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Phase III, randomised, double-blind, placebo-controlled, multicentre trial to evaluate the efficacy and safety of rhGAD65 to preserve endogenous beta cell function in adolescents and adults with recently diagnosed type 1 diabetes, carrying the genetic HLA DR3-DQ2 haplotype: the DIAGNODE-3 study protocol

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ABSTRACT

Introduction Type 1 diabetes (T1D) is an autoimmune disease leading to the destruction of the insulin-producing beta cells resulting in insulin deficiency and hyperglycaemic. Today, no approved therapy exists to halt this detrimental immunologic process. In a recent phase 2b study, intralymphatic administration of recombinant human glutamic acid decarboxylase 65 kDa (rhGAD65) adsorbed to Alhydrogel adjuvant to individuals recently diagnosed with T1D and carrying the HLA DR3-DQ2 haplotype showed promising results in preserving endogenous insulin secretion, confirming the results of a large meta-analysis of three randomised placebo-controlled trials of subcutaneous rhGAD65. The aim of the current precision medicine phase 3 study is to determine whether intralymphatic administration of rhGAD65 preserves insulin secretion and improves glycaemic control in presumed responder individuals with recently diagnosed T1D carrying HLA DR3-DQ2.

Methods and analysis Individuals ≥12 and <29 years recently diagnosed with T1D (<6 months) will be screened for the HLA DR3-DQ2 haplotype, endogenous insulin production estimated by fasting C-peptide and presence of GAD65 antibodies. 330 patients are planned to be randomised to 3 monthly intralymphatic injections of rhGAD65 or placebo (both accompanied by oral vitamin D supplementation), followed by 22 months of follow-up. The study is powered to detect a treatment effect in the two coprimary endpoints; change from baseline in $AUC_{(0-120min)}$ C-peptide levels during a mixed meal tolerance test, and change from baseline in glycaemic control estimated by haemoglobin A1c at 24 months. Secondary endpoints include effects on glucose patterns collected by masked continuous glucose monitoring, proportion of patients in partial remission and number of episodes of severe hypoglycaemia and/or diabetic ketoacidosis.

Ethics and dissemination The trial is approved by Ethics Committees in Poland (124/2021), the Netherlands (R21.089), Sweden (2021-05063), Czech Republic (EK-1144/21), Germany (2021361) and Spain (21/2021). Results will be published in international peer-reviewed scientific journals and presented at national and international conferences.

Trial registration number EudraCT identifier: 2021-002731-32, NCT identifier: NCT05018585.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The current study is a large, international, multicentre, randomised double-blind placebo-controlled trial.
⇒ The study is adequately powered to detect a treatment effect on two clinically important coprimary endpoints; preservation of beta cell function and glycaemic control (haemoglobin A1c).
⇒ The total study duration of 24 months should allay concerns about confounding from the so-called honeymoon period in recently diagnosed type 1 diabetes (T1D).
⇒ This is the first phase 3 trial in T1D using a precision medicine approach, limiting recruitment to the identified HLA DR3-DQ2 responder population to recombinant human glutamic acid decarboxylase 65 kDa treatment.
⇒ To fully understand the magnitude of a possible beneficial treatment effect, additional follow-up over several years might be needed to see the benefits of even minimum residual beta cell function.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disorder in which the immune system attacks the insulin producing beta cells in the pancreas. By the time an individual is diagnosed with T1D, 70%–90% of beta cell function has generally been lost. The destruction of the pancreatic beta cells in T1D is associated with cellular immune responses towards the pancreatic islet cells.[1] Autoantibodies directed against glutamic acid decarboxylase (GAD) with a molecular mass 65 kDa (GAD65A), insulinoma-associated protein 2 (IA-2A), insulin (IAA) or zinc transporter antigen T8 (ZnT8A) precede the clinical onset of the disease.[1]

T1D treatment consists of lifelong administration of exogenous insulin, which does not satisfactorily prevent neither acute nor long-term complications. The disease has a devastating impact on the quality of life (QoL) of the affected person and their family due to the constant stress of adjusting blood sugar and the common acute and life-threatening consequences of imperfect control—diabetic ketoacidosis (DKA) and severe hypoglycaemia.[2–4] In addition, many individuals with T1D experience over the long term both macrovascular and microvascular complications affecting the heart, nerves, eyes and kidneys, putting them at risk of blindness, kidney failure and myocardial ischaemia.[5 6] A recent article[7] concluded that patients who received their diagnosis before the age of 10 years had a shortened lifespan by 14 years for males and 18 years for females. Early-onset T1D was also found to be associated with 30 times increased risk of serious cardiovascular outcomes and for women, this risk was 90 times higher compared with non-diabetic control persons.[7] Even with good long-term blood glucose control (haemoglobin A1c, HbA1c ≤52 mmol/mol), the risk of premature death for any T1D patient is found to be twice as high as for healthy individuals and up to eight times higher for patients with poor glycaemic control.[8]

There is currently no approved treatment preventing the destruction of beta cells. Insulin replacement therapy is the standard-of-care treatment, and despite the development of new insulins, new technologies for insulin administration and blood glucose diagnostics, patient targets for long-term blood glucose are currently met less frequently than 5 years ago in some populations in the USA.[9] Any intervention which can stop or delay the loss of beta cell function would likely provide protection against hypoglycaemia and ketoacidosis, improve metabolic control, decrease blood glucose fluctuations, facilitate treatment and delay and/or reduce microvascular and macrovascular complications of diabetes.[10–13] In addition, decreasing the autoimmune destruction of beta cells could allow for beta cell replenishment, either through regeneration or transplantation.

The most efficient immune therapy for preservation of beta cell function is so far treatment with anti-CD3 monoclonal antibodies (teplizumab).[14 15] TNF-alfa inhibitors,[16] anti-thymocyte globulin,[17] alefacept[18] and rituximab[19] have also demonstrated some efficacy in preserving beta cell function, but often these therapies have adverse events (AEs), serious risks and impose a heavy treatment burden, including, for example, several days of intravenous infusions. An alternative approach is treatment with autoantigen immunotherapies, even though most clinical trials with autoantigen immunotherapies have failed to meet their primary endpoints or shown inconclusive results.[20–23]

Over the last two decades, however, an important development in the field has meant a shift away from a one-size-fits-all approach to T1D pathophysiology towards a more individualised, precision medicine approach that recognises inter-individual heterogeneity in T1D.[24] The concept of disease heterogeneity has recently been extended to the concept of endotypes; that is, subtypes of T1D with distinct underlying pathobiological mechanisms, which should be considered in the design of clinical trials.[25] For instance, the appearance of GAD65 autoantibodies (GADA) as the first autoantibody is linked to the juman leucocyte antigen (HLA) DR3-DQ2 haplotype, while the emergence of insulin autoantibodies as the first antibody is linked to HLA DR4-DQ8.[25 26] As a consequence, applying the same intervention targeting a specific pathophysiological mechanism across an entire population ignores the fact that subgroups of patients whose disease is driven by the targeted mechanism may respond particularly well, while others show no response, resulting in apparently absent treatment effects across the entire population.

GAD antigen-specific immunotherapy to preserve endogenous insulin secretion

GAD65 is a major autoantigen in autoimmune diabetes, and clinical administration of purified recombinant human GAD65 rhGAD65 aims to intervene in the autoimmune process in T1D. The rhGAD65 is adsorbed to Alhydrogel (aluminium hydroxide particles) and formulated in phosphate buffer with mannitol. The intended mode of action is to slow or prevent autoimmune destruction of pancreatic beta cells by modulation of immune responses to GAD65. Inconsistent results observed in trials testing subcutaneous administration of rhGAD65 spurred the evaluation of alternative approach to improve treatment efficacy.[20–22 27] DIAGNODE-1,[28 29] a phase 1/2a open-label pilot combination trial evaluated an alternative administration route, with three doses of 4 µg rhGAD65 administered directly into inguinal lymph nodes, in combination with oral vitamin D in 12 patients (12–30 years of age) recently diagnosed with T1D. All patients were followed for 30 months. The positive results of the DIAGNODE-1 trial[29] supported further development in a Phase 2b trial (DIAGNODE-2), a randomised and placebo-controlled trial testing the intralymphatic administration in 109 patients (12–24 years of age) recently diagnosed with T1D. Importantly, based on the concept of heterogeneity of disease mechanism, a meta-analysis of three previous Randomized Control Trials testing subcutaneous rhGAD65 was performed. The analysis showed that

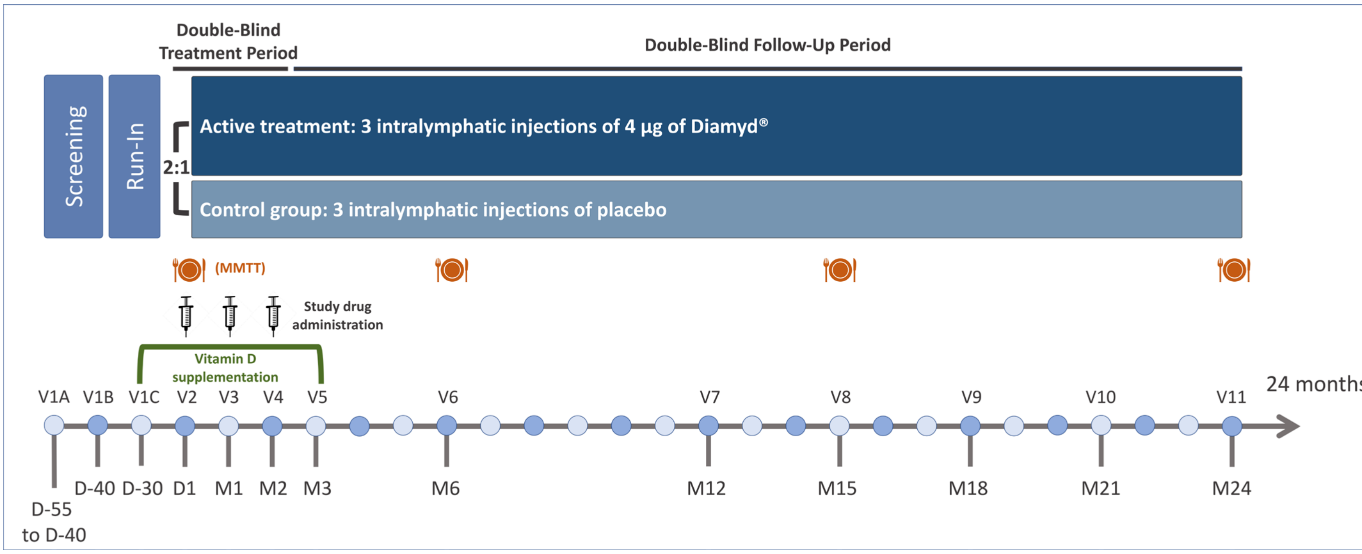

Figure 1 Schematic overview of the study design.

clinical efficacy is mainly seen in patients with HLA DR3-DQ2, and an even more pronounced treatment effect was seen in those individuals with HLA DR3DQ2 without HLA DR4-DQ8 though no clinical efficacy was observed in the full population.[30] Due to the identification of HLA DR3-DQ2 patients as the responder population, the statistical analysis plan of the then ongoing DIAGNODE-2 study was amended before database lock to include analyses of the primary and secondary endpoints in the HLA DR3-DQ2 subgroup in the topline results. At 15 months of follow-up in DIAGNODE-2, rhGAD65 treatment showed a significant positive treatment effect in the prespecified genetic subgroup of patients positive for HLA DR3-DQ2 of 55.7% (p=0.0078), that is, that on average, the primary end point stimulated C-peptide secretion Area Under the Curve $(AUC)_{mean\ 0\text{-}120\ min}$ declined by 55.7% less in patients treated with rhGAD65 compared with patients treated with placebo. For patients positive for HLA DR3-DQ2, C-peptide $AUC_{mean\ 0\text{-}120\ min}$ declined approximately 28% over 15 months in the rhGAD65 group compared with about 58% for placebo.[31] There were corresponding trends, though not statistically significant, in improvement in the secondary efficacy variables HbA1c, IDAA1c and exogenous insulin use after 15 months in the HLA DR3-DQ2-positive patients treated with rhGAD65 when compared with placebo.

Results from an updated meta-analysis (manuscript in preparation) which added data from DIAGNODE-2 to the previous meta-analysis[30] showed that in patients carrying the HLA DR3-DQ2-haplotype, a statistically significant treatment effect on change in AUC C-peptide of 48.3% for the subjects receiving 3 or 4 injections of rhGAD65 (p<0.0001) and 36.1% for the 2–4 injections (p=0.0316) . In addition to this, a statistically significant treatment effect on change in HbA1c of −4.789 mmol/mol for the subjects receiving 3 or 4 injections of rhGAD65 (p=0.0044) and −3.120 mol/mol for subjects receiving 2–4 injections (p=0.032). The data also reconfirmed previous findings that an even more pronounced treatment effect (on both change in AUC C-peptide and HbA1c) was seen in those individuals with HLA DR3DQ2 without HLA DR4-DQ8. Intralymphatic rhGAD65 injections were well tolerated and considered safe, consistent with prior clinical trial findings.[31]

Objectives

The primary objective is to evaluate the effect of three doses of rhGAD65 compared with placebo in terms of[1] beta cell function; and[2] glycaemic control in adolescents and adults recently diagnosed with T1D, who carry the HLA DR3-DQ2 haplotype. Secondary objectives are to compare the effect of rhGAD65 to placebo treatment with respect to the effects on other diabetes disease management indicators and long-term safety.

METHOD AND ANALYSIS

Overall study design

DIAGNODE-3 is a phase III randomised, double-blind, placebo-controlled, international, multicentre, parallel-arm, 24-month trial in adolescents and adults with recently diagnosed T1D, carrying the HLA DR3-DQ2 haplotype. Overall study design is shown in figure 1. The study is registered on Clinicaltrials.gov (NCT05018585). The study is expected to take 4.5 years to complete. This includes an intervention with follow-up for 24 months.

Throughout the study duration, all patients will receive standard-of-care routine treatment for their diabetes according to ADA guidelines (amended as appropriate to reflect local standard of care).

Screening and run-in period

Patients deemed eligible and/or their parent(s)/legal guardian(s) will have the study explained to them and will receive written patient information. After having had the time to review the study, they will have the opportunity to ask questions to the investigational team. After this, if the patient agrees to participate, they will sign and date the written informed consent form and for patients who are minors, both age-appropriate assent (according to local regulations) and parent's/caregiver's consent is collected. Patients and their parent(s)/legal guardian(s), when applicable, will provide written informed consent before any study-related procedures are performed. The patient and/or their parent(s)/legal guardian(s) will then receive a copy of the signed and dated patient information. Detailed study assessments are shown in table 1. HLA genotyping of the patient is performed at the first screening visit after preliminary eligibility is confirmed. If the patient is carrying the HLA DR3-DQ2 haplotype, the patient will attend the second screening visit (visit 1B) to perform the remaining screening procedures. After screening, patients deemed eligible will proceed to the run-in period (beginning at visit 1C) undergo masked CGM for 14 days, receive diabetes education and collect self-reported diabetes information in their eDiary.

Patients with a screening vitamin D level <100 nmol/L (40 ng/mL) will start oral vitamin D supplementation (2000 IU daily) beginning at visit 1C, 30 days prior to randomiszation. During the period of supplementation, vitamin D should be discontinued temporarily if the level exceeds 125 nmol/L (50 ng/mL) and may be resumed when levels are <100 nmol/L (40 ng/mL).

Double-blind treatment period and long-term follow-up

At visit 2, patients will be randomised 2:1 to one of the following two treatment groups:

Treatment group 1:	3 intralymphatic injections of 4 µg (0.1 mL) of rhGAD65 administered on days 0, 30 and 60
Treatment group 2:	3 intralymphatic injections of 0.1 mL placebo administered on days 0, 30 and 60

Randomisation will be performed by an Interactive Web Response Systems and stratified by HLA subgroup (presence or absence of HLA DR4-DQ8) and by region. The maximum number of adults (>18 years) recruited into the trial is 160. rhGAD65 or placebo injections will be administered in the inguinal lymph node by qualified personnel with the help of ultrasound. Vitamin D levels will be monitored throughout the trial. Vitamin D oral supplementation (2000 IU daily) will be administered from day −30 (Visit 1C) to day 90 for a total of 120 days for patients with a level<100 nmol/L (40 ng/mL) atscreening. All patients will continue to receive intensive insulin therapy via multiple daily injections or via CSII. Safety will be assessed via physical examinations, neurological assessments, vital signs, clinical laboratory assessments, injection site reactions and AEs. After the double blinded treatment period of 2 months, patients will be followed in a blinded manner for 22 months. An independent DSMB will be appointed to review unblinded safety data (at least twice a year).

Study population

Individuals between ≥12 and <29 years old, will be eligible for enrollment if they have been diagnosed with T1D within the previous 6 months at the time of screening, positive for the HLA DR3-DQ2 haplotype, fasting C-peptide is ≥0.12 nmol/L (0.36 ng/mL) and seropositive for GADA. Full list of inclusion and exclusion criteria is shown in tables 2 and 3.

Study assessments

Demographics and study procedures

Demographics, baseline data medical history and family history of T1D will be collected at screening. Medical examinations (ie, physical, neurological and vital signs) will be performed at all on site visits. Patients will also be provided with an eDiary to collect self-reported data on daily insulin dose, injection site reactions, significant glucose events (mild/moderate/severe hypoglycaemic events and DKAs), as well as mealtimes and physical activity. Patients and caregivers (if applicable) will answer the Paediatric Quality of Life Inventory questionnaires at four visits between baseline and month 24 to assess QoL. Timings of all assessment can be found in table 1.

Clinical laboratory assessments

Laboratory assessments of diabetes status

The timing of all study assessments is presented in table 1 All laboratory parameters will be analysed at a central laboratory. A 2-hour mixed meal tolerance test (MMTT) following an overnight fast (>10 hours) will be performed at baseline and at month 6, month 15 and month 24. Meal stimulated plasma glucose and C-peptide levels will be assessed throughout the MMTT.[32]

Patients should come to the study site following an overnight fast (>10 hours) and have a plasma glucose level between 3.5 and 12 mmol/L (63–216 mg/dL) at home in the morning. Patients are allowed to take basal-insulin day/night before, but not in the morning before the MMTT. Patients should also not administer any short/direct acting insulin within 6 hours before the MMTT. Patients with CSII (insulin pump) must continue with their basal dose insulin, but not add any bolus dose during the last 6 hours before the MMTT.

Samples for HbA1c will be analysed at a National Glycohemoglobin Standardization Program (NGSP) certified central laboratory. Results will be reported in both International Federation of Clinical Chemistry (mmol HbA1c/mol Hb) and NGSP (% HbA1c) units. Serum samples for fasting glucose and fasting C-peptide levels will also be collected and analysed throughout the trial.

Safety and other laboratory assessments

All patients will undergo HLA class II genotyping to assess the presence of HLA haplotypes DR3-DQ2 and DR4-DQ8 during the screening procedure. Samples will also be collected for clinical chemistry, haematology, urinalysis, lipids (total cholesterol, LDL-C, HDL-C and triglycerides), vitamin D, thyroid stimulating hormone,

Table 1 Schedule of assessments in diagnode-3

	Screening		Run-In	Double-blind treatment period			Double-blind follow-up period								
Visit	**1A**	**1B**	**1C**	**2 (Baseline)**	**3**	**4**	**5 ☎**	**6**	**7**	**8**	**9**	**10 ☎**	**11 EoS**	**ET**	**UNS Visit**
Trial day	**−55 to −40**	**−40**	**−30**	0	30	60	90	180	360	450	540	630	720		
Trial month	**-2**		**-1**	1	1	2	3	6	12	15	18	21	24		
Visit window (days)		±5	±5	0	**±5**L	**±5**L	±14	±14	±14	±14	±14	±14	±14		
Informed consent	X														
Informed consent for genomic sub-study									X						
Review eligibility criteria	X	X	X	X											
Demographic information	X														
Medical history	X	X													
Family history of T1D	X														
Examinations															
Physical examination		X		X	X	X		X	X	X	X		X	X	X
		Complete		Limited	Limited	Limited		Limited	Limited	Complete	Limited		Complete	Complete	Limited
Neurological assessment		X		X	X	X		X	X	X	X		X	X	X
Tanner staging (if <18 years)				X				X	X		X		X		
Vital signs		X	X	X	X	X		X	X	X	X		X	X	X
Height and weight		X		X	X	X		X	X	X	X		X	X	X
Trial activities															
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diabetes education			X			X			X		X				
Adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X
Vitamin D supplementation if <100 nmol/L (40 ng/mL)			X	X	X	X	X								
			Start				End								
MMTT[j]				X				X		X			X		
Continuous glucose monitoring (blinded)			X					X		X			X		
Randomisation				X											
Intralymphatic study drug administration				X	X	X									
Injection site inspection, site staff				X	X	X									
Monitoring of target and adjacent lymph nodes using ultrasound				X	X	X									
eDiary															
Distribution of eDiary			X												
Review of eDiary				X	X	X	X	X	X	X	X	X	X	X	X
Glucose events collection in eDiary			X	X	X	X	X	X	X	X	X	X	X		X
Review of injection site inspection, eDiary					X	X	X								
Patient-reported activities in eDiary			X					X		X			X		

Continued

Table 1 Continued

Visit	Screening		Run-In	Double-blind treatment period			Double-blind follow-up period								
	1A	1B	1C	2 (Baseline)	3	4	5 ☎	6	7	8	9	10 ☎	11 EoS	ET	UNS Visit
Daily insulin collection in eDiary			X	X	X	X	X	X	X	X	X	X	X		
Blood samples for diabetes status															
Fasting C-peptide		X		X	X	X		X	X	X	X		X	X	
MMTT-induced C-peptide				X				X		X			X		
MMTT-induced glucose				X				X		X			X		
HbA1c		X		X	X	X		X	X	X	X		X	X	
Fasting plasma glucose		X		X	X	X		X	X	X	X		X	X	
Blood and urine sampling for safety, genetics, vitamin D levels and immunology															
Haematology		X		X				X	X	X	X		X	X	
Clinical chemistry		X		X	X	X		X	X	X	X		X	X	
Lipid panel (fasting)				X						X			X		
TSH (reflex Free T4), TPO		X								X			X		
Transglutaminase antibody titre				X						X			X		
IA-2 antibody titre				X						X			X		
Vitamin D[s]		X		X	X	X		X	X	X	X		X		
Pregnancy testing, (FOCBP only)		X		X	X	X		X	X	X	X		X		
		Serum		Urine	Urine	Urine		Urine	Urine	Urine	Urine		Urine		
FSH, LH		X		X		X				X	X		X		
Microalbuminuria (UACR)				X				X	X	X	X		X		
GAD65A titre		X		X	X	X		X	X	X	X		X	X	
HLA class II genotyping	X														
COVID-19 antibody testing				X						X			X		
Other immunologic parameters				X	X	X		X		X			X		
DNA sample collection for genomic substudy									X						
Quality of life assessments															
PedsQL questionnaires				X				X		X			X		

eDiary, electronic diary; EoS, end of study; ET, early termination; FOCBP, females of childbearing potential; FSH, follicle stimulating hormone; GAD65A, glutamic acid decarboxylase with molecular mass 65 kDa antibody; HbA1c, haemoglobin A1c; HLA, human leucocyte antigen; IA-2, insulinoma-associated protein 2; LH, luteinising hormone; MMTT, mixed meal tolerance test; PedsQL, Paediatric Quality of Life Inventory; T4, thyroxine; T1D, type 1 diabetes; TPO, thyroid peroxidase antibody; TSH, thyroid stimulating hormone; UACR, urine albumin to creatinine ratio; UNS, unscheduled.

Table 2 Inclusion criteria in the diagnode-3 study

Patients are eligible to be included in this study if all the following criteria apply	
1.	Must be capable of providing written, signed and dated informed consent; and for patients who are minors, age-appropriate assent (performed according to local regulations) and parent/caregiver consent.
2.	Males and females aged ≥12 and <29 years old at the time of screening.
3	Diagnosed with T1D (according to the American Diabetes Association (ADA) classification) ≤6 months at the time of screening.
4	Possess the HLA DR3-DQ2 haplotype (all patients will be tested; prior genetic testing results will not be accepted).
5	Fasting C-peptide ≥0.12 nmol/L (≥0.36 ng/mL) on at least one occasion (maximum two tests on different days during the screening period).
6	Possess detectable circulating GAD65 antibodies (lowest level of detection defined by the method used by the central laboratory).
7	Possess HbA1c levels between 35 to 80 mmol/mol (5.4 to 9.5%) on at least one occasion prior to randomisation (maximum one additional test within 1 month from Visit 1B).
8	Be on a stable insulin dose or insulin dosing regimen for 1 month prior to inclusion with limited fluctuation of daily insulin requirement based on investigator's assessment. For example, if the average insulin dose/kg/24 hours over a 7 day period compared with the previous 7 day period does not vary more than approximately 15% and/or if the daily insulin dose does not vary more than 0.1 U/kg/24 hours, the dose can be considered stable. Individuals that are diagnosed with T1D according to the ADA classification but are not taking insulin are eligible to participate.
9	i. Females of childbearing potential (FOCBP) must agree to avoid pregnancy and have a negative pregnancy test performed at the required study visits. FOCBP must agree to use highly effective contraception, during treatment and, until 90 days after the last administration of study medication. ii. Male patients must agree to remain abstinent from heterosexual sex during treatment and for 90 days after treatment or, if sexually active, to use two effective methods of birth control (eg, male uses a condom and female uses contraception) during and for 90 days after treatment.

GAD65, glutamic acid decarboxylase 65 kDa; HbA1c, haemoglobin A1c.

thyroid peroxidase antibody, transglutaminase antibody, IA-2 antibody, GAD65 antibody, SARS-CoV-2 antibody and for females; human chorionic gonadotropin, follicle stimulating hormone and luteinising hormone. Timing of the assessments can be found in table 1 and all samples will be analysed at a central laboratory.

Immunology assessments

Timing of immunological assessments is indicated in table 1. GAD65 antibody titres will be measured at a central laboratory. Additional variables to evaluate the influence of treatment on the immune system include GAD65 antibody isotypes, IA-2 antibodies and cell-mediated immune response by proliferation and cytokine secretion on GAD65 stimulation of PBMC.

Continuous glucose monitoring

CGM will be performed for 14 days during the run-in period (following visit 1C) and at three other timepoints during the trial. The timing of the distribution of the glucose monitoring system and assessments is presented in table 1. The FreeStyle Libre Pro/Pro iQ devices are intended for use only by healthcare professionals, with the patients being blinded to the CGM sensor readings. The devices will be used for data collection during the clinical trial, but not to inform decisions on diabetes management and therapy adjustments. Patients will be allowed to use an unblinded CGM device to manage their diabetes and adjust the therapy based on the glucose levels registered.

Time period and frequency for collecting AE and serious AE information

Any worsening in the patient's condition after administration of study drug and up to the end of study or early termination visit should be considered an AE. All AEs will be collected throughout the whole study period (starting from visit 1C), reviewed and assessed for causality by the investigators at the time points specified in table 1. Injection site reactions will be collected during the 7 days following study drug injections (visits 2, 3 and 4), starting the day after the injection. Injection site reactions persisting after 7 days should be reported as an AE.

Statistical considerations

Sample size and power

The primary efficacy analysis will be performed in the full analysis set. The primary efficacy variables will be (1) change from baseline to Month 24 in log-transformed C-peptide $AUC_{mean\ 0\text{-}120\ min}$ during an MMTT and (2) change from baseline to Month 24 in mean HbA1c. The coprimary endpoints will be tested in both the overall population and in the subgroup of patients who carry the HLA DR3-DQ2 haplotype and simultaneously do not carry the DR4-DQ8 haplotype (hereafter the HLA DR4-DQ8-negative subgroup). The overall two-sided 5% type

Table 3 Exclusion criteria in the diagnode-3 study

	Patients are not eligible to be included in this study if any of the following criteria apply
1	Participation in any other trial aimed to influence beta cell function from time of diagnosis of T1D.
2.	Treatment with any oral or non-insulin injectable anti-diabetic medication within 3 months prior to screening.
3.	History of maturity-onset diabetes of the young.
4.	Pancreatic surgery, chronic pancreatitis or other pancreatic disorders that could result in decreased beta cell capacity (eg, pancreatogenous diabetes).
5.	History of DKA or severe hypoglycaemia requiring hospitalisation within 1 month before screening or severe episodes of hypoglycaemia requiring third party assistance within 1 month before screening.
6.	Signs or symptoms suggesting very poorly controlled diabetes for example, ongoing weight loss, polyuria or polydipsia.
7.	Haematological condition that would make HbA1c uninterpretable including: a. Haemoglobinopathy, with the exception of sickle cell trait or thalassaemia minor, or chronic or recurrent haemolysis. b. Donation of blood or blood products to a blood bank, blood transfusion or participation in a clinical study requiring withdrawal of >400 mL of blood during the 8 weeks prior to the screening visit. c. Significant iron deficiency anaemia. d. Heart malformations or vaso-occlusive crisis leading to increased turnover of erythrocytes.
8.	Treatment with marketed or over-the-counter vitamin D at the time of screening and unwilling to abstain from such medication during the 120 days when the patient will be supplemented with the study-provided vitamin D. A patient currently taking vitamin D at the time of screening must be willing to switch to the study-provided vitamin D treatment and to administer it per the study requirements.
9.	Any clinically significant history of an acute reaction to a vaccine or its constituents (eg, Alhydrogel).
10.	Treatment with any (live or inactive) vaccine, including influenza vaccine and COVID-19 vaccine, within 4 weeks prior to planned first study dose of study drug; or planned treatment with any vaccine up to 4 weeks after the last injection with study drug.
11.	Any acute or chronic skin infection or condition that would preclude intralymphatic injection.
12.	Recent (past 12 months) or current treatment with immunosuppressant therapy, including chronic use of glucocorticoid therapy. Inhaled, topical and intranasal steroid use is acceptable. Short courses (eg, ≤5 days) of oral or intra-articular injections of steroids will be permitted on trial.
13.	Continuous/chronic treatment with prescribed or over-the-counter anti-inflammatory therapies. Short-term use (eg, <7 days) is permissible, for example to treat a headache or in connection with a fever.
14.	Known or suspected acute infection, including COVID-19 or influenza, at the time of screening or within 2 weeks prior to screening. After confirmed recent COVID-19 infection, a negative PCR test will be required before randomisation.
15.	A history of epilepsy, head trauma or cerebrovascular accident, or clinical features of continuous motor unit activity in proximal muscles.
16.	Known diagnosis of HIV, hepatitis B or hepatitis C infection. Patients with previous hepatitis C infection that is now cured may be eligible.
17.	Any clinically significant concomitant medical condition
18.	History of significant hepatic disease
19.	Estimated glomerular filtration rate calculated by Chronic Kidney Disease Epidemiology Collaboratio for those >18 years old, and by the Schwartz equation for those 12–18 years old, <90 mL/min per 1.73 m or rapidly progressing renal disease.
20.	Patients with hypothyroidism or hyperthyroidism must be on stable treatment for at least 3 months prior to screening (with normal free thyroxine(T4) levels if hypothyroid).
21.	Any clinically significant abnormal findings during screening, and any other medical condition(s) or laboratory findings that, in the opinion of the investigator, might jeopardise the patient's safety or ability to complete the trial.
22.	History of malignancy not in remission within the last 5 years other than adequately treated basal cell or squamous cell skin cancer or cervical carcinoma in situ.
23.	Patients with any mental condition rendering him/her unable to understand the nature, scope and possible consequences of the trial, and/or evidence of poor compliance with medical instructions at screening or showing non-compliance during the run-in period.
24.	A history of alcohol or drug abuse or dependence within the past 12 months based on Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria.
25.	Current or previous participation in a trial of Diamyd.

Continued

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Table 3 Continued

Patients are not eligible to be included in this study if any of the following criteria apply	
26.	Participation in a clinical trial involving administration of an investigational drug in the past 3 months or five half-lives (whichever is longer) prior to first dosing of study drug or during the trial.
27.	Females who are breast feeding, pregnant or plan to become pregnant during the trial.
28.	Patients who in the opinion of the investigator will not be able to follow instructions and/or follow the study procedures, or patients that are unwilling or unable to comply with the provisions of this protocol.
29.	An employee or immediate family member of an employee of Diamyd Medical AB.

DKA, diabetic ketoacidosis; HbA1c, haemoglobin A1c; T1D, type 1 diabetes.

I error rate will be controlled using a fallback procedure. A two-sided 4% alpha will be assigned to the primary efficacy analysis in the overall population. The remaining 1% alpha will be assigned to the primary efficacy analysis in the HLA DR4-DQ8-negative subgroup.

A total sample size of 288 patients is planned for a 2:1 randomisation to the rhGAD65 and placebo arms, respectively. This achieves 90% power to detect a clinically relevant difference of 40% in geometric mean ratio C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at month 24 between the rhGAD65 arm and placebo arm using a two-sided test at the 4% significance level. This is based on a t-test employing natural log transformation of C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at month 24 and assumed CV of 0.95 based on simulations of EoS (month 15) results in the placebo group of the phase 2b study DIAGNODE-2. Allowing for 12% drop-out to month 24, approximately 330 patients will be randomised.

Negligible measurement error is expected, which is assumed to affect all patients, time points and treatment groups equally. The total variance used in the sample size calculation is constructed from the within-subject and the between-subject component of variation. Based on the assumption that the measurement error is the same for everyone, it is therefore accounted for in the total variance estimate.

The coprimary endpoints will be tested sequentially, meaning that C-peptide is tested first, and, if significant, HbA1c is tested. Both co-primary endpoints need to meet the statistical significance criterion. The fallback procedure described by Wiens and Dmitrienko[33] will be used to test the primary endpoints in the overall population and in the HLA DR4-DQ8-negative subgroup.

If either of the coprimary endpoints is not statistically significant in the overall population at a two-sided significance level of 0.04, the coprimary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.01 level of significance in an analogous manner to the primary analysis in the overall population. If both coprimary endpoints in the overall population are statistically significant at the two-sided 0.04 level, then the coprimary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.05 level of significance in an analogous manner to the primary analysis in the overall population. The analysis of secondary and exploratory endpoints will be described in a statistical analysis plan (see online supplemental appendix 1) which will be finalised before the first patient is enrolled.

Statistical analyses

Primary efficacy analysis

Change from baseline in the coprimary endpoints will be analysed using a restricted maximum likelihood-based repeated measures approach (MMRM). The model for analysis will include fixed, categorical effects of such as for treatment and stratification variables, as well as interactions effects such as between baseline value-by-visit and the fixed continuous covariates, such as baseline age. Patient identification number will be included as a categorical random effect. An unstructured covariance matrix will be assumed. If this analysis fails to converge, compound symmetry will be tested. The (co)variance structure converging to the best fit, as determined by Akaike's information criterion, will be used as the primary analysis. The Kenward-Roger approximation will be used to estimate denominator df. Additional methods of sensitivity analyses may be performed in the event that the proportion of missing values is greater than 5%. These analyses will compare the results from the MMRM model, which assumes that values are missing at random, with analyses assuming the values are missing not at random such as: return-to-baseline using multiple imputation and tipping point analysis. All sensitivity analyses will be regarded as exploratory, thus no formal adjustment for multiplicity will be performed.

Patient and public involvement

Patients were not involved in the study design. Patients and Patient organisations (in Sweden Barndiabetesfonden) support recruitment through dissemination of information and participation in press conferences. Participating patients and caregivers will be informed about the outcome of the trial via webcast, letter and personal communication on the completion of the trial.

Ethics and dissemination

The trial will be performed in accordance with International Council for Harmonisation guidelines, Good Clinical Practice (GCP) and principles of the Declaration of Helsinki. The study has been approved by Ethics

Committees in Poland (ref number: 124/2021), the Netherlands (ref number: R21.089), Sweden (Ref number: 2021-05063), Czech Republic (ref number: EK-1144/21) Germany (ref number: 2021361) and Spain (ref number: 21/2021). Recruitment of participants is planned to start during 2022. Once the trial is completed, results will be published in international peer-reviewed scientific journals and presented at national and international conferences. The main paper will include the primary and secondary outcomes. The manuscript will be submitted to an international peer-reviewed journal, and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be registered at ClinicalTrials.gov, in EudraCT and will be disseminated to the public.

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Contributors JL conceived the idea and wrote the protocol for the DIAGNODE-1 trial on which the current trial is based . Thus, the design of DIAGNODE-3 is based on the ideas of JL, with further support from UH, MW and AL. The protocol is written by JL, UH, LE, CN, PFT, MW, AL. JL, UH, LE, CN, PFT, MW, AL, RC and ML have taken part in writing and reviewing the manuscript. All authors have approved the manuscript for publication.

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Competing interests JL has received unrestricted grants from Diamyd Medical, and honoraria as consultant from Dompé International and Provention Bio. ML has received research grants from Eli Lilly and NovoNordisk and been a consultant or received honoraria from Astra Zeneca, Boehringer Ingelheim, Eli Lilly and NovoNordisk. LE, CN, PFT, MW, AL and UH are all employees of Diamyd Medical. CN, PFT, MW and UH own shares in Diamyd Medical.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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