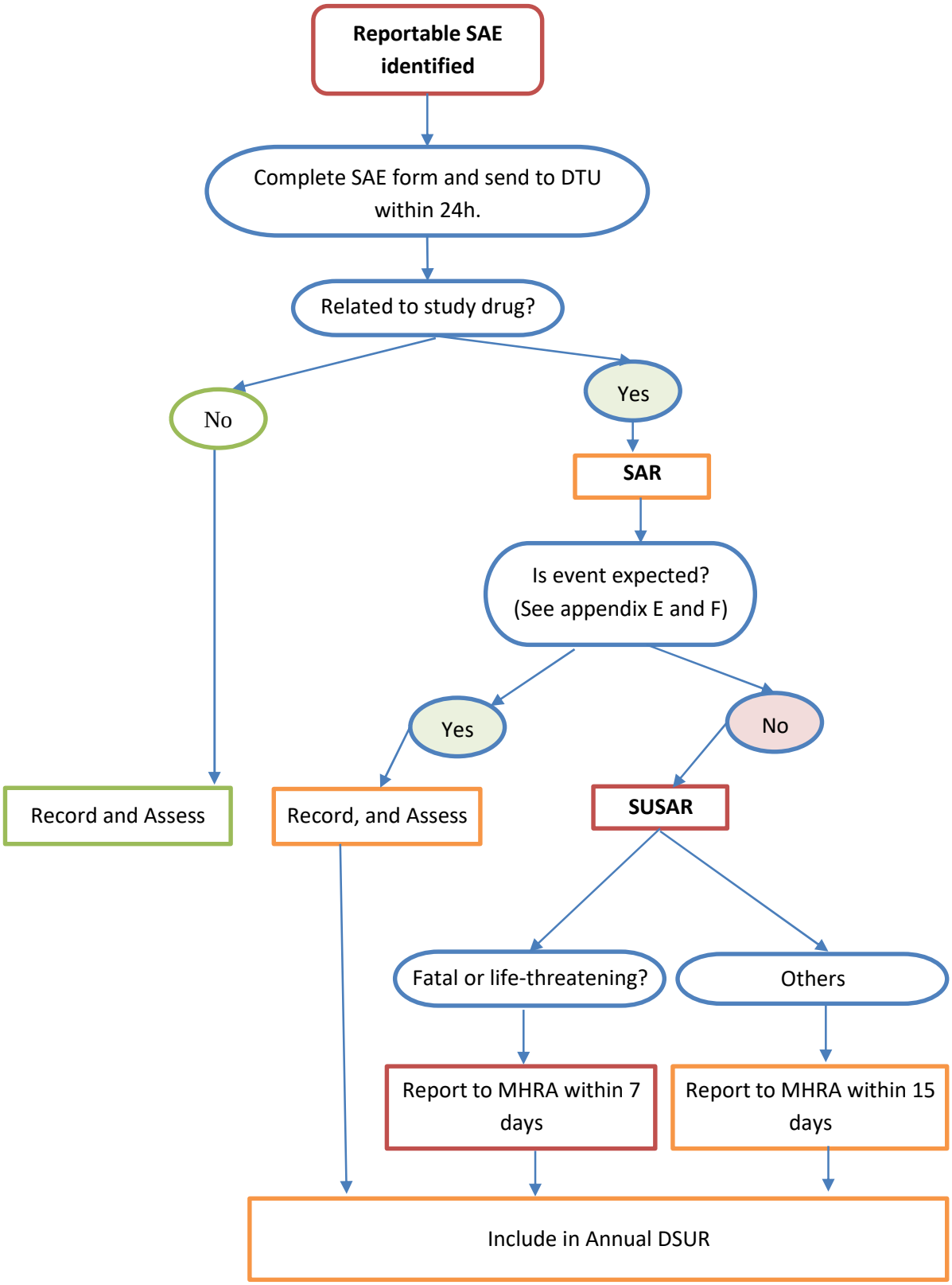
24. APPENDIX C: Schedule of Procedures (non-DM group)

Procedures		**Study visits**	
	Screening visit	**1**	**Follow-up**
	Screening		
Informed consent	x		
Demographics	x		
Observations: weight (visit 1), blood pressure, heart rate	x	x	
Medical history	x		
Concomitant medications	x		
Laboratory tests:	x		
Blood: HbA1c, FBC*, U&E, LFTs.	x		
Urine pregnancy test (if applicable)	x	x**	
Hypo clamp (see 28APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp) for blood tests)		x	
Eligibility assessment	x		
Telephone follow-up			x

**Haemoglobin, white cell count, haematocrit and platelets*

***Required only if >28 days since initial screening visit*

25. APPENDIX D: SAE REPORTING FLOW CHART



26. APPENDIX E: Expected adverse reactions with Amglidia

The SmPC for Amglidia will be used to check the expectedness of any adverse reactions.

27. APPENDIX F: Expected adverse reactions with dapagliflozin

The SmPC for Forxiga will be used to check the expectedness of any adverse reactions.

28. APPENDIX G: Stepped hyperinsulinaemic hypoglycaemic clamp (hypo clamp)

This will be performed by a delegated member of the clinical research team, and will be take place at the Churchill Hospital, Oxford. It will take an average of 4 hours per procedure. Participants will be fasted overnight, and those with T1D will be asked not to take their quick acting (meal-time) insulin on the morning of the tests (but they can take their basal insulin as usual).

The standard operating procedure for conducting a stepped hyperinsulinaemic hypoglycaemic clamp will be followed, as previously described (21). As a guide, a standard gauge cannula will be placed into an antecubital fossa vein for infusion of insulin and glucose, and an additional cannula will be inserted into a hand vein for blood sample collection. Participants will receive a primed, continuous infusion of insulin, with 20% glucose infused at a variable rate to achieve two steps of glycaemic plateau.

Timepoint are used for illustration purposes. Each plateau (euglycaemia and hypoglycaemia) will be maintained for 40 min (as indicated in **Figure *2***), however the time required to reach the plateau may vary between participants. As a guide, we will aim to achieve the target blood glucose level (6.0mmol/L for euglycaemic phase and 2.5mmol/L for hypoglycaemic phase) within 40 min, but this may take longer; if it is achieved earlier then the plateau phase will begin at that point.

After the hypoglycaemic phase, the insulin infusion will be discontinued and glucose will be infused as required so that all participants enter into a recovery phase with plasma glucose >4.0mmol/L.

0 – 40min: set-up, aim to reach 6.0mmol/L by 40min
40 – 80min: **euglycaemic plateau phase**, maintain plateau at 6.0mmol/L (+/-0.5mmol/L)
80 – 120min: drop phase, aim to reach 2.5mmol/L by 120min
120 – 160min: **hypoglycaemic plateau phase**, maintain plateau at 2.5mmol/L (+/-0.5mmol/L)
160 – 200min: recovery to euglycaemia

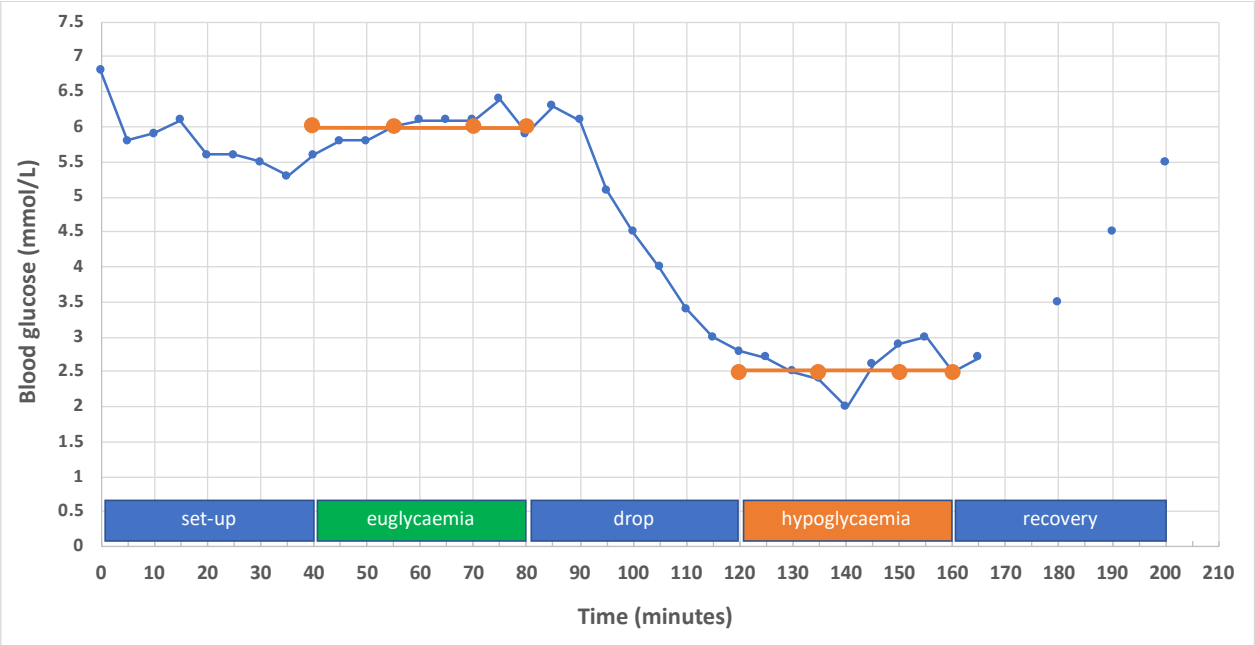


Figure 2 Illustration of hypoglycaemic clamp. Blue trace: simulated data, orange trace: targets.

Blood samples: approximately 10ml / time-point = 100ml per clamp (around 6 tablespoons) + approximately 2ml per Hemocue sample (estimated 30-40 samples throughout the clamp). Total amount of blood per clamp = approximately 180ml (around 10 tablespoons).

	Timepoints:														
Approximate time from start	0	…	40	55		70	80	…	120	135		150	160	…	200
Phase	set-up		euglycaemia					drop	hypoglycaemia					recovery	
Time from reaching target	**(start)**		**0**	**15**		**30**	**40**		**0**	**15**		**30**	**40**		**(end)**
Blood samples:															
Glucose	x		x	x		x	x		x	x		x	x		x
Glucagon	x		x	x		x	x		x	x		x	x		x
C-peptide (T1D clamp-1 only)	x														
Somatostatin (clamp-1 and clamp-dapa only)	x		x	x		x	x		x	x		x	x		
POC Hemocue	every 5 minutes throughout clamp														

29. APPENDIX H: Assessment of hypoglycaemia symptoms

For participants with T1D, the Gold score (22) will be included as part of the medical history assessment during the screening visit. The wording will be as follows:

"On a scale of 1 to 7, 1 being you absolutely always know when your blood sugar starts going below 4mmol/L and 7 being you absolutely never know when your blood sugar starts going below 4mmol/L, where would you put yourself?".

For the self-reported hypoglycaemia symptoms, a list of symptoms will be presented to the participants, grouped as Autonomic, Neuroglycopenic or Non-specific (23):

Autonomic	**Neuroglycopenic**	**Non-specific**
Sweating	Inability to concentrate	Tingling around the mouth
Trembling	Confusion	Dry mouth
Flushing	Tiredness	Blurred vision
Anxiety	Feeling tearful	Headache
Pounding heart	Difficulty in speaking	Nausea
Hunger	Odd behaviour	
	Incoordination	
	Weakness	
	Drowsiness	

Participants will be asked to mark when they start experiencing one of the symptoms, and the corresponding blood glucose value (as measured by Hemocue) will be documented.

We will give each participant details of the management of hypoglycaemia (as per normal clinical care using the TREND Diabetes patient leaflet: https://trenddiabetes.online/portfolio/diabetes-why-do-i-sometimes-feel-shaky-dizzy-and-sweaty-hypoglygaemia-explained/), but these are also summarised below:

> *Symptoms of hypoglycaemia vary from person to person but can include: feeling shaky, sweating, hunger, tiredness, blurred vision, lack of concentration, headaches, irritability and going pale. These symptoms can be managed by immediately having a glass of fruit juice (or a sugary drink), then having something to eat such as a banana, a slice of toast or your normal meal.*

30. APPENDIX I: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2.0	09/07/2024	Nkemjika Abiakam	Extension of study recruitment and end date
2	2.0	09/07/2024	Nkemjika Abiakam	Amendment to reflect the possibility to combine consecutive study visits
3	3.0	26/09/2024	Nkemjika Abiakam	Extension of study recruitment and end date
4	4.0	19/02/2025	Jennifer Lawson	Clarification of timepoints required for Secondary Objective A3.

List details of all protocol amendments here whenever a new version of the protocol is produced.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee, HRA (where required) or MHRA.