Enhanced Epidermal Antigen Specific Immunotherapy
Against Type 1 Diabetes

Protocol Title:

Enhanced Epidermal Antigen Specific Immunotherapy trial -1 (EE-ASI-1): A Phase 1a study of gold nanoparticles administered intradermally by microneedles to deliver immunotherapy with a proinsulin derived peptide in Type 1 diabetes.

Short title:

EE-ASI – 1.

MIDATECH PHARMA

NanoPASS TECHNOLOGIES LTD.

LINKÖPINGS UNIVERSITET

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1 LIST OF ABBREVIATONS

ADDQoL: Audit of diabetes-dependent quality of life
AE: Adverse Event
AGA: Antigen-induced activation and gene array assay
AICD: Activation-induced cell death
APC: Antigen presenting cell
AR: Adverse reaction
ASI: Antigen-specific immunotherapy
CI: Chief Investigator
CSRI: Client Service Receipt Inventory
CRF: Case Report Form
CU: Cardiff University
DSMB: Data Safety Monitoring Board
DTSQc: Diabetes treatment satisfaction questionnaire, change version
DTSQs: Diabetes treatment satisfaction questionnaire
ELISPOT: Cytokine enzyme-linked immunospot assay
GAD-65: Glutamic acid decarboxylase-65
GCP: Good Clinical Practice
GNP: Gold nanoparticles
HbA1c: Glycated haemoglobin
HFS: Hypoglycaemia fear survey
HLA class II: Human Leukocyte Antigen class II molecule
IB: Investigator's brochure
i.d.: Intradermal
IA-2: Insulinoma-associated antigen-2
IFN-γ: Interferon-γ
IL: Interleukin
LN: Lymph node
IMP: Investigational Medicinal Product
IMPD: Investigational Medicinal Product Dossier
MAGE: Mean amplitude of glucose excursion
MHRA: Medicines & Healthcare Products Regulatory Agency
MMTT: Mixed meal tolerance test
MPA: Medical Products Agency
NIHR: National Institute for Health Research
NISCHR CRC: National Institute for Social Care & Health Research Clinical Research Centre
PBMC: peripheral blood mononuclear cells
PI: Principal Investigator
PIS: Patient information sheet
PIT: Peptide immunotherapy
REC: Research Ethics Committee
RERB: Regional Ethical Review Board

RIES: Research, Innovation and Enterprise Services
SAE: Severe Adverse Event
SAR: Severe Adverse Reaction
s.c.: Subcutaneous
SPC: Summary of product characteristics
STU: Swansea Trials Unit
SUSAR: Suspected Unexpected Serious Adverse Reaction
TGF-β: transforming growth factor-β
Th1, Th2: T helper 1, T helper 2
TMG: Trial Management Group
TNF-α: tumour necrosis factor-α
TSC: Trial Steering Committee
Treg: Regulatory T lymphocytes
UAR: Unexpected adverse reaction
UCPCR: Urine C-peptide creatinine ratio
ZnT8: Zinc transporter 8

2 TRIAL MANAGEMENT

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3 BACKGROUND INFORMATION

3.1 Overview of the intervention in this protocol

This protocol describes the use of the investigational medicinal product (IMP) C19-A3 GNP in a first-in-man study of safety of administration in patients with Type 1 diabetes. C19-A3 GNP comprises gold nanoparticles (GNPs) of size less 5nm covalently coupled to an 18-amino acid human peptide the sequence of which is identical to the residues from position 19 in the C-peptide of proinsulin through to position 3 on the A-chain of the same molecule (GSLQPLALEGSLQKRGIV or using 3-letter designation: H-Gly-Ser-Leu-Gln-Pro-Leu-Ala-Leu-Glu-Gly-Ser-Leu-Gln-Lys-Arg-Gly-Ile-Val-OH). The peptide is synthesised with a linker to facilitate binding to the GNPs: 3-Mercaptopropionyl-Gly-Ser-Leu-Gln-Pro-Leu-Ala-Leu-Glu-Gly-Ser-Leu-Gln-Lys-Arg-Gly-Ile-Val-OH) 2 acetate salt (Disulfide bond). GNP C 19-A3 will be delivered via CE marked microneedles made by NanoPass.

3.2 Background to approach to immunotherapy explored in the protocol.

The challenge of glycaemic control with insulin in Type 1 diabetes and the need for immunotherapy.

Type 1 diabetes is an autoimmune disease in which the insulin-secreting β-cells of the endocrine pancreas are destroyed to a sufficient extent that the patient relies on injection of exogenous insulin to adequately control blood glucose levels for the remainder of his/her life.

Most patients with Type 1 diabetes are unable to achieve levels of long-term metabolic control sufficient to avoid long-term complications, including loss of sight, renal failure and premature cardiovascular disease. Even with the most sophisticated insulin regimes, such as sensor augmented pump therapy, used in the setting of clinical trials, less than 30% of patients achieve an HbA1c < 7.0% (53 mmol/mmol)l(1, 2) and in the "real world" rates are typically less than 16%(3). The personal effort required to achieve these glycaemic targets is so high (4) that compliance is poor especially amongst young people (5). Although advances in insulin delivery systems continue to be made, there is little indication that they will be able to match the level of metabolic control and flexibility that can be achieved by functioning pancreatic beta cells(6). This is well illustrated by data from islet transplantation, in which restoration of even a small amount of beta cell function, results in complete correction of hypoglycaemia and excellent levels of HbA1c (< 7.0%, 53mmol/mol)(7). At diagnosis of Type 1 diabetes, 90% of adults and 80% of children have sufficient residual beta cell function (peak insulin c-peptide > 200pmol/L)(8) to achieve these goals. Prospective studies show that this level of beta cell function, even if it declines or is lost over the next few years, is nevertheless associated with a 50% reduction in hypoglycaemia and a 40% reduction in the development of long-term complications over 9 years(9). Furthermore, evidence suggests that the "legacy" of such a period of good glycaemic control will result in a 50% relative reduction in retinopathy even 30 years later(10). Clearly, if this level of beta cell function were maintained for longer, the health gains would be even greater. Hence slowing the autoimmune process and thereby preserving the residual beta cell function present at diagnosis of type 1 diabetes, even for a few years, has the potential to deliver very major quality of life improvements (hypoglycaemia reduction) and long-term health gains (complications reduction). Importantly, since metabolic control delivered by functioning beta cells does not require high levels of compliance from patients, it is likely that these benefits could be realised in almost all individuals, at lower personal cost and inconvenience to patients than insulin delivery systems.

An effective immunological therapy which could preserve beta cell function by preventing immune-mediated damage in type 1 diabetes would therefore represent an important advance in achieving improved glycaemia control. Such a therapy might also have application in individuals with long-standing diabetes by protecting new or regenerating beta cells from destruction by the immune system. Furthermore, such a therapeutic could also be used in "pre-diabetes" to prevent the development of clinical diabetes in individuals identified by autoantibody screening to be at high risk(11).

Immunosuppression. Studies in the 1980s demonstrated that immunosuppressive treatment with cyclosporine can slow the loss of endogenous insulin production in newly diagnosed patients (including children) with type 1 diabetes but the toxicity was unacceptable (12-14). Since that time, two approaches have been taken to reduce toxicity: "smarter" immunosuppression and targeted immunoregulation. Of the immunosuppressive approaches, phase 2 efficacy has recently been reported for B- cell depletion with anti-CD20 (rituximab) monoclonal antibody therapy(15), T cell modulation with anti-CD3 (otelixizumab, teplizumab)(16, 17), blockade of co-stimulation using CTLA-4 Ig (abatacept)(18) , LFA-2

(alefacept)(19), as well as antityhymocyte globulin and G-CSF(20). Although phase 2 studies with limited follow-up reported that these treatments were well tolerated, they are expensive, are likely to have to be given repeatedly and have the potential long-term risks associated with immunosuppression including opportunistic infection and increased cancer incidence(21). It should be noted that not all immunosuppressive regimes have met with success: recently negative results have been reported with the combination of mycophenolate mofetil and anti-CD25 (daclizumab) (22). Induction of remission has also been reported for the use of autologous stem cell transplantation (23), but controlled studies using the conditioning agents alone and replication of this approach in different centres are awaited.

Immunoregulation. In recent years we have demonstrated the presence of T cells in humans specific for islet-derived peptides that have a regulatory (protective) phenotype (Treg). These cells secrete IL-10 or a combination of IL-10 and interferon gamma, are more prevalent in healthy controls and first-degree relatives than in patients with type 1 diabetes (24, 25), and have been shown to mediate immunoregulation (26). Hence expansion of these T cells or subsets with similar function would be expected to result in a slowing of the autoimmune process and preservation of beta cells. Amongst approaches to achieve such an immunoregulatory outcome, beta cell preservation with non-depleting anti-CD3 monoclonal antibody therapy has been extensively studied. The exact mechanism of action is uncertain(27) but the induction of CD8 regulatory cells has been postulated(28). Despite very promising results from initial phase II studies and evidence of efficacy over 4 years (16, 29, 30), more recent results attempting to lower the dose to reduce toxicity or use alternative regimes have been less encouraging (31, 32). In parallel with these studies, others have taken a cellular approach to trying to increase levels of Tregs that might slow the autoimmune process. Bluestone and colleagues are currently reinfusing purified and expanded CD4+CD127lo/-CD25+ polyclonal Tregs into patients with newly-diagnosed type 1 diabetes (NCT01210664). Trucco and colleagues are exploring the administration of autologous dendritic cells stabilised in a tolerogenic state by antisense oligonucleotides in patients with type 1 diabetes(33). However, these studies are at an early stage, and even if they proved effective would require very substantial investment in facilities for cellular manipulation to enable patients to be treated in this way.

Antigen-specific immunotherapy (ASI). In animal models, there is extensive evidence that antigen-specific immunotherapy (ASI) is possible (34, 35). In this approach, beta cell antigens, or peptides comprising T cell epitopes derived from them, are administered to animals either intranasally, subcutaneously or intraperitoneally, with the aim of inducing a regulatory response targeted to the beta cells, without immunosuppression(35-40). Evidence for the induction of antigen specific Tregs has been obtained in several experimental systems (35, 41-44). In the majority of cases one, or a short series of treatments, was sufficient to protect the animal from disease for the rest of its life and it was also possible to reverse established disease at the time of disease onset (35, 37). Advantages of ASI over immunosuppressive

treatments or non-antigen targeted immunoregulatory approaches include lower long-term risk of infection and malignancy, lower systemic toxicity and lower cost; hence several groups have attempted to develop this approach for use in humans (reviewed in(44)). Many different approaches have been used including whole antigen alone or in adjuvant(45) (46) (47-49), given by the mucosal route (orally or nasally), (50, 51) (52, 53) , DNA vaccination (54, 55) (http://www.bayhilltx.com)(55), the use of peptide epitopes or altered peptide ligands (56) (57), modified peptide epitopes that bind directly to HLA molecules without modification ("apitopes", www.apitope.com*)* or preformed MHC peptide complexes(58), although the latter two approaches have not yet been applied to human type 1 diabetes. These approaches have generally proved safe and very well tolerated, but sufficient efficacy to reliably reverse established disease in humans has proved difficult to generate(48, 51, 52, 59). Analysis of the antigen specific trials to date, suggests that this is less likely to be due to the particular antigen used, the dose or the route of administration, and more related to the context in which the antigen is presented to the immune system.

Enhancing the efficacy of antigen specific immunotherapy – recruiting the potential of tolerogenic dendritic cells. Studies in animal models suggest that to achieve and maintain remission in established autoimmune disease via antigen-specific therapy, it is important to efficiently generate regulatory T cells specific for the antigen target(35). In recent years, much work has been directed to determining the optimal conditions for the generation of regulatory T cells (60). It has become apparent that the status of the antigen presenting cells (APCs) that activate T cells is pivotal in this process. If the APCs express increased levels of MHC class II and costimulatory molecules (e.g. CD80, CD86) as well as key immunostimulatory cytokines such as IL-12 and IL-23, they generate T cells with proinflammatory properties(61). By contrast, expression of immunoregulatory cytokines such as IL-10 by APCs, promotes the induction of regulatory T cells (62-65). This effect can be further enhanced by the expression of certain surface molecules, such as ILT3(66), by the upregulation of Indoleamine-Pyrrole 2,3,-Dioxygenase (IDO)(67) and surface CD155 activation by the T cell ligand TIGIT(68), as well as by increased levels of TGFβ and retinoic acid signalling(62). As a result, some groups are currently generating tolerogenic APC ex-vivo (from blood derived dendritic cells) and re-infusing these into patients as a tolerogenic therapy (44, 69, 70). While this may prove very effective, this therapy will inevitably be costly and require GMP cell handling facilities in each clinical setting to deliver.

Targeting antigen presentation through the superficial layers of the skin improves efficiency of antigen delivery. The skin has repeatedly been shown to be not only the most convenient but also the most efficient site for delivery of antigen to the immune system due to the very high density and specialised nature of its antigen presenting cells(71, 72). Recent studies have shown that not only is there an interlinked network of relatively immobile Langerhans Cells at the dermo-epidermal junction, but this is supplemented by very motile dermal dendritic

cells present in the superficial layers of skin just below the epidermis(73). The density of APCs reduces rapidly below this level, and hence antigen presentation is more efficient, the more superficially it is delivered in the skin. Consistent with this, intradermal immunisation requires less antigen than subcutaneous administration to achieve the same level of immunisation(74). Traditionally, intradermal immunisation has been achieved using angled hypodermic needle injection. However, a wide range of ultrashort needles (typically 650micrometres in length or less) have now been developed which can deliver antigen into the superficial layers of the skin reliably with direct (perpendicular) injection. This approach has the advantage that it ensures intradermal rather than subcutaneous delivery ensuring high efficiency - recent trials have confirmed that 10 fold less antigen than subcutaneous administration is required to achieve similar levels of immunisation(75).

The use of gold nanoparticles to enhance uptake of antigen by dendritic cells and reduce inflammation. Dendritic cells are particularly active in taking up particulate material and hence there has been increasing interest in the use of microparticles or nano-scale particles to further target antigen to DCs (76). For the current application, it is important that any particles used are both safe in humans and inert, since the generation of inflammation is likely to antagonise the development of regulatory T cells (76). Gold is an inert metal which has an extensive safety record dating back to its use as a therapy for rheumatoid arthritis. However, safety can be further enhanced by the use of very small nanoparticles (< 5nm) as these can be excreted through the kidney reducing the potential for accumulation with repeated therapy. Gold nanoparticles of this size have also been reported to have low toxicity in cellular assays(77).

Antigen delivery to be used in the current protocol. In the current protocol, we will explore the safety of antigen specific immunotherapy using very small (< 5nm) gold nanoparticles coupled to a peptide of proinsulin C19-A3 which we have identified as being a naturally processed and presented T cell epitope of proinsulin that is frequently targeted in patients with type 1 diabetes who carry the susceptibility molecule, HLADRB1*0401 (~35% of the patient population)(24, 78). We have completed a phase 1A study of intradermal delivery of this peptide (alone) in patients with type 1 diabetes (24) up to doses of 100ug and no evidence of toxicity was observed (79). At the lower dose tested (10mcg) potentially protective IL-10 responses to the peptide were induced in 5/18 subjects (79). We have also recently completed a phase 1b study of this peptide in new-onset type 1 diabetes patients giving more doses (12 versus 3), more frequently (every 14 days rather than monthly) with no evidence of toxicity other than transient local red reactions seen previously. The small size and high solubility of this peptide allows it to be easily coupled to small nanoparticles. In addition, it does not have the bioactivity of insulin and hence is free from the risk of hypoglycaemia associated with the intact molecule. In a future study, we plan to study the additional effect of linking delivery of these particles to IL-10 to reduce inflammation and promote the generation of regulatory T cells. We have termed the overall approach, “Enhanced Epidermal – Antigen Specific Immunotherapy” (EE-ASI).

Preclinical data

Gold nanoparticles (GNP) were formulated with a thiol three amino-acid linker to covalently attach to the peptide proinsulin C19-A3 (C19-A3 GNP). Addition of C19-A3 GNP to human monocyte-derived immature dendritic cells did not induce maturation of these cells. However, following exposure to a maturation signal (lipopolysaccharide, LPS), dendritic cells treated with C19-A3 GNP released less cytokines – notably less TNF, IL-12 as well as IL-10. Presentation of the peptide in C19-A3 GNP particles to peptide specific T cells by these dendritic cells was as efficient as non-conjugated peptide.

Intradermal injection of fluorescent nanoparticles via NanoPass 600micron microneedles into ex-vivo human skin resulted in accumulation of fluorescence in the upper dermis. Injection of GNP resulted in a more widespread distribution, including "reflux" into the epidermis. Flow cytometry of cells extracted from the epidermis following injection of fluorescently conjugated GNP into human skin, showed that over 98% of dendritic cells took up GNP as compared to 38% of non-dendritic cells.

In murine studies, intradermal injection of peptide coupled GNPs resulted in antigen presentation in draining and distant lymph nodes and spleen within 3 days, as compared to peptide alone which was only presented in the draining lymph node. Studies with CCR7 deficient mice, in which dendritic cells do not migrate from the skin to the draining following local stimuli, suggested that this distant dissemination of GNPs at least in part requires migration of dendritic cells, and is not wholly a fluid phase process.

Taken together, this data provides evidence that the coupling of peptide to GNPs results in efficient uptake and antigen presentation by dendritic cells, increased diffusion into the surrounding skin including epidermis (where there is a high density of dendritic (Langerhans) cells with regulatory potential), an anti-inflammatory effect on local DCs and enhanced dissemination of the antigen to distant lymphoid tissue. All of these features may be expected to increase the capability of GNP coupled peptide to induce a regulatory T cell response not only in skin at the site of administration but at distance sites including the pancreas draining lymph nodes. In addition, C19-A3 GNP has the potential in future studies to carry a dual cargo, for example a combination of peptide antigen with a "tolerogenic adjuvant".

3.3 Route of administration, dosage, regimen, and treatment period

C19-A3 GNP will be administered intradermally via CE marked Nanopass 600nm microneedles. 3 doses will be given at 4 weekly intervals. The dose given will be equivalent to 10ug of C19-A3 peptide.

3.4 Sample size and population to be studied

8 subjects aged 16 to 40 years with a diagnosis of Type 1 diabetes and some residual endogenous insulin (c-peptide) production will be included in the study. The Cardiff site will recruit subjects from the age of 18 years only and the Swedish site will recruit subjects from the age of 16 years, as the PI is an experienced paediatrician. Patients will be required to possess the HLA DRB1*0401 genotype and residual c-peptide production as evidenced by a stimulated urine c-peptide: creatinine level > 0.2 nmol/mmol or random serum sample level

of > 60 pmol/l. Residual c-peptide production is required to be able to monitor for disease exacerbation (ie unexpectedly rapid loss of insulin c-peptide production).

3.5 Conduct of trial

The trial will be conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (2013), the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended in 2006, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The Protocol will be submitted for approval by an NHS Research Ethics Committee (REC), UK and Regional Ethical Review Board (RERB), Sweden.

4 TRIAL OBJECTIVES AND DESIGN

4.1 Trial Objectives

The primary objective of the study is to examine the risk of C19-A3 GNP administration in terms of general safety and induction of hypersensitivity.

Secondary objectives are:

- To study the feasibility of delivering C19-A3 GNP via microneedles to humans.
- To study the size and nature of immune responses to C19-A3 GNP generated in blood and the draining (axillary) lymph node.

4.2 Trial Design

The study is a two centre, open-label, uncontrolled single group phase 1A study of C19-A3 GNP peptide (10 µg peptide equivalent content) administered via Nanopass microneedles every 28 days for 8 weeks (3 doses), with follow-up for 12 weeks (20 weeks in total from first dose). Treatment will be given into the arm at a volume of 50ul.

4.3 Primary endpoint

Assessment of the safety of C19-A3 GNP administration in terms of adverse events at the dose used.

4.4 Secondary endpoints

- T cell responses to C19-A3 GNP as determined by changes from baseline of interferon gamma and IL-10 ELISPOT responses to this peptide in blood following treatment at weeks 0, 9 and 14.
- T cell responses to C19-A3 GNP as determined by changes from baseline of interferon gamma and IL-10 ELISPOT responses to this peptide in draining axillary lymph node before treatment and following the last treatment administration.
- Changes in additional immunological biomarkers (e.g. flow cytometry profiles, T reg assays, autoantibodies, beta cell and T cell free DNA markers) from baseline at week 0, 9 and 14.

- Effects on residual insulin requirement and c-peptide secretion at week 14 as compared to baseline as assessed by a mixed meal tolerance test and a stimulated urine c-peptide test.
- Effects on glycaemic control assessed by blood sugar profiles and HbA1c at week 14 as compared to baseline
- Questionnaires on quality of life and diabetes self-management.

Trial Flowchart

The schedule of visits for the study is shown in Table 1. Figure 1 shows the steps from ascertainment to first injection.

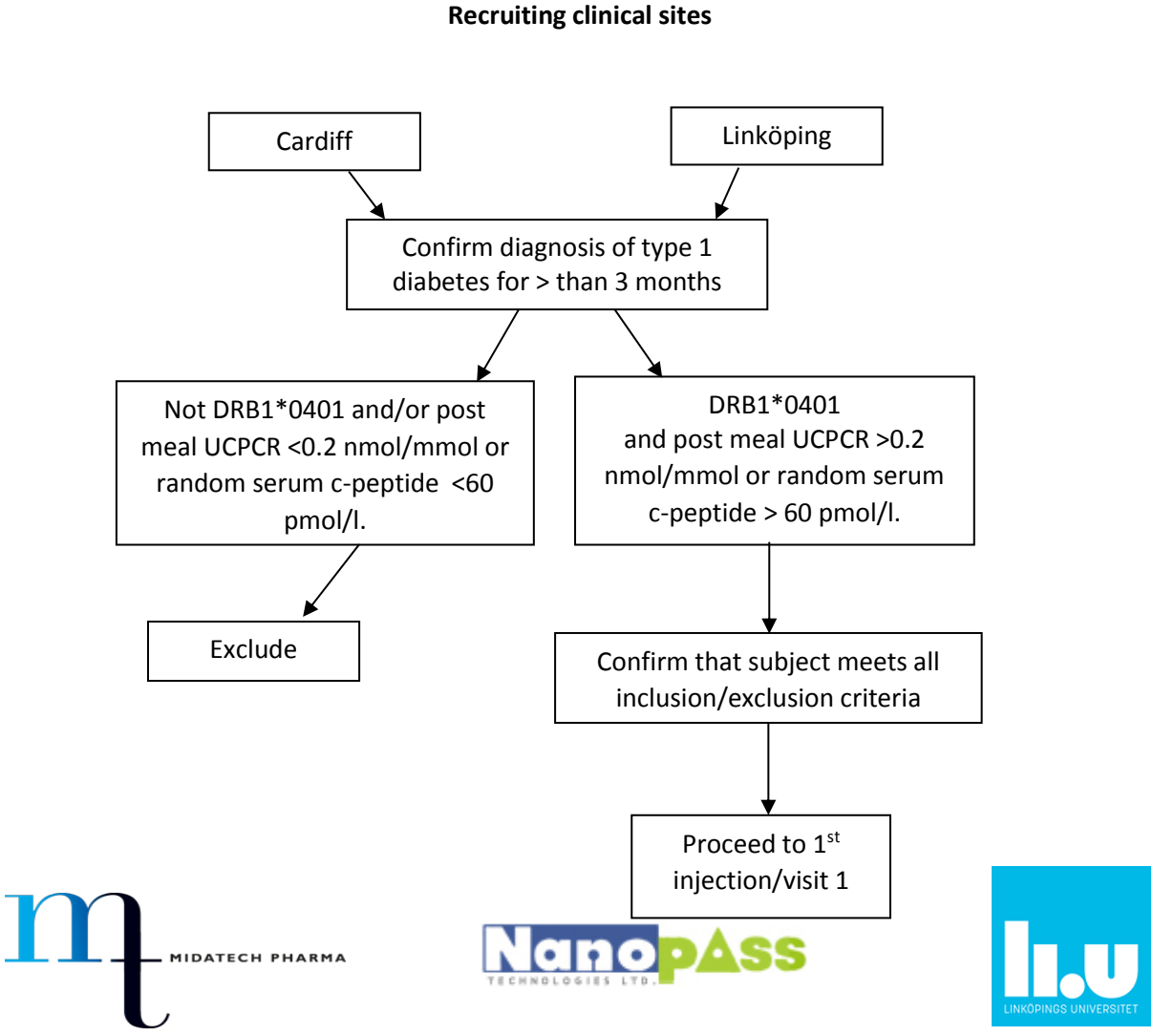
Table 1: Schedule of study visits

	Screening			**Treatment**							**Follow up**				**Optional**	
Visit number	-2	-1	Pre V1	1	1b	1C	2	3	3b	3C	4	Pre V5	5	6	7	any time post dosing
Weeks	These visits can be combined. Treatment must start within 3 months of screening commencing[1]		A minimum of 72 hours prior to visit 1	0	1 day post visit1	3 to 5 days post visit 1	4	8	1 day post visit3	3 to 5 days post visit 3	9	A minimum of 72 hours prior to visit 5	14	20	52	
Days				0			28	56			63 (or one week post final injection)		98	140 (85 days post final dose)	366 (311 days post. final dose +/- 14 days)	
Informed consent	X															
Study drug administration				X			X	X								
Follow up telephone call 24 hours post IMP				X			X	X								
Physical Exam & ECG	X			X			X	X					X		X	
[2]Serum for AAbs and immune markers				X							X		X		X	
DRB1*0401 genotype	X															
MMTT				X									X		X	
Photographs at injection site				X			X	X			X		X	X	X	
Blood sample to accompany skin studies (optional)								X								
Stitch removal from biopsy site (optional)										X						
urine or random serum c-peptide post meal		X		X									X		X	
72 hour continuous glucose monitoring			Attach CGM	X								Attach CGM	X		X	
Serum for Cystatin C				X							X		X	X	X	
Urinalysis for blood, protein, and beta-2 microglobulin		X		X			X				X		X	X	X	
Serum and urine (morning) sample to measure gold concentrations				X pre dose	X[2]				X[2]		X		X	X	X	
T cell (immune biomarker) assays (blood)				X				-			X		X		X	
[3]LN aspiration 0/3-5d				X		X		X		X						
[3]Safety blood samples		X		X			X				X		X	X	X	
HbA1c measurement		X		X			X				X		X		X	
Vital signs	X	X		X			X	X			X		X	X	X	
Adverse Event assessment/con meds		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine pregnancy test		X		X			X	X					X	X		
Record of insulin use	X			X			X	X					X		X	
[3]Blood glucose profile/insulin usage				X			X	X					X	X	X	

Assessment of glycaemic control/review blood sugar recordings				X			X	X					X		X	
Skin biopsy (optional)[5]																X
Contact hypersensitivity testing (optional)[4,5]																X
Suction blister (optional)[5]																X
XHFS survey				X									X			
ADDQoL				X									X		X	
DTSQs				X									X		X	
DTSQc													X			

Screening can be up to 3 months
Treatment visits 1, 2 and 3 should be every 28 days +/- 3 days and follow up visit 5 should be at 14 weeks +/- 1 week
[1]If dosing is delayed beyond 3 months, all test other than genotype should be repeated.
[2]Samples to be taken post dose.
[3]Details supplied in outcome assessment section 6 and safety assessment section 10.
[4] Contact hypersensitivity testing will be done once post dose. It involves 3 visits (the 2nd visit can be done at home).
[5] These tests will be done in subjects with a persistent reaction (optionally, post-dose).

Figure 1. Flow chart of ascertainment, recruitment and screening through to IMP administration.



5 TRIAL MEDICATION

5.1 IMP

The IMP will be delivered as sterilised solution in small vials produced by Baccinex SA (Courroux, CH), and stored, QP released and shipped to the clinical sites by PharmaKorell GmbH (Lörrach, Germany).
The vials should be stored refrigerated at 2-8 °C.
The IMP will be 10ug of C19-A3 peptide Gold NPs injected by Nanopass microneedles, delivered intradermally into the upper arm, in 50ul volume.

5.2 Dosing regimen

C19-A3 GNP will be administered intradermally via CE marked Nanopass 600nm microneedles. 3 doses will be given at 4 weekly intervals. The dose given will be equivalent to 10ug of C19-A3 peptide. Thus the cumulative dose will be 30ug.

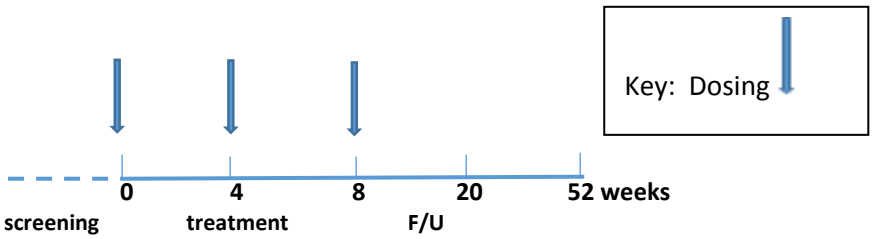
5.3 Drug accountability

The will arrange transfer of IMP to local IMP Storage and Supply Sites. The local Site Pharmacy will then take responsibility for IMP accountability by ensuring that: the IMP is stored in a secure location, segregated from other medicines; used and returned medication is kept separate from unused medication; storage conditions are monitored and recorded; IMP is dispensed to participants in accordance with the trial protocol; unused medication is returned to the study team or disposed of as authorised by the study team. Full accountability records will be kept for all aspects of IMP handling. IMP accountability records will be monitored by the Sponsor.

5.4 Subject compliance

Patients will be allowed 28 days +/- 3 days between treatments, but every effort should be made to dose every 28 days. Where a visit is > 3 days late it should be documented as a protocol violation and scheduled as soon as possible, with subsequent dosing visits recommenced on a 28 day schedule after this. Where the interval between 2 doses is > 42 days, the dose will be considered to have been missed and the subsequent dose should be given. Subject attendance and compliance will be recorded. If a treatment is missed the subject will continue in follow-up but an additional subject will be recruited, so that a total of 8 subjects will receive all 3 doses.
Patients will be allowed to attend for follow up visit 4 (week 9 or one week post final injection) +/- 1 day of schedule, visit 5 (week 14), +/- 1 week of visit schedule and visit 6 (week 20) must be a minimum of 85 days post final dose/+2 weeks. There will also be an optional visit 7 (week 52) at a minimum of 311 days post final dose +/- 2 weeks. There will be an optional skin blister, an optional skin biopsy and an optional contact hypersensitivity testing performed anytime post-dose (referring to post final dose). Any visits outside of this guidance will be considered a protocol deviation.

Figure 2 Chart indicating dosing schedule



5.5 Dosing strategy:

This is an open label uncontrolled early phase safety study, so no blinding or randomisation will be performed. In keeping with standard phase 1 study designs, no placebo or control group is included as the primary aim is to establish whether there are any major unexpected safety issues in the use of this IMP for the first time in man.

5.6 Concomitant medication

Patients will be supported to receive optimal care during the study, to include daily insulin injections. Routine vaccinations (flu and others) should be avoided for the duration of the study unless clinically essential (eg during periods of high risk flu exposure). Use of β-cell stimulants (e.g. sulphonylureas), glucagon-like peptide-1 agonists, dipeptidyl peptidase-IV inhibitors, insulin sensitisers (e.g. metformin, thiazolidinediones) that may affect insulin production, and the use of immunosuppressive or immunomodulatory therapies (including systemic but not topical or inhaled steroids) which may modulate immune responses is not permitted. Use of other drugs with potential effects on the immune system, insulin sensitivity or β-cell function, where these are considered clinically essential for treatment of concomitant medical conditions, will be discussed with the Chief Investigator. See also 6.3

5.7 Concomitant health issues

Study drug should not be administered if a subject has had a febrile illness within the last 3 days as this may activate T cells non-specifically. Under these circumstances, the missed study visit should be delayed to the earliest next feasible date (but no longer than 14 days) and the study continued.

Females will be advised not to become pregnant within 85 days following the date of their final treatment of IMP and will be advised to continue using adequate contraception until this time point See section 6.2
Males will be advised not to father a child within 85 days following the date of their final treatment of IMP.

5.8 Measures to avoid bias

The study will be open label. Assessment of immune responses will be conducted blinded from metabolic parameters in the first instance. All samples will be labelled with a study ID only, and laboratory staff will know whether samples are from the same subject but not the identity of the subjects.

5.9 Timescales

5.9.1 Proposed Trial Start: July 2016

5.9.2 Projected Trial Completion (amended): January 2019

5.9.3 Trial Duration: 26 months

5.9.4 Duration of Each Patient's Participation: 12 months (52weeks excluding screening), the last visit (visit 7) is optional and if the participants choose not to take it the duration would be 5 months (20 weeks excluding screening), there will be three optional tests performed post final dose

6 SELECTION AND WITHDRAWAL OF SUBJECTS

6.1 Study population

8 subjects with a diagnosis of Type 1 diabetes will be included in the study. Autoimmune diabetes will be confirmed by measurement of islet cell autoantibodies. Patients will be required to possess the HLA-DR4 (*B1*0401*) genotype.

6.2 Inclusion criteria

1. Clinical diagnosis of type 1 diabetes for > 3 months (dated from the first insulin injection).
2. Commenced on insulin treatment within 1 month of diagnosis.
3. Age 16 to 40 years

4. 2 hour post-meal UCPCR > 0.2 nmol/mmol or random serum sample > 60 pmol/l on at least one occasion (maximum 3 tests on different days)
5. Possession of 0401 allele at the *HLA-DRB1* gene locus
6. The following birth control methods should be used (considered highly effective with a failure rate of less than 1% per year when used consistently and correctly]:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- _ intrauterine device (IUD)
- _ intrauterine hormone-releasing system (IUS)
- _ bilateral tubal occlusion
- _ vasectomised partner (provided that the partner is the sole sexual partner of the trial participant and that medical assessment of azoospermia has been confirmed)
- Sexual abstinence (defined as refraining from hetrosexual intercourse during the duration of the trial)

7. Written and witnessed informed consent to participate.

6.3 Exclusion criteria

1. HbA1c > 86mmol/L (10%).
2. Females who are pregnant, breast-feeding or not using adequate forms of contraception.
3. Previous diagnosis of renal disease including glomerulonephritis or nephropathy.
4. Raised serum creatinine or abnormal urine albumin/creatinine ratio (ACR) (values above the laboratory reference range). If the initial ACR is raised, this should be repeated on two further occasions as first morning samples. The subject can be included if both of these samples are negative (within the reference range).
5. Use of immunosuppressive or immunomodulatory therapies, including systemic steroids within 1 month prior to receiving the IMP and any monoclonal antibody therapy given for any indication. Note that previous exposure to proinsulin peptide C19-A3 in a clinical trial is an exclusion criterion.
6. Use of cannabis within one month prior to trial entry.
7. Use of any hypoglycaemia agents other than insulin, for more than 6 weeks, at any time prior to trial entry.
8. Use of inhaled insulin.
9. Known alcohol abuse, drug abuse, HIV or hepatitis.

10. Allergies to drug components or any excipients.
11. Any other medical condition which, in the opinion of investigators, could affect the safety of the subject's participation or outcomes of the study, including immunocompromised states and autoimmune conditions.
12. Subjects should not have had immunisations (flu and others) for 1 month prior to trial entry and should not receive any during their time in the trial – see section 5.6.
13. Recent subject's involvement in other research studies which, in the opinion of investigators, may adversely affect the safety of the subjects or the results of the study.
14. Abnormal ECG findings.

6.4 Recruitment and informed consent

Subjects will be recruited into the study according to the following steps:

1. Patients with type 1 diabetes will be identified by their local diabetes teams (hospital doctors or diabetes nurses), from clinic records, during clinic visits or inpatient admissions.
2. Some patients may self-refer and contact the trial team directly, from seeing internet information, news articles or posters regarding participating in diabetes research, or be identified by the ADDRESS-2 registry of recently diagnosed patients with type 1 diabetes. Patients who self-refer will have their details passed to the local coordinator of the study or research nurse/doctor and will be contacted initially by phone, email or in person by the local centre research team who will outline and explain the aim of the study and provide copies of the Participant Information Sheet (PIS).
3. For subjects identified by health records or clinical contacts, the study will be discussed with the identified patients by a member of their clinical care team and, with the subject's agreement, details will be passed to the local coordinator of the study.
4. Subjects who have consented to be on the ADDRESS2 registry and consented to be contacted regarding research that they may be eligible to take part in, will be contacted initially by the lead centre.
5. Subjects will then be contacted by phone, email or in person by the local centre research team who will outline and explain the aim of the study and provide copies of the Participant Information Sheet (PIS).
6. Subjects will have a minimum of 24 hours to consider this information and usually at least 5-7 days. Subjects will be invited to call the local research nurse or Local Principal Investigator for any queries about the PIS and to indicate if they wish to take part.
7. An appointment will then be made for interested subjects, at which the Local Principal Investigator or their appointed deputy can check the subject's understanding of the study and obtain written consent. Subjects may then take part in assessments required for the first screening visit (visit -2).

8. If the subject has the HLA *DRB1*0401* genotype then the local research team will arrange an appointment with him/her for the second screening visit (visit -1) which will include a post meal UCPCR or random serum c-peptide, blood samples and urine pregnancy test (if female).
9. If appropriate, screening visits -2 & -1 can be combined.
10. Subjects who fulfil all inclusion criteria without exclusion criteria will be informed of their screening results, and arrangements made for enrolment and the first study visit.
11. A schedule of study visits and procedures carried out at each study visit are shown in table 1.
12. Where the start of dosing is delayed more than three months beyond screening visit -1, all the tests and physical assessment required at screening (excluding genotyping) should be repeated to confirm continues eligibility.

6.5 General assessments

General assessments for this protocol are:

- Inclusion/exclusion criteria
- Medical history
- Physical examination & ECG recording
- Concomitant medications
- Adverse event recording
- Record of insulin usage
- Assessment of glycaemic control
- Quality of life questionnaires
- Photographs at injection sites at each visit

6.6 Follow up assessments

The primary outcome measure for the trial is the safety (adverse event) profile of this investigational agent.

The key components of the follow-up visits are:

1. Diabetes assessments;
2. Adverse events;
3. Any changes in medications;
4. Review of clinical safety blood tests.

6.7 General laboratory tests

A study specific laboratory manual will be provided and the following general laboratory assessments will be performed during the study:

- Full blood count; urea, electrolytes and creatinine; liver function tests (prothrombin time, total bilirubin, total protein, albumin, AST (SGOT), SGPT (ALT), alkaline phosphatase; thyroid stimulating hormone; immunoglobulins (G, A, M); calcium; magnesium, phosphate, lipid profile (total cholesterol, LDL, HDL, triglyceride). These will be tested in the local laboratories of the study sites.
- Urine pregnancy test (females only, at all trial visits)
- Urinalysis for pH, blood and protein by dipstick urinalysis and laboratory analysis for albumin/creatinine ratio, urine beta-2-microglobulin and cystatin-C.
- Autoantibodies against insulin, GAD-65, IA-2 and ZnT8 (performed in the Diabetes Research Unit Cymru Laboratories, Swansea University)
- Anti-drug (GNP-peptide C19-A3) antibodies

6.8 Study Drug Administration

Study subjects will attend a clinical research facility or hospital ward (Linkoping) for receiving the IMP. Staff will be experienced in CTIMPs and facilities for managing a severe reaction will be to hand (see section 10.2). The drug will be prepared and injected intradermally in the upper arm of the patient in a 50ul volume, according to the schedule (see figure 2).

All subjects will be observed 6 hours after their first dosing/injection, however the first subject will be dosed at least 24 hours before subsequent subjects as per sentinel dosing strategy (see risk mitigation strategy section 7). If no SAEs are observed after this first dose, all subjects will be observed for a minimum of one hour after each injection and up to 2 hours if there are any signs of hypersensitivity. All local and systemic reactions will be documented.

6.9 Metabolic outcome assessments

Metabolic assessments will consist of:

- Glucose records and reports of hypoglycaemia (clinical care measurement)
- Insulin dose (clinical care measurement)
- HbA1c level (laboratory test)
- Urine or random serum post-meal c-peptide/creatinine ratio (laboratory test)
- Mixed meal tolerance test (MMTT) (laboratory test)

Laboratory measurements for these will be performed in the laboratories of the Diabetes Research Unit Cymru laboratory, Swansea University.

6.10 Mechanistic immunological studies

Mechanistic assessments will consist of:

- Ultra-sound guided Axillary LN aspiration and ELISPOT data before and 3-5days after administration of the first dose and the final dose of IMP (total of 4 aspirations for each patient)

- T cell assays to include enzyme-linked immunospot (ELISPOT) to measure T cell responses to PPI C19-A3 and other islet cell autoantigenic peptides using LN cells and fresh whole heparinised blood sent by courier to the assay laboratory
- Storage of peripheral blood mononuclear cells (PBMCs) for subsequent analysis of T cell responses (measurement of Tregs and antigen-specific T cells by tetramer and Diab-Q-kit)
- Storage of DNA for subsequent analysis of gene-phenotype interactions
- Storage of serum for islet autoantibody assessments and cell free DNA measurements
- Additional mechanistic assays by the T1D UK Consortium Mechanistic Core

6.11 General guidance on glycaemic control during the study

Glycaemic control will be maintained according to clinical guidelines and conducted in collaboration with the patient's diabetes health care team.

- HbA1c will be measured every 1-3 months with a target value set by agreement with the patient and their clinical care team. Where this target is not met, advice will be given as clinically required.
- Glycaemic control will be reviewed at every study visit.
- Where insulin requirements fall to less than a total of 8 IU per day, continuation of insulin therapy should be discussed with the Chief Investigator: continuation of a low dose of insulin where possible is considered preferable.

6.12 Ultrasound guided Fine Needle Aspiration of axillary lymph node

Ultrasound guided Fine Needle Aspiration of axillary lymph node (US-guided FNA) will be performed by an experienced consultant radiologist, before and after Visits 1 and 3.

The procedure is as follows: Under aseptic technique and ultrasound guidance and following local anaesthetic, a 21G needle is inserted into an axillary lymph node and few drops of fluid aspirated and sent for immunological studies (as in section 6.10).

6.13 Optional Skin biopsy and blister sampling.

To determine how long the gold nanoparticles stay in the skin and the immunological effects, subjects will be invited to undergo an optional skin biopsy and an optional suction blister. In order not to disturb the systemic effects of nanoparticle administration, skin sampling will not be performed until after visit 3 (post final dose). For the optional skin biopsy, a 4mm skin biopsy using standard dermatological techniques will be taken at the site of the first injection (ie 2 months after the final dose) to determine residual gold distribution. For the optional suction blister, a standard suction blister sample (82,83) will be taken from the site of the second injection (ie 1 month after the final dose) to study any infiltrating lymphocytes (83).

Skin biopsies will be taken by a 4mm standard punch technique. The material obtained will snap frozen for immunohistochemistry and gold staining and part stored for RNA extraction and PCR/RNA seq analysis.

Skin suction blisters will be performed by gradually applying negative pressure (up to 50 kPa) from a suction pump machine VP25 (Eschmann, Lancing, UK) through a suction blister cup with a 15-mm hole in the base for 2–6 hr until a unilocular blister had formed within the cup. The blister fluid is then aspirated after blister formation and spun to obtain any infiltrating lymphocytes. These will be analysed for reactivity to the administered peptide by ELISPOT and for surface molecule expression by cytospins and fluorescence microscopy if sufficient sample in obtained.

6.14 Photographs at injection sites

After the injection it is possible, in some patients, to see the gold as a small grey mark under the skin less than 5mm across and this may still be there for 3 months or more after the injection. In some individuals there may be a painless red area around the grey mark which may also stay there for 3 months or more. We intend to take photographs of the injection sites at each visit to compare the marks at subsequent visits.

6.15 Contact hypersensitivity or skin patch testing

Contact hypersensitivity testing or patch testing is to determine delayed Type 4 hypersensitivity to allergens including gold for this study. We would like to check for development of hypersensitivity to gold in patients who received trial medication. This is an optional test performed anytime post-dose (referring to post final dose). The test itself can involve 3 visits. For the participant's convenience, visit 2 can done at home by the patient. These visits include day 0 (patches applied to back or arm of patient), day 2 (patches taken off (home or clinic) and first reading (if in clinic)) and final reading (day 4 or day 7). We are looking for red, raised, itchy, vesicular reactions to detect contact allergy and these results are graded according to internationally agreed standards. The allergens applied have to be diluted to correct dilutions considered suitable by experts for patch testing and application to patients' backs. The patient needs to keep the back (or other site of application for example the arm) dry during the whole test period and be prepared to attend all 3 visits to perform the test. Participants will be asked for blood sample (5ml) to measure serum gold concentration before the test.

6.16 Sample management

Sample management will be carried out according to GCP and according to the approval of the Research Ethics Committee.

6.17 Specimen handling

Samples for local laboratory assessment may be taken at a time convenient for same day processing by the local laboratory. Samples for immunologic or metabolic studies, HLA typing or autoantibodies will be drawn before 12.00 and sent by approved courier to the appropriate laboratory or stored locally for later analysis. At dosing visits blood draw will take place prior to administration of the IMP.

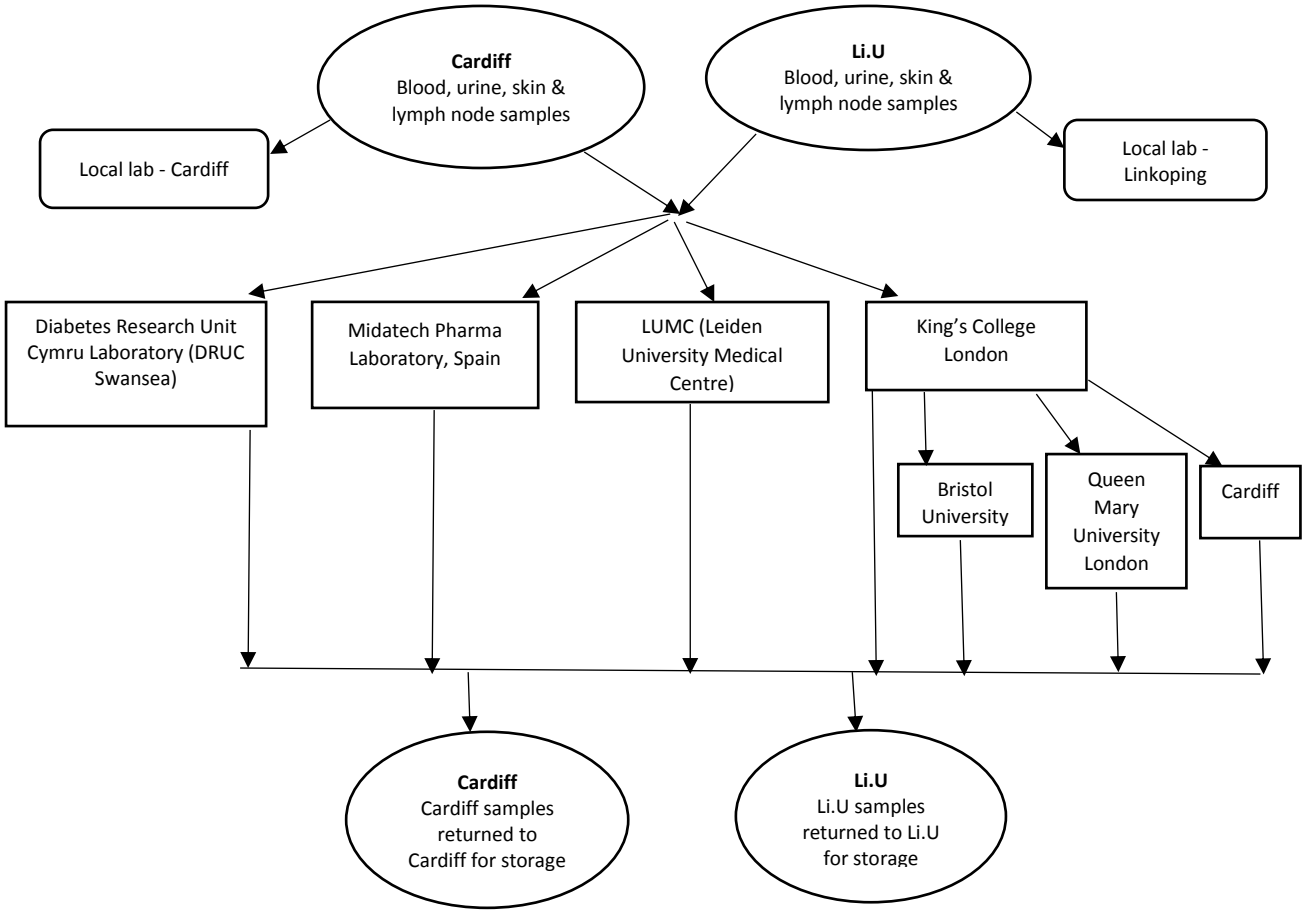
Figure 3 Blood draw schedule:

	Screening		Treatment					Follow-up				
Visit no:	-2	-1	1	1b	2	3	3b	4	5	6	7	post
Weeks			0		4	8		9	14	20	52	final
Days			0	1	28	56	57	63	98	140	366	dose
Safety blood samples HbA1c & glucose		11			11			11	11	11	11	
DRB1*0401 and A*02	2											
Serum for Aabs	2											
Serum for insulin cell free DNA	8											
Serum for: Aabs, cystatin-c, insulin cell free DNA, other			10					10	10	10	10	
MMTT			25						25			
Blood to accompany skin biopsies												20
Serum for gold concentrations (morning urine also to be sent)			1	1			1	1	1	1	1	
PBMC's T reg assay, T cells, CD4 & CD8 tetramers, Elispots & biobank storage.			64					64	64*		64	
Flow cytometry			2					2	2		2	
Serum for gold concentration before contact hypersensitivity testing												1
Total	***12***	***11***	***102***	***1***	***11***	***20***	***1***	***88***	***113***	***22***	***113***	***1***

**in subjects with immune responses of interest visit 4, an additional blood sample up to 70mls (35mls for under 18s) may be requested between visit 4 and visit 6.*

Volumes for the study within an 8-week period coincide with guidelines of the National Institute of Health (ie blood collection should be <550ml per 8-week period for adults, and no more than 5ml/kg in a single day and no more than 9.5ml/kg in any 8 week period for under 18's) and are those used by the Type 1 diabetes clinical trial consortium, Type 1 Diabetes Trial Net (www.diabetestrialnet.org).

Figure 4 Sample management flowchart



6.18 Laboratory procedures

6.18.1 Stimulated secretion of C-peptide

For screening, a post-meal random serum sample in the clinic or urine c-peptide/creatinine ratio will be measured. Patients are provided with a suitable container (boric acid preservative) and asked to void their bladder (and discard this urine) prior to their first meal of the day. They are then asked to substitute their first meal of the day with Ensure Plus and asked not to pass any urine until 2 hours after the Ensure Plus meal and take a sample of urine into the container at this time. These samples will be assayed in the laboratory in Diabetes Research Unit Cymru Laboratories in Swansea for urine c-peptide and creatinine.

During the study secretion of c-peptide will also be tested using the Mixed Meal Tolerance Test method (MMTT). Briefly, the test is performed in the morning (between 7 and 10 AM). The participant must be fasting and have had no food or drink (with the exception of water), and no smoking is permitted from 12 (midnight) or 8 hours prior to the start of the test. Tests should be conducted only if fasting value by capillary blood glucose meter is between 3.9-11.1mmol/L. Participants should withhold taking long acting insulin on the morning of the test. They can take very short acting insulin (e.g. Humalog, Apidra, or Novorapid) up to 2 hours before the test. They can take regular insulin up to 6 hours before the test. If the subject is using an insulin pump, they should be advised to continue their basal regime but not have a bolus.(80)

The procedures are as follows. Start IV line. Obtain baseline blood samples at 0 minutes, and any other test samples required at that visit (e.g. HbA1c, T cell studies, PBMCs islet cell autoantibodies). For each time point blood samples are 1.2 ml fluoride tube for glucose and 1.2 ml serum tube for C-peptide. In addition, for each test the first sample (0 minutes) and the last sample (120 minute for MMTT) will be used to obtain a meter ketone reading, which is recorded. A first blood sample is drawn immediately before the participant starts drinking the liquid meal (formal “baseline samples”). The patient is given the standardized liquid meal: Ensure Plus 6 ml/kg (Maximum 360ml) to be ingested within 5 minutes. Blood samples are drawn at times: 15, 30, 60, 90 and 120 minutes, after the end of ingestion of Ensure Plus (note: time runs from the start of ingestion). After the test is completed, the participant eats and receives insulin as appropriate and prescribed by the local investigator. A urine sample at the 120 minute time point will also be collected for c-peptide creatinine ratio as an alternative marker of insulin production.

6.18.2 Mechanistic assays for the assessment of immune biomarkers

6.16.2.1 Cytokine ELISPOT

Cytokine ELISPOTS for the detection of IFN-γ and IL-10 producing CD4 T cells in response to PPI C19-A3 and other islet autoantigenic peptides will be performed as previously described (24, 79, 81). These assays provide a means to monitor potentially favourable changes in the immune response to PPI C19-A3 (eg a shift from IFN-γ to IL-10 secreting cells). These will be performed on both peripheral blood mononuclear cells and LN aspirate cells as well as on skin derived leukocytes where an optional skin blister aspiration sample is provided.

6.18.2.2 Storage of peripheral blood mononuclear cells for additional T and B cells assays and immune gene profiling

Blood samples will be taken at 0, 9 and 14 weeks and the peripheral blood mononuclear cells cryopreserved as well as samples stored for later DNA analysis. These will be performed by the T1D UK Mechanistic Core laboratories.

6.18.2.3 Storage of serum for islet autoantibody and cell free DNA studies.

Blood will be taken at 0, 9 and 14 weeks and spun down for serum which will be aliquoted and frozen at -80C. Sample will be stored for later islet autoantibody and cell free DNA

analysis. Islet autoantibodies to GAD, IA-2 and ZnT8 will be measured in the Diabetes Research Unit Cymru Laboratories, Swansea University and cell free DNA assays (for beta cell and T cell apoptosis) will be performed via the T1D UK Mechanistic Core laboratories.

6.18.3 Analysis of skin biopsies.

In individuals who agree to provide a skin biopsy from the first injection site anytime post-dose, this material will divided for immunohistology/gold distribution analysis and RNA extraction for RNA seq/QPCR.

7 Risk Mitigation Strategy

Communication between trial sites. The clinical research teams of the two sites involved will be in weekly telephone contact (teleconference) during the recruitment and dosing periods. All adverse reactions will be discussed and information shared.

All serious adverse events will be shared within 24 hours with the other recruiting site.

Sentinel dosing. The first patient dosed with GNP-peptide will be observed for 6 hours post dose. Temperature, blood pressure and heart rate will be recorded at the following intervals post injection: 15 mins, 30 mins, 45 mins, 1 hour, 1 hour 30mins, 2 hours, 2 hours 30 mins, 3 hours, 4 hours, 5 hours and 6 hours. The injection site will be observed at the following intervals post injection: 5mins, 10 mins, 15 mins, 30 mins, 45 mins and 1 hour. A follow-up phone call will be made to the subject 24 hours after the first dose. No additional patients will be able to be recruited until this 24 hour period is completed ("lock-out period").

If no serious adverse events are observed, subsequent subjects will also be observed for 6 hours after their first injection. If no serious adverse events are observed, then subjects will be observed for a minimum of 1 hour post dose, or 2 hours if there are any symptoms of signs of hypersensitivity. This time period has been chosen as the only anticipated severe reactions is one of hypersensitivity which, if systemic, would be apparent within 60 mins of intradermal dosing. See section 10.2 for further discussion of hypersensitivity.

Each subject will be contacted by telephone 24 hours after each dose.

The trial manager will communicate with clinical staff by email and telephone to keep both sites up to date with screening and recruitment activity. Weekly teleconferences between sites will take place during the screening and recruitment phase and the trial manager will inform clinical staff at both sites when the first patient is going to be dosed and report to them afterwards regarding whether any AE's were experienced. This will negate the possibility of two subjects starting treatment on the same day. Close communication between sites will be maintained throughout the trial and details of any adverse reactions will be communicated to the other trial treatment site by email, in addition to the standard pharmacovigilance procedures.

Conditions for interruption of dosing to individuals.

If there is evidence of hypersensitivity (i.e. wheal as well as flare), subjects can have paracetamol and antihistamines and continue in the trial as per the study schedule.

Injections and treatment will be immediately discontinued (but follow up will continue) in any subject who:

1. Experiences a worsening local wheal or flare reaction at the injection site exceeding 5 cm.
2. Experiences a serious adverse reaction or an allergic event suggestive of systemic hypersensitivity requiring more than local therapy (e.g. requiring steroids, bronchodilators, or adrenaline).
3. Becomes pregnant (see section 8 withdrawal/exit criteria).

Conditions for suspending further dosing to all subjects. If any individual in the study experiences a SUSAR further dosing of all subjects at all sites will be suspended pending review by the Data Safety Monitoring Board and discussion with the CI and Sponsor. Advice will be sought from the DSMB regarding re-starting the study.

A substantial amendment application will be submitted to the Regulatory Agencies to resume the trial.

8 WITHDRAWAL/EXIT CRITERIA

8.1 At any time

- Patient withdrawal of consent

8.2 After receipt of first dose

- Potential Serious Adverse Event attributable to the IMP.

8.3 Subject becomes pregnant

- No further doses will be given and the subject will be invited to attend trial follow up visits, to obtain outcome data in accordance with the planned analysis.
- The pregnancy will be recorded on a pregnancy notification form and the subject will be followed up until child birth.

8.4 Treatment of Withdrawn Subjects

8.4.1 Follow-up of Withdrawn Subjects

Care for withdrawn subjects will return to their referring diabetologist, however they will also be invited to attend trial follow up visits, to obtain outcome data in accordance with the planned analysis.

8.4.2 Management of Data from Withdrawn Subjects

All withdrawn patients will be invited to attend follow up visits to provide outcome data for the purposes of the planned analysis.

9 END OF STUDY

REC, the MHRA, MPA and the Regional Ethical Review Board in Linköping will be notified of end of study within 60 days of the last visit of the last participant taking part in the trial.

10 PHARMACOVIGILANCE

10.1 Specification, timing and recording of safety parameters

The major associated safety parameters are evidence of induction of hypersensitivity and exacerbation of β-cell specific autoimmunity with accelerated β-cell loss. To address general safety concerns at screening and 0, 4, 8 and 14 weeks a physical examination will be conducted. A review of AEs will be performed at all visits and blood will be drawn at screening, weeks 4, 9, 14 & 20 to examine the full blood count; urea, electrolytes and creatinine; liver function tests; (prothrombin time, total bilirubin, total protein, albumin, AST (SGOT), SGPT (ALT), alkaline phosphatase; thyroid stimulating hormone; immunoglobulins (G, A, M); calcium; magnesium, phosphate, lipid profile (total cholesterol, LDL, HDL, triglyceride). Urinalysis for pH blood, protein, urine beta-2-microglobulin and albumin/creatinine ratio will be done at screening and visits 1, 2, 4, 5 and 6 and urine for cystatin-c will be collected at visits 1, 4, 5 & 6. A urine pregnancy test will be completed on females only, at all trial visits. At visits 1, 1b, 3b, 4, 5 and 6 blood and urine samples will be taken for gold concentrations to enable assessment of gold excretion. Laboratory measurements for these will be performed at the Midatech Pharma laboratory, Spain.

The safety of the investigational medicinal product (IMP) C19-A3 GNP has been extensively studied in pre-clinical toxicity studies. No signs of toxicity and no significant adverse effects in the organs and the >40 tissues examined were seen, not even at doses that were up to 500-fold higher than the intended dose in this clinical study, and given daily during 14 consecutive days. The studies revealed that the major organ of accumulation and excretion of the gold nanoparticles is the kidney, and to a much lesser extent the liver and spleen. Significant quantities of gold were only detectable in kidneys, which collected on average 30 % of the total dose administered (in rats) over the 14 day period, with a half-life of elimination of circa

17 days. Because the kidney was the most exposed vital organ in the animals used for the pre-clinical safety studies, a number of markers of kidney function will be monitored in the patients. Electrolytes, urea, creatinine, calcium, phosphate, and magnesium levels will be tested in blood. Urinalysis for blood, protein (cystatin-c) and beta-2-microglobulin will be performed in addition. A final analysis will be performed 12 weeks after the last dosing. There will also be an optional analysis at 12 months/ 52 weeks post last dose. For safety reasons, there will be an optional contact hypersensitivity/ skin testing to be performed anytime post final dose.

10.2 Hypersensitivity

Induction of hypersensitivity to C19-A3 GNP will be assessed by a period of observation of subjects and during the immediate period after peptide injection. Local hypersensitivity may manifest as an increasing wheal and flare reaction at the site of peptide injection. The first patient dosed with GNP-peptide will be observed for 6 hours post dose – see section 7.3. Any adverse reactions will be communicated to the other trial treatment site by email, in addition to the standard pharmacovigilance procedures. Subsequent subjects will also be observed for 6 hours. If no serious adverse events are observed post first dose, subsequent visits will have an observation period of a minimum of 1 hour post dose, or 2 hours if there are any symptoms of signs of hypersensitivity. This time period has been chosen as the only anticipated severe reactions is one of hypersensitivity which, if systemic, would be apparent within 60 mins of intradermal dosing.

Observation will be by inspection of the injection site at 5 minute intervals for the first 15 minutes and then 15 minute intervals for a further 45 minutes. If any reaction is present and not resolving over this period, observation will be extended to 15 minute intervals until the reaction is reducing and < 1cm in diameter. Maximum size of any redness or swelling at the injection site will be recorded at each visit at which study drug is given (visits 1-3). Systemic hypersensitivity may manifest as shortness of breath; skin rash; tissue swelling; collapse. Subjects will be inspected for such signs at 5-minute intervals for the first 15 minutes and then at 15 minute intervals for a further 45 minutes. Pulse and blood pressure will be recorded at 15 minute intervals over this time. Any adverse events during this observation period will be recorded. To minimise any risk that might result from hypersensitivity:

- IMP administration should ONLY performed in a clinical research environment appropriate for phase 1 studies in which resuscitation facilities are immediately available.
- Facilities for treatment of a major hypersensitivity reaction, adrenaline, oxygen, intravenous infusion, head-down position, blood pressure and pulse monitoring should be available in the immediate environment of the subject, with access to hydrocortisone and antihistamines.
- Subjects should not be treated by a lone-worker unless he/she has immediate access to clinically trained support.
- Subjects should be directly observed for a minimum of 60 minutes after IMP administration and for up to an additional 2 hours or more if there are symptoms/signs of hypersensitivity.

There is an optional contact hypersensitivity testing or patch testing that can be performed anytime post final dose. This test was amended in per suggestion of the Data Steering Monitoring Board as a safety measure. This test determines delayed Type 4 hypersensitivity to allergens including gold for this study. This test can involve 3 visits (visit 2 is flexible and can be done at home) for the patient including day 0 (patches applied to back or arm of patient), day 2 (patches taken off and first reading- this can be done at home by the patient) and final reading (day 4 or day 7).

10.3 Disease exacerbation

Laboratory tests for exacerbation of β-cell specific autoimmunity are (i) measurement of islet cell autoantibodies (against insulin, GAD-65, IA-2 and ZnT8) at 0 and 14 weeks; and (ii) measurement of pro-inflammatory β-cell specific T cell responses at 0, and 14 weeks.
Clinical manifestation of disease exacerbation will be monitored by measurement of secreted C-peptide AUC after MMTT and stimulated urine c-peptide at 0 and 14 weeks ; HbA1c levels (0, 4, 8 and 14 weeks.); and the record of blood sugars and insulin use at each study visit. Disease specific quality of life questionnaires including hypoglycaemia fear (HFS), treatment satisfaction DTSQ and quality of life ADDQoL will be performed at 0 and 14 weeks.

11 ADVERSE EVENTS

AE collection will be recorded from the time the participant receives their first dose of IMP until one month after the patient's last trial visit.
Adverse events will be monitored, reported in the trial publications and to the Trial Sponsor, Research Ethics Committee (REC), Medicines and Healthcare products Regulatory Agency (MHRA), Medical Products Agency (MPA) and the Regional Ethical Review Board in Linköping if appropriate.

Definitions used will be those under the Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 as follows.

11.1 Definitions

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

An Adverse Reaction (AR) is any untoward and unintended response, in a participant, to an IMP which is related to any dose administered to that participant. An Adverse Reaction is an Adverse Event with a causal relationship to the IMP.

Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Serious Unexpected Suspected Adverse Reaction (SUSAR) is defined as an Adverse Event, Adverse Reaction or unexpected Adverse Reaction that:

- results in death;
- is life-threatening;
  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;
- Requires hospitalisation or prolongation of existing hospitalisation. Hospitalization is defined as an inpatient admission, regardless of the length of stay, even if the hospitalization is a precautionary measure, for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened or elective procedures does not constitute an adverse event;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect; or
- is medically significant;
  Other events that may not result in death are not life-threatening, or do not require hospitalisation may be considered as 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.

Pregnancy in either a participant or the partner of a participant taking trial medication will be recorded on a pregnancy notification form and the subject will be followed up until child birth.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an adverse reaction that is both serious and unexpected.

11.2 Eliciting Adverse Events

All trial subjects will have an enquiry about co-morbidities, medications and new or unexpected symptoms at each follow-up visit, as well as diabetes assessment.

11.3 Principal Investigator/Trial Centre Responsibilities

The PI will be required to report all AEs/SAEs which occur during the Trial on an Adverse Event Form and keep a record in the case report form (CRF). AEs will be collected from the time the participant receives their first dose of IMP until one month after the last trial visit (visit 6, week 20). The AEs will be reported at agreed intervals to the DSMB and if there is any concern a DSMB meeting will be convened.

11.4 Assessing AEs

The PI should assess the AE for seriousness and causality. The PI should exercise medical judgement in deciding whether an Adverse Event/Reaction is serious in other situations. All SAEs should be reported to STU by the PI immediately (within 24hrs) of becoming aware of the event. The PI will be asked to grade all AEs in relationship to the study treatment according to their clinical judgement as follows:

Causality:

- **Not related** – temporal relationship of the onset of the event, relative to administration of the product, is not reasonable or another cause can by itself explain the occurrence of the event.
- **Unlikely to be related** – temporal relationship of the onset of the event, relative to administration of the product, is likely to have another cause which can by itself explain the occurrence of the event.
- **Possibly related** – temporal relationship of the onset of the event, relative to administration of the product, is reasonable but the event could have been due to another, equally likely cause.
- **Probably related** – temporal relationship of the onset of the event, relative to administration of the product, is reasonable and the event is more likely explained by the product than any other cause.
- **Very likely** – temporal relationship of the onset of the event, relative to administration of the product, is reasonable and there is no other cause to explain the event, or a re-challenge is positive.

11.5 Reporting procedures

All SAEs must be reported immediately (within 24 hrs of being made aware of the event) by the PI to STU within 24 hours of receipt.

Initial reports should be submitted as soon as the following minimum criteria are met:

- A suspected SAE is identified;
- An identifiable participant (e.g. trial participant code number);
- An AE assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship;
- An identifiable reporting source (e.g. clinical research fellow).

Following the Initial Report, all SAEs should be followed to resolution. Following the initial report the PI may be requested to provide further information. The participant should only be identified by trial number, date of birth and initials. The participant's name should not be used on any correspondence. The PI is also responsible for reporting AEs to their NHS Trust as per their local NHS Trust procedures.

11.6 Sponsor and Chief Investigator Responsibilities

The Sponsor (or delegate – STU) is responsible for ensuring all SAEs, SARs and SUSARs (except those specified in this protocol as not requiring reporting) will be reported in the appropriate timescale to the MHRA, REC, MPA and the Regional Ethical Review Board (RERB) in Linköping.

STU is responsible for ensuring all SUSARs from Cardiff and Linkoping (except those specified in this protocol as not requiring reporting) will be reported in the appropriate timescale to the MHRA, REC. MPA and RERB.

Once an SAE is received by STU, the SAE will be sent to the CI (or appropriate delegate) for clinical review (assessment of causality and expectedness). Fatal and life threatening SAEs should be assessed by the CI within 24 hours of receipt. Non-fatal or non-life-threatening SAEs should be assessed by the CI within 4 days of receipt.

Only SUSARs should be expedited to MHRA, MPA, RERB and REC. STU (UK) and Karolinska Trial Alliance (Sweden) will report according to the following timelines:
Fatal and life threatening SUSARs not later than 7 days after receipt; Non-fatal or non-life-threatening SUSARs not later than 15 days after receipt. Follow up information should be reported within 8 days of receipt of the follow up information. A copy of the SUSAR report should be provided to the Sponsor.

In addition to reporting to the relevant REC, RERB, MHRA and MPA, SUSARs will also be reported to all members of the TMG. The CI shall ensure that all co-investigators receive regular safety updates of SAE's and SUSARs that occur in relation to the IMP in the trial.

Dose interrupting and suspension of dosing will be managed as per the risk mitigation, see section 7.

The CI is responsible for assessing expectedness
No events other than those listed in section 10.2 are expected.

11.7 Urgent Safety measures

Urgent safety measures which are implemented immediately by the PI should be notified to the Trial Manager immediately, along with the reasons for the measure being taken.
The CI (or delegate – STU) shall be responsible for reporting urgent safety measures to MHRA, MPA, RERB and REC. This should include an initial contact with a medical assessor at the MHRA/MPA to discuss the urgent safety measure, followed by a detailed written report within 3 days. The report should include details of the urgent safety notification, the measures taken and the reason for the measures and details of any discussion with the MHRA and MPA medical assessor.
See section 8 for treatment stopping and withdrawal criteria.

11.8 Safety Report/ Line Listing

One year following the granting of a CTA, and thereafter annually, the CI (or delegate – STU) should compile an annual safety report & DSUR for submission to the MHRA and REC, UK and the MPA and the Regional Ethical Review Board in Linköping.

12 ASSESSMENT OF EFFICACY

Although not primarily a study designed to assess efficacy, laboratory and clinical measurements will be made that may indicate beneficial therapeutic trends associated with C19-A3 GNP administration. These have been specified as secondary endpoints.

12.1 Efficacy Parameters

12.1.1 Metabolic outcomes

Residual insulin production will be assessed by measurement of stimulated C-peptide production during the MMTT using the area under the curve (AUC) approach. The normalized AUC will be calculated for each subject at 14 weeks and compared versus baseline (visit 1). Stimulated urine c-peptide creatinine ratio will also be measured as an alternative measurement.

Likewise, change in glycated haemoglobin (as measured by % HbA1c levels), daily insulin usage, and mean amplitude of glucose excursions will be measured/recorded as specified in the schedule of visits and compared with baseline.

For objective assessment of hypoglycaemia continuous glucose monitoring will be conducted for 3 days at baseline and prior to the final follow up visit. Hypoglycaemia frequency and standard deviation and interquartile range of glucose variability will be calculated from this data.

12.1.2 Immunological outcomes

Change in level or quality of T lymphocyte biomarkers of β-cell specific immune response will be assessed at 14 weeks (8 weeks for lymph nodes sampling) versus baseline, Levels of T lymphocyte biomarkers are measured as the numbers of cytokine-producing T cells responding to PPI C19-A3 or other β-cell autoantigenic peptides and the numbers of cytokine and other immune gene transcripts produced in response to stimulation with PPI C19-A3 or other β-cell autoantigenic peptides in vitro. A potentially important biomarker of therapeutic efficacy would be an increase in the number of responder T cells synthesizing the immune regulatory cytokine IL-10, or a decrease in the number of responder T cells synthesizing the pro-inflammatory cytokines IFN-γ.

Changes in the quality of the immune response can also be measured by assays that specifically detect the ability of post-treatment CD4 T cells to regulate pre-treatment pro-inflammatory responses.

Changes in the level or quality of islet cell autoantibody biomarkers of β-cell specific immune response will be measured at 0, 14 weeks and compared with levels at baseline and between

groups. A potentially important biomarker would be a reduction in levels of islet cell autoantibodies. Changes in serum levels of cell free DNA derived from beta cells and T cells will be measured to provide potentially important biomarkers of beta cell and T cell death.

12.2 Procedures for Assessing Efficacy Parameters

12.2.1 Insulin secretion

C-peptide production in the MMTT assay will be assessed using the AUC approach. Levels of glucose, and C-peptide will be assessed from blood samples taken during the test. Stimulated C-peptide will be the area under the (C-peptide/time) curve from time 0 to 120 minutes, and this will be calculated by the trapezoidal rule. The AUC will be normalized for time interval by dividing it by the number of minutes over which AUC was determined; the number is expected to be 120 minutes but may differ in some cases. A urine sample after 120 minutes will be taken for urine C-peptide creatinine ratio which has been proposed as a surrogate marker of insulin secretion.

12.2.2 Insulin usage

Subjects will record the daily insulin usage in their capillary blood glucose testing meters supplied for the study. Insulin usage will be recorded daily from the screening stage through to the end of the study. In particular, insulin usage should be recorded accurately for at least 7 consecutive days during the 2 weeks prior to visits at 0, 4, 8 weeks. The mean daily insulin usage over the 7 consecutive days (IU units/kg body weight/day) will thus be calculated for each subject.

12.2.3 Blood glucose variability

Mean amplitude of glucose excursion (MAGE) will be calculated at 0 and 14 weeks using records from 72 hour CGM recordings.

12.2.4 Immunological outcomes

See sections 6.15 and 12.1.

13 STATISTICS

The primary aim of this study is provide patient safety data.
The study will be open label.

14 SAMPLE SIZE

The single group study provides 8 patients on active treatment from whom it will be possible to collect safety data. This is considered optimal to achieve sufficient data to inform future study designs and on which to base future sample size calculations.

15 PLANNED ANALYSIS

15.1 Final

Data cleaning and preparation process will be carried out prior to analysis.
All patients enrolled will be followed up and included unless they withdrew from the study before the administration of the first dose. An intention to treat analysis will be carried out. Per protocol analyses may also be carried out alongside the intention to treat analysis if necessary. The analyses will mostly consist of tables of data such as mean values (or geometric means if the data is log normal) together with standard deviations and ranges. For data such as scores, medians, and ranges may be used instead, and binary outcome variables will use percentages. Comparisons against baseline will be made using Student's t-tests for paired and unpaired samples; Mann-Whitney U test; Wilcoxon matched pairs test; p values <0.05 will be considered significant.
Since the primary aim of the study is to provide patient safety data and guide the development of an antigen specific immunotherapy programme, it may be a requirement for strategic planning of subsequent stages of clinical study development, that early analysis of trial data is conducted.

16 QUALITY ASSURANCE, SITE MONITORING AND DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections (where appropriate) by providing direct access to source data and other documents (ie patients' case sheets, blood test reports, X-ray reports, histology reports etc). Monitoring of this trial to ensure compliance with Good Clinical Practice and scientific integrity will be conducted by STU for the Cardiff site and Forum Östergötland at Linkoping who will have direct access to source data.

Source documents produced for this trial will be kept in the patient's hospital records and source data will be transcribed into trial-specific Case Report Forms (CRFs) at the end of each patient visit. These CRFs will be coded with the study number and will not include patients' names and addresses.

Selected anonymised data will also be stored in electronic format. Paper records will be kept in a locked cabinet in secure premises at all times when the record is not in use for a study visit. Access to the records will be restricted to researchers working on the study, Sponsor representatives and representatives of regulatory authorities required to audit the conduct of the research study.

Relevant data will be transferred (with double data entry/data checking) from the paper record in the trial office to an electronic database which will be stored and regularly backed up on a Cardiff University server. Identifiable data including the link between the patients'

names and the study number will be stored separately from other data. All files will be password protected. Electronic data containing personalised information will be saved on Cardiff University computers only in password protected files and backed up regularly to hard copy on secure remote Cardiff University servers. Participant data will be anonymised by the use of study numbers. A copy of the study number code identifying subjects will be kept in a secure cabinet at local study sites accessible to the investigators at all times. Analysis will be conducted by the study team. Analysis will only be conducted on anonymised data.

The Chief Investigator, Professor Colin Dayan will act as custodian of the data, however for practical purposes this role will be delegated to STU. Personal data will be stored for a minimum of 15 years. Access will be controlled by Professor Dayan who will continue to act as custodian for all data held by the Sponsor and will permit trial related monitoring, audits, REC review, and regulatory inspections (where appropriate) by providing direct access to source data and all other documents (i.e. patients' case sheets, blood test reports, etc).

17 ETHICS AND REGULATORY APPROVALS

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (2013), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted via the Integrated Research Application System for review by a Research Ethics Committee (REC), Regional Ethical Review Board (RERB) and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation and to the Medical Products Agency (MPA) for approval.

The Chief Investigator will submit annual progress and safety reports and a final report at conclusion of the trial to the REC and the MHRA within the timelines defined in the Regulations.

18 FINANCIAL AND INSURANCE ASPECTS

Support for this trial has been granted by the European Commission FP7 programme.

Cardiff University has arranged clinical research insurance to cover the legal liability of the University for negligent harm. In addition the study doctors hold substantive or honorary NHS contracts, giving them the protection of the NHS clinical negligence arrangements. Swedish patients will be covered by Patientskadeförsäkringen.

19 PATIENT INVOLVEMENT

Involving patients from the outset strengthens the application by showing that the research is patient-centred and that patient participation has been welcomed, encouraged and facilitated. Our patient representative(s) will be involved in:

- Trial management meetings;
- Trouble shooting to support recruitment and retention of participants;
- Reporting the final results to the trial participants;
- Preparing information for web site inclusion.

The study will be registered on the NIHR INVOLVE and HCRW Involving People (Cynnwys Pobl) databases once ethical approval is granted. These are national advisory groups that support greater public involvement in NHS, public health and social care research. INVOLVE provides a public information pack, newsletters and public networking to facilitate public involvement. Locally, Involving People provides excellent training and support for public involvement via local conferences, newsletters and public network.

Patient involvement, will also facilitate dissemination of the study findings to as wide an audience as possible and will help set the agenda for future research in this field. Training and support for our patient representatives will be flexible and tailored to their individual needs. It will include training on the study background, methods and outcomes and where indicated IT training will be provided. Patients will be reimbursed in line with HCRW's AcoRD guidance (Attributing the costs of Health and Social Care Research & Development).

20 PUBLICATION POLICY

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. Written feedback will also be provided to the study participants.

21 TRIAL MANAGEMENT

21.1 Trial Steering Committee (TSC)

1. For a small phase 1 trial such as this, it is not considered that a TSC is required.

21.2 Trial Management Group (TMG)

The project will be run by a Trial Management Group comprising PI's, trial manager, clinical research fellow, statistician (CTU staff), chief investigator and patient representatives and Sponsor representation. The TMG will oversee the day to day trial management and will meet in person or by teleconference at a minimum 2 monthly for the duration of the study. The TMG will overview and provide guidance on all aspects of regulatory approval, set-up, recruitment, protocol deviations, adverse events, data management, data analysis and dissemination. The TMG will report at agreed intervals to the DSMB and to the study sponsor.

21.3 Data safety Monitoring Board (DSMB) members:

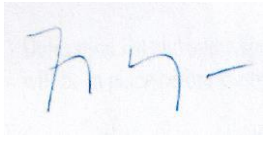
An independent Data Safety Monitoring Board (DSMB) will be convened for the duration of the trial comprising a consultant diabetes physician, a clinical immunologist and a consultant rheumatologist. The DSMB will meet (face-to-face or by teleconference) at agreed intervals throughout the trial and receive reports of all adverse events from the Chief Investigator and/or other members of the trial team, as well as assessing progress in recruitment. The chairman of the DSMB may also call additional meetings as necessary. The DSMB will have responsibility for suspending or terminating treatment of one or a group of subjects if subject safety is considered at risk.

22 SIGNATURE PAGE

Investigator's Statement of Agreement: I have read, understand, and will conduct the study according to this protocol and Good Clinical Practice

Chief Investigator
Professor Colin Dayan

Date: 18th July 2018



20th July 2018

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Principal Investigator
Professor Johnny Ludvigsson

Date

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