Clinical Study Report

Non-Commercial Sponsor

1 TITLE PAGE

Study Title: A Randomised, Double Blind, Placebo-Controlled Trial of Intranasal Insulin in Children and Young Adults at Risk of Type 1 Diabetes: Intranasal Insulin Trial II

Study Acronym: INIT II

Investigational drug(s)	DV001 (human recombinant insulin aqueous nasal spray)
Indication studied	Prevention of type 1 diabetes
Sponsor name and address	Melbourne Health Royal Melbourne Hospital Grattan Street, Parkville Victoria 3052 Australia
Protocol number	INIT/002
Development phase of study	Phase 2
Study initiation date	February 7, 2007
Study early termination date	May 31, 2016
Principal investigator(s)	Professor Leonard C Harrison Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Victoria, Australia Professor Peter Colman Royal Melbourne Hospital, Parkville 3052, Victoria, Australia
Sponsor signatory	Professor Ingrid Winship Executive Director for Research Melbourne Health Phone: +613 9342 8530 Fax: +613 9342 4267 Email: ingrid.winship@mh.org.au
Good Clinical Practice (GCP), including	The study was conducted in accordance with the general ethical principles outlined in the

archiving of essential documents	Declaration of Helsinki. The review of the protocol by the relevant Human Research and Ethics Committees and the performance of all aspects of the study, including the methods used for obtaining informed consent, were in accordance with the principles of the Declaration of Helsinki, Notes for Guidance on Good Clinical Practice (ICH GCP. annotated with comments by the Therapeutic Goods Administration, Australia), the National Australian Statement of Ethical Conduct in Research Involving Humans and the New Zealand Regulatory Guidelines for Medicines. All essential documents have been archived.
Date of report	April 18, 2017
Previous reports	Dec 4, 2013: DSURDV001-2.00 Dec 10, 2014: DSURDV001-3.00 Jan 31, 2016: DSURDV001-4.00 Jan 17, 2017: DSURDV001-5.00
Applicable EudraCT numbers	2009-017329-20

2 SYNOPSIS

Name of Sponsor/Company: Melbourne Health Royal Melbourne Hospital Grattan Street, Parkville Victoria 3052 Australia	**Individual Study Table Referring to Part of the Dossier** **Volume:** **Page:**	*(For National Authority Use only)*
Name of Finished Product: Aqueous nasal insulin spray		
Name of Active Ingredient: Human recombinant Insulin, manufactured by recombinant DNA technology in *Saccharomyces cerevisiae* by NovoNordisk A/S Denmark.		
Title of Study: A randomised, double-blind, placebo-controlled trial of intranasal insulin in children and young adults at risk of type 1 diabetes: intranasal insulin trial II. Protocol Version: Amendment 9, dated 23 June 2014 Protocol amendments: Original protocol – 22 November 2004 Amendment 1 – 25 August 2005 Amendment 2 – 30 March 2006 Amendment 3 – 16 November 2006 Amendment 4 – 15 January 2007 Amendment 5 – 29 January 2008 Amendment 6 – 13 July 2009 Amendment 7 – 11 January 2010 Amendment 8 – 14 July 2010 Amendment 9 – 23 June 2014		
Investigators: Leonard C Harrison and Peter G Colman (Melbourne, AUS), Anette-Gabriele Ziegler (Munich, GER), Craig Jefferies (Auckland, NZ), Jenny Couper (Adelaide, AUS), Elizabeth Davis (Perth, AUS), Kim Donaghue (Sydney, AUS), Andrew Cotterill (Brisbane, AUS)		
Study centre(s): Australia: Royal Melbourne Hospital, Centre for Children's Health Research, The Children's Hospital at Westmead, Perth Children's Hospital, Women's and Children's Hospital New Zealand: University of Auckland; Germany: Forschergruppe Diabetes der TU Műnchen		

Publication (reference): None	
Studied period (years): 8.75 years **Date first dose administered:** March 21, 2007 **Date study terminated by DSMB:** December 18, 2015.	**Phase of development:** Phase 2
Objectives: To determine if insulin administered in the form of a nasal spray to the mucous membranes of the naso-pharynx would decrease the rate of development of diabetes in children and young adults at high risk of type 1 diabetes (T1D). The primary endpoint was diabetes, defined by American Diabetes Association criteria. Secondary endpoints were: 1) beta-cell function assessed by plasma glucose and insulin responses in the oral glucose tolerance test (OGTT), 2) insulin resistance derived as the homeostasis of model assessment-resistance (HOMA-R), 3) concentrations of autoantibodies to the islet antigens insulin, glutamic acid decarboxylase, molecular weight 65,000 isoform (GAD) and tyrosine phosphatase-like insulinoma antigen 2 (IA2). The rationale was based on published evidence that 1) in the non-obese diabetic (NOD) mouse model of spontaneous autoimmune disease (1), and in children developing the disease that insulin is a major target autoantigen that drives the autoimmune attack on the insulin-producing beta cells in the islets of the pancreas, 2) intranasal or aerosol insulin administered to NOD mice induces regulatory T cells that protect against the development of T1D, and 3) intranasal insulin in humans at risk for T1D has no significant side-effects and induces immune tolerance to insulin. A body of literature stretching back for nearly a century indicated that, in the absence of absorption-promoting agents, intranasal insulin was not absorbed into the bloodstream and had no effect on blood glucose concentration. However, published data showed that it acted locally as an antigen to activate T cells in the mucosal immune system.	
Methodology: Relatives of people with T1D were recruited through a variety of means (diabetes educators, doctors, media campaigns) and, following consent, screened for serum autoantibodies to islet antigens (insulin, GAD65 and IA-2). Based on published data, it was expected that 2.0-2.5% would be positive for at least two autoantibody specificities. The actual proportion was 2.2%. Following screening, autoantibody-positive relatives underwent 'staging' to evaluate beta-cell function, with an OGTT and an intravenous glucose tolerance test (to measure first phase insulin response, FPIR). Participants who fulfilled criteria for beta-cell function (see below) were invited to enter the randomised, double blind, placebo-controlled trial of intranasal insulin. Initially, the trial comprised two insulin dose (1.6mg and 16mg) arms and a placebo insulin carrier arm. However, because recruitment was slower than expected ongoing recruitment into the 'low dose' 1.6mg dose arm was curtailed in 2009, but those participants already enrolled in this 'low dose' arm (18/40)	

continued to be followed.

Treatment was self-administered by participants as two 100µl spray doses per each nostril, daily in the morning (7-10am) for 7 consecutive days, then on one day each week for one year.

Number of participants (planned and analysed): Initially, it was planned to screen approximately 12,240 T1D relatives to identify approximately 260 islet autoantibody-positive potential participants for recruitment into the randomised intervention trial. At the time the DSMB terminated the study on the basis of futility, 110 participants had been randomized.

Diagnosis and main criteria for inclusion: Trial participants: 1) had a first- or second-degree relative with T1D diagnosed before age 40; 2) were aged 4-30 years if a first-degree relative or 4-20 years if a second-degree relative; 3) had evidence of sub-clinical pancreatic islet autoimmunity, namely confirmed serum antibodies to two or more islet autoantigens (insulin, GAD65, IA-2); 4) had a first-phase insulin response (FPIR) to intravenous glucose at or above a threshold allowing allocation to a Primary Stratum = ≥ 10^{th} percentile for siblings, offspring and second-degree relatives (≥ 100uU/ml if aged ≥ 8 years or ≥ 60uU/ml if aged < 8 years) or Secondary Stratum = ≥ 1^{st} <10^{th} percentile for siblings, offspring and second-degree relatives (≥ 50uU/ml<100uU/ml if aged ≥ 8 years or ≥ 20uU/ml < 60uU/ml if aged < 8 years); provided written informed consent directly or via parents/guardians.

Test product, dose and mode of administration, batch number: Bulk recombinant human insulin active (26.7 IU/mg) was supplied by Novo Nordisk A/S Novo Allé 2880 Bagsvaerd Denmark. Insulin (4 or 40mg/ml) was formulated by IDT Ltd Melbourne in a sterile solution of benzalkonium chloride (0.06mg/ml) and glycerol (16mg/ml) in water, and loaded into 7ml sterile, multi-dose brown glass bottles (Pfeiffer) to which sterile plastic actuator pumps were then attached. Each single spray dose was 100µl, containing either 0.4 or 4 mg of insulin. The reference placebo (insulin carrier solution containing benzalkonium chloride and glycerol) was identical in appearance to the active formulation. Participants self-administered two 100µl spray doses to each nostril (total 1.6mg or 16mg), daily in the morning (7-10am) initially for 7 consecutive days, then once daily each week for one year. NOTE: the 1.6 and 16mg doses were referred to as 40 and 440IU, following formulation by IDT. While these activity concentrations are slightly inaccurate based on the specific activity of 26.7 IU/mg they appeared in documentation and have been retained.

Batch numbers: DB741001, DB741201, DB741301, DB740601

Duration of treatment: Participants were to be followed up three monthly in the first year while on treatment, then six monthly for a further four years, or until they developed diabetes or dropped out. Thus, overall the follow-up would be for five years after the last participant had been

randomized (i.e. one year of treatment and four years of follow-up).

Reference therapy, dose and mode of administration, batch number: See placebo composition described and administration above.

Name of Sponsor/Company:	**Individual Study Table Referring to Part of the Dossier**	*(For National Authority Use Only)*
Melbourne Health Royal Melbourne Hospital Grattan Street, Parkville Victoria 3052 Australia	**Volume:** **Page:**	

Name of Finished Product: Aqueous nasal insulin spray

Name of Active Ingredient: Human recombinant Insulin, manufactured by recombinant DNA technology in *Saccharomyces cerevisiae* by NovoNordisk A/S Denmark.

Criteria for evaluation:

Efficacy: Efficacy was to be determined primarily by a statistically significant decrease in the development of diabetes over 5 years (see below).

Safety: Local and systemic adverse effects were elicited by direct questioning of the participant and/or parent/guardian. Adverse events (AEs) and serious AEs (SAEs) were documented. SAEs that met or exceeded the requirements of the ICH Guidelines for Good Clinical Practice included events that result in death, are life-threatening (when the participant is, in the view of the investigator, at immediate risk of death), develop during the study and require inpatient hospitalization or prolongation of existing hospitalization, are permanently disabling or incapacitating or cause a severe or permanent disruption of a person's ability to carry out normal life functions or daily activities.

An independent Data and Safety Monitoring Board (DSMB) was established to review study progress, safety and outcomes.

Statistical methods:

The sample size was based on the following:

• A 5-year risk for relatives with at least two islet autoantibodies, FPIR above 1st percentile threshold and normal OGTT, for progression to diabetes of 40%.

• An expectation of reducing diabetes development by 50%, i.e. a 5-year risk for progression to diabetes of 20%

• A power of 85% to detect 50% reduction at $p<0.05$, one-sided

For a 2-arm study, 48 observed events would be expected requiring 102 participants or 51

participants for each arm, assuming accrual over 5 years, 5 years of follow-up from the time the last participant was randomized, and a 5% loss to follow-up over the course of the study.

The primary endpoint is analysed by constructing Kaplan-Meier survival curves of time until diabetes and using the logrank test to compare them. Statistical analyses of secondary objectives is by longitudinal data modeling of rates of change with corrections for repeated measures and intra-participant correlations.

SUMMARY-CONCLUSIONS

EFFICACY RESULTS: After an interim statistical analysis (with the investigators blinded), the DSMB formally directed, on December 18, 2015, that the study be terminated on the basis of futility. At this time, 110 participants had been randomized, 18 to the 'low-dose' insulin arm that had been terminated in 2009, and 92 to the placebo and 'high-dose' insulin arms. A final analysis by one of the DSMB's statisticians, Dr. Jim Sockler, employed by the study's data management organization, DataPharm, revealed that survival in the placebo arm equated to the assumed 60% at 5 years, with no statistical difference between the two insulin treatment arms and the placebo arm.

SAFETY RESULTS: Twenty-three SAEs, including ten cases of new-onset T1D, and 1437 treatment emergent AEs, were reported.

CONCLUSION: For the primary endpoint, intranasal insulin in the dose-schedule employed had no effect on the natural history of development of clinical T1D in children and young adults at increased genetic risk (having an affected close relative) and with autoantibodies to at least two islet antigens.

For the secondary endpoints, differences overall between participants treated with intranasal insulin versus placebo were not significant. Intranasal insulin led to an increase in serum InsAb concentrations, which was dose-related (higher with 440IU than 40IU dose) but then decreased over several months after cessation of treatment. However, changes in InsAb or other antibodies were not associated with the incidence of diabetes.

Date of the report: April 18, 2017