BRIEF REPORT

Survival of Transplanted Allogeneic Beta Cells with No Immunosuppression

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SUMMARY

The need to suppress a patient's immune system after the transplantation of allogeneic cells is associated with wide-ranging side effects. We report the outcomes of transplantation of genetically modified allogeneic donor islet cells into a man with long-standing type 1 diabetes. We used clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 12b (Cas12b) editing and lentiviral transduction to genetically edit the cells to avoid rejection; the cells were then transplanted into the participant's forearm muscle. He did not receive any immunosuppressive drugs and, at 12 weeks after transplantation, showed no immune response against the gene-edited cells. C-peptide measurements showed stable and glucose-responsive insulin secretion. A total of four adverse events occurred, none of which were serious or related to the study drug. (Funded by the Leona M. and Harry B. Helmsley Charitable Trust; EudraCT number, 2023-507988-19-00; ClinicalTrials.gov number, NCT06239636.)

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The discovery and clinical adoption of the calcineurin inhibitor cyclosporin A was a major milestone for the transplantation field and allowed the successful routine transplantation of allogeneic organs. However, the toxicity of immunosuppression leads to considerable morbidity and mortality among patients receiving transplants.

Exogenous insulin has been used in the treatment of type 1 diabetes mellitus for more than 100 years. Intensive insulin therapy delays the onset and slows the progression of long-term complications[1] and improves life expectancy.[2] However, insulin remains a treatment and is not a cure; persons with early-onset type 1 diabetes continue to have a reduced quality of life, an elevated risk of serious cardiovascular outcomes, and a shortened life span.[3]

We have shown previously that transplantation of islet cells obtained from a deceased human donor or from a rhesus monkey and edited to be hypoimmune cured diabetes in allogeneic, diabetic, humanized mice[4] and in an allogeneic, diabetic cynomolgus monkey, respectively, without the use of immunosuppression. Here, we report the results of a proof-of-concept study of the transplantation of gene-edited, hypoimmune platform (HIP) islet cells in a patient with long-term type 1 diabetes, without the use of immunosuppression. The depletion of HLA class I and II protects against adaptive T-cell rejection but renders the engineered cells susceptible to innate immune-cell killing through a mechanism known as missing-self recognition. Overexpression of CD47 inhibits such innate killing through

the inhibition of macrophages and natural killer cells. The study was an investigator-initiated, first-in-human, open-label study of UP421, a therapeutic product composed of gene-edited human HIP islet cells that has been approved by the Swedish Medical Products Agency and the Swedish Ethical Review Authority (see the Supplementary Appendix, which, along with the protocol, is available with the full text of this article at NEJM.org).

CASE REPORT

A 42-year-old man with a 37-year history of type 1 diabetes provided written informed consent to participate in the study. He fulfilled eligibility criteria. No additional patients were enrolled in this study. The participant had a glycated hemoglobin level of 10.9% (96 mmol per mole), undetectable endogenous insulin production (i.e., no measurable C-peptide), and detectable glutamic acid decarboxylase and islet antigen 2 autoantibodies (indicators of an autoimmune cause of his disease) and was receiving a daily insulin dose of 32 units.

A blood type O–matched pancreas from a 60-year-old donor with a glycated hemoglobin level of 6.0% (42 mmol per mole) became available, and islets were isolated after 5 hours 11 minutes of cold ischemia at Uppsala University Hospital. These islets had a glucose-stimulated insulin secretion index of 16.7 and a purity of 87%. The islets were shipped to Oslo University Hospital for gene editing. At the manufacturing facility of the hospital, the islets were dissociated into single cells, and the genes *B2M* (encoding a component of class I HLA) and *CIITA* (encoding a master regulator of class II HLA transcription) were inactivated with the use of the nuclease Cas12b (clustered regularly interspaced short palindromic repeats [CRISPR]–CRISPR-associated protein 12b) and guide RNAs. The cells were then allowed to recluster and rest before they were again dissociated and transduced with a lentiviral vector containing *CD47* complementary DNA. Of the edited islet cells, 85.8% were negative for HLA class I, 100% were negative for HLA class II, and 46.4% had high CD47 expression (Fig. 1A). The final cellular product (UP421) thus contained fully edited HLA-depleted HIP islet cells with high CD47 expression, some HLA class I and II double-knockout cells with endogenous CD47 levels, and islet cells with retained HLA expression (wild type) and varying CD47 levels (Fig. 1B). Gene editing did not change the composition of the islets. Approximately 66% of the islet cells in the study were beta cells (Fig. 1C).

The engineered islet cells were then shipped to Uppsala University Hospital for implantation. With the participant under general anesthesia, a small (3.8 cm) skin incision was made over the participant's left brachioradialis muscle (Fig. 1D). A total of 79.6 million engineered HIP islet cells were prepared into 17 syringes and delivered through 17 injections into the muscle. In each injection, the islets were distributed in a linear pearl-on-string pattern while the syringe was slowly pulled back. The participant remained hospitalized overnight to monitor for immediate complications and was discharged the following day. The participant did not receive any glucocorticoids or antiinflammatory or immunosuppressive medications.

We monitored the participant's immune response against each of the islet-cell subpopulations over 12 weeks. The wild-type islet cells remaining in the graft induced strong T-cell activation and killing, which peaked on day 7 (Fig. 2A and 2B) but were spared by innate immune cells (natural killer cells and macrophages) (Fig. 2C and 2D). The wild-type islet cells induced an immediate IgM response (evident at day 7), and a subsequent immunoglobulin class switch to IgG occurred between days 14 and 21 (Fig. 2E). Cell killing mediated by complement-dependent and antibody-dependent cellular cytotoxicity was observed at each time point after the transplantation (Fig. 2F and 2G). The wild-type islet cells were killed when incubated with the participant's peripheral-blood mononuclear cells (PBMCs) combined with his serum containing antibodies and complement to simulate his comprehensive immune response (Fig. 2H). The double-knockout islet cells remaining in the graft induced a strong innate immune response and were killed by the participant's innate natural killer cells and macrophages (both cell types can sense HLA deficiency[6]) and by the participant's PBMCs and serum (Fig. 2C, 2D, and 2H). In spite of the ongoing immune responses against wild-type and double-knockout cells, HIP islet cells were not killed by the participant's immune cells, did not induce antibodies, and survived when incubated

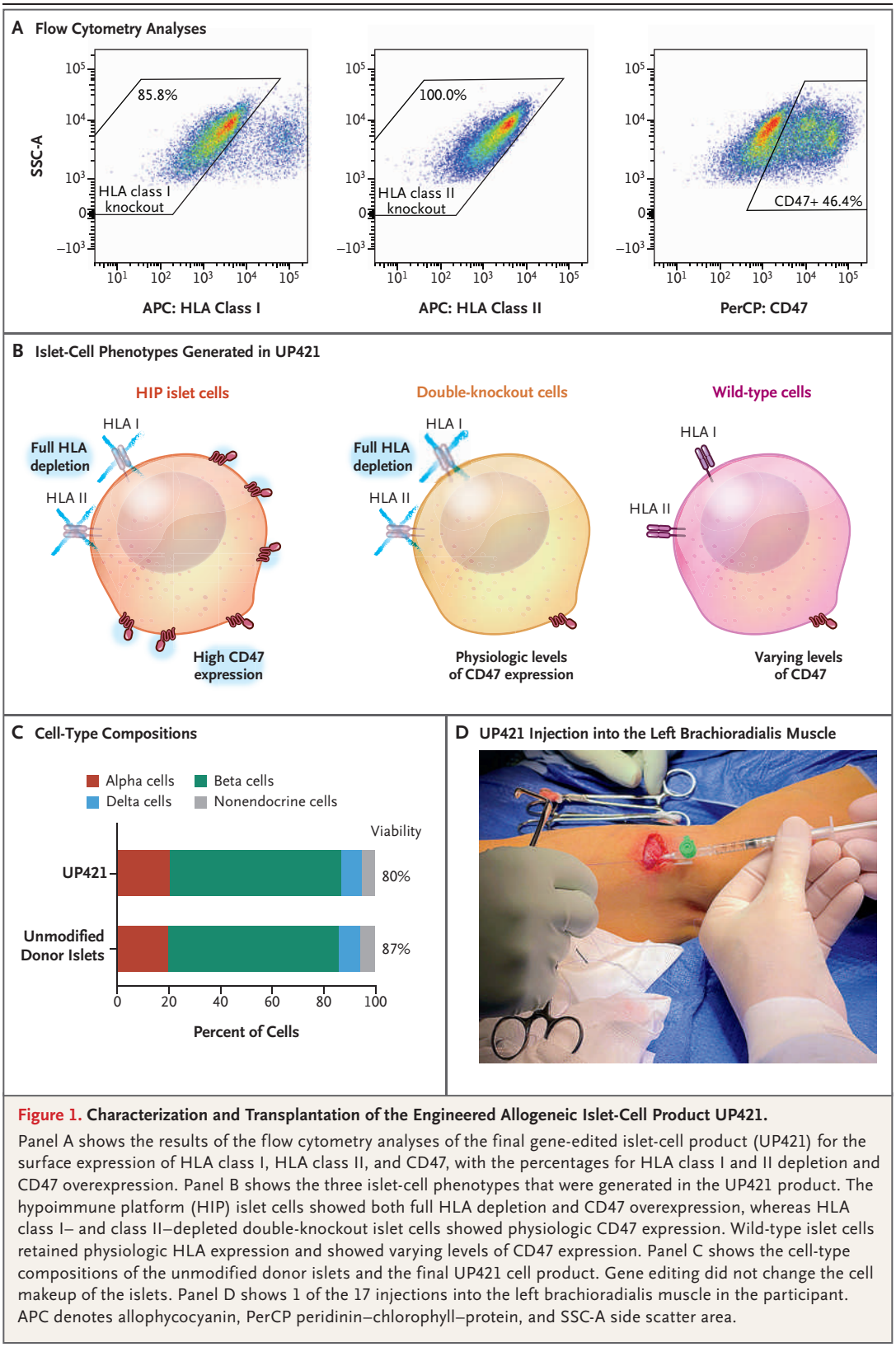

Figure 1. Characterization and Transplantation of the Engineered Allogeneic Islet-Cell Product UP421.

Panel A shows the results of the flow cytometry analyses of the final gene-edited islet-cell product (UP421) for the surface expression of HLA class I, HLA class II, and CD47, with the percentages for HLA class I and II depletion and CD47 overexpression. Panel B shows the three islet-cell phenotypes that were generated in the UP421 product. The hypoimmune platform (HIP) islet cells showed both full HLA depletion and CD47 overexpression, whereas HLA class I– and class II–depleted double-knockout islet cells showed physiologic CD47 expression. Wild-type islet cells retained physiologic HLA expression and showed varying levels of CD47 expression. Panel C shows the cell-type compositions of the unmodified donor islets and the final UP421 cell product. Gene editing did not change the cell makeup of the islets. Panel D shows 1 of the 17 injections into the left brachioradialis muscle in the participant. APC denotes allophycocyanin, PerCP peridinin–chlorophyll–protein, and SSC-A side scatter area.

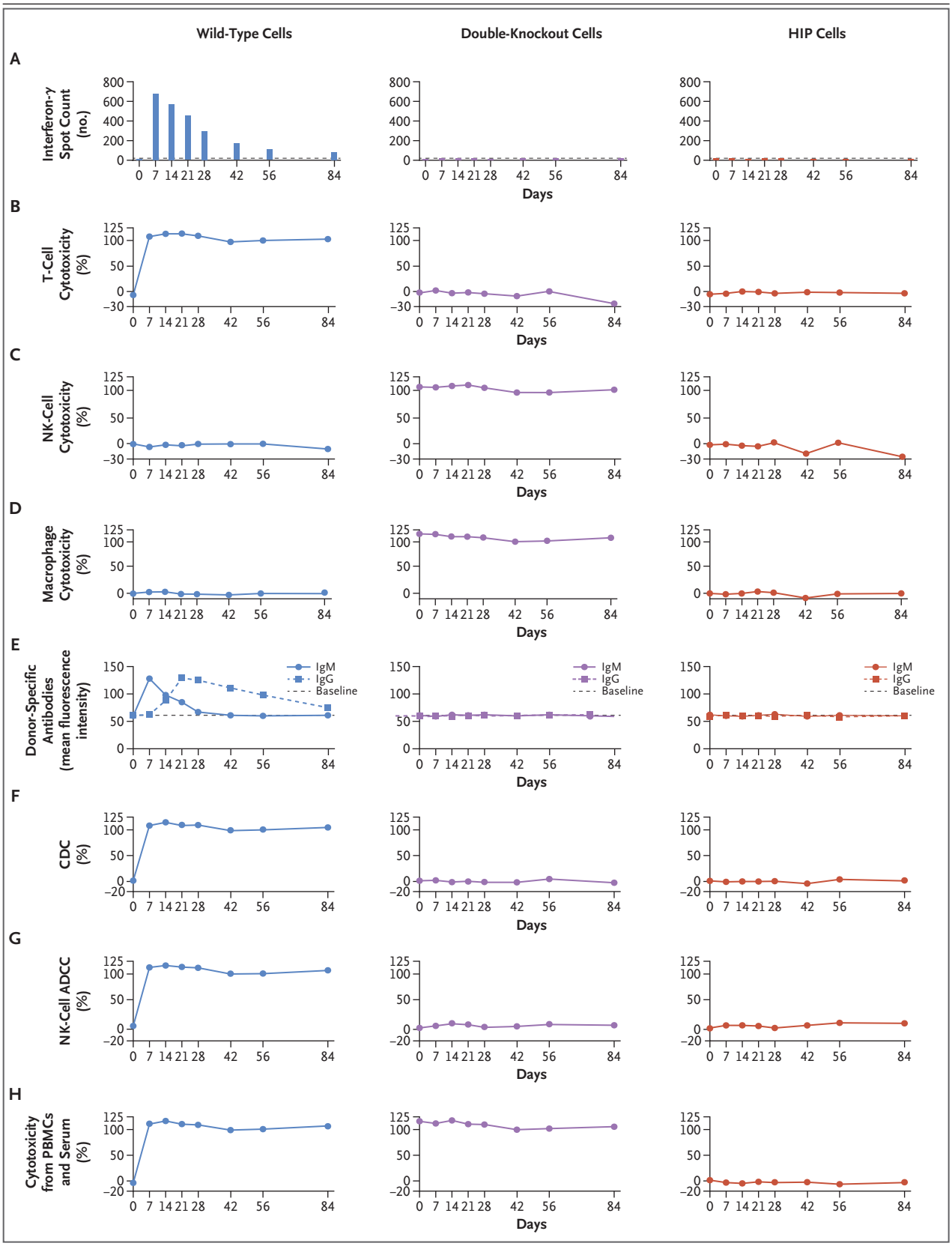

Figure 2 (facing page). Cellular and Humoral Immune Components in the Participant over 12 Weeks.

Panel A shows interferon-γ enzyme-linked immunosorbent spot (ELISpot) data from the assessment of the participant's T-cell activation on restimulation with one of the three islet-cell subpopulations. Strong T-cell activation that peaked at approximately 7 days was seen only against wild-type cells. Panel B shows the results of corresponding T-cell cytotoxicity assays. The participant's T cells only killed wild-type cells. Panels C and D show the results of innate immune-cell killing assays with natural killer (NK) effector cells and macrophages, respectively. Only double-knockout cells were killed. Panel E shows mean fluorescence intensity of antibody binding against the UP421 wild-type cell, double-knockout cell, or HIP cell subpopulations. Antibodies were only generated against wild-type cells, with IgM antibody binding peaking early (circles with solid line), followed by a class switch to IgG antibodies (squares with dashed line). No antibodies against double-knockout or HIP islet cells were generated (the dashed lines show the background staining). Panels F and G show antibody-mediated complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) with NK effector cells, respectively. Only wild-type islet cells, against which antibodies were measurable, were killed in these assays. Panel H shows the participant's comprehensive immune response orchestrated by peripheral-blood mononuclear cells (PBMCs) and serum containing antibodies and complement. In this assay, both the wild-type islet cells and the double-knockout cells were killed, but the HIP islet cells survived because they escape all the immune components in the participant.

with the participant's PBMCs and serum (Fig. 2A through 2H). Thus, we did not detect any immune response targeting the HIP islet cells over the course of the study.

We assessed allograft function in the participant by means of serial high-sensitivity C-peptide measurements and observed that the levels were stable between 7 days and 12 weeks (Fig. 3A). At screening, the participant had no detectable C-peptide levels during a mixed-meal tolerance test, but at 4, 8, and 12 weeks, C-peptide levels increased in response to a liquid meal beverage containing fats, protein, and carbohydrates (Fig. 3B). The participant's exogenous insulin dose (in terms of both total dose and dose per kilogram of body weight) was increased after islet transplantation to prevent hyperglycemic spikes that could have harmed the fresh islet graft (Fig. 3C and 3D). His glycated hemoglobin level decreased by approximately 42% over the 12-week follow-up period (Fig. 3E), probably entirely in response to exogenous insulin. Persistence of the islet allograft was confirmed by magnetic resonance imaging (MRI) at 4 and 8 weeks. The MRI findings showed several punctate signals at each injection site and no inflammation or evidence of pathologic changes (Fig. S1A in the Supplementary Appendix). Positron-emission tomography and MRI (PET-MRI) at 12 weeks showed specific sites of high radiotracer uptake in the areas of the islet allografts, which could be delineated from surrounding muscle (Fig. S1B). The use of a glucagon-like peptide 1 receptor (GLP-1R)–targeting PET tracer has been shown to permit the visualization of functional pancreatic islet grafts, which express high levels GLP-1R, in the brachioradialis muscle.[7] Standardized uptake value plots support tracer accumulation in the graft areas and washout in the adjacent muscle tissue (Fig. S1C).

After 12 weeks, the participant continues to do well. A total of four adverse events occurred, none of which were serious. Mild thrombophlebitis developed in the participant at the site of the peripheral intravenous catheter, and he had paresthesia in his left lower arm, which was possibly related to the surgical procedure (see the Supplementary Appendix).

DISCUSSION

The results of this first-in-human study are consistent with immune evasion by allogeneic, hypoimmune-engineered islet cells. These cells, transplanted and engrafted in the forearm muscle of a person with type 1 diabetes, did not induce an immune response and escaped the alloimmune responses induced against nonedited or partially edited cells. The results are also consistent with stable beta-cell function over a 12-week period. Our findings are encouraging in consideration of the reported association between early graft function and long-term clinical outcomes.[8] Although T-cell–mediated immunity and donor-specific antibody surges peaked during the first 3 weeks, the HIP islet cells did not induce a measurable immune-cell or antibody-mediated response throughout the 12-week follow-up period. The absence of an alloimmune response against the HIP islet cells is consistent with our earlier findings in a diabetic cynomolgus monkey that received allogeneic hypoimmune-engineered islet allografts.[5] C-peptide levels in the monkey were stable throughout the 6-month follow-up period.

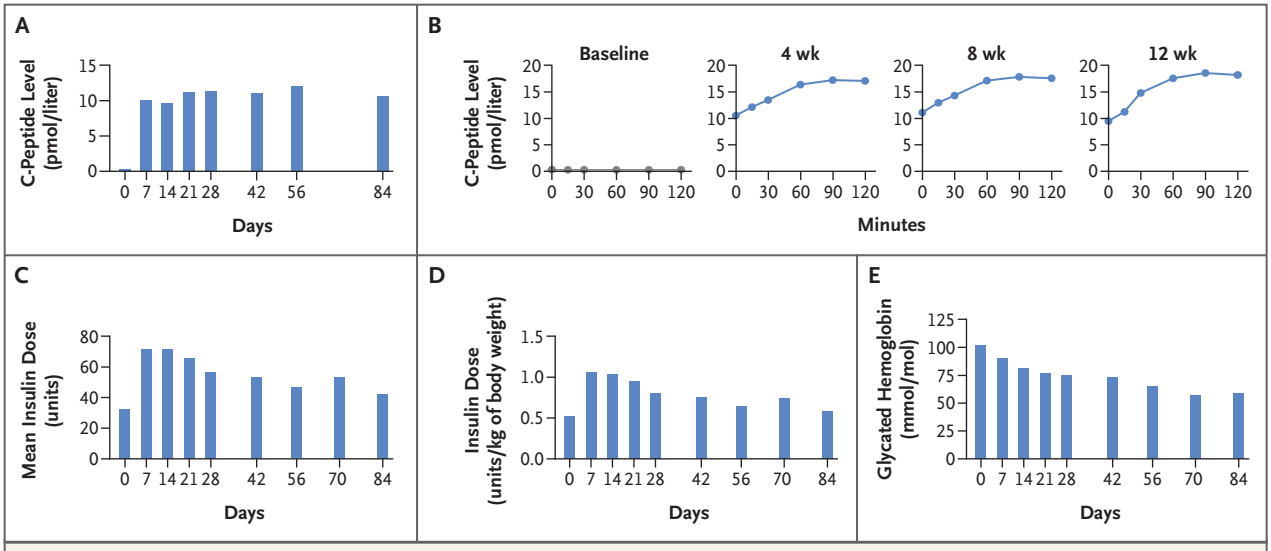

Figure 3. Postoperative Persistence and Function of HIP Islet-Cell Allografts.

Panel A shows that the participant did not have measurable C-peptide levels before receiving the UP421 islet-cell product. C-peptide levels were measured over the 12-week follow-up period with a high-sensitivity assay, which showed that the levels remained stable near or above 10 pmol per liter. Panel B shows that the participant did not have any C-peptide response to a mixed-meal tolerance test before transplantation, but at 4, 8, and 12 weeks after transplantation, an increase in the C-peptide level was observed, a finding suggestive of functional beta-cell grafts. Panels C and D show the mean insulin doses (the total dose and the dose per kilogram of body weight, respectively) that were administered during the study period. Panel E shows glycated hemoglobin levels over the 12-week follow-up period.

Moreover, a previous study in humanized mouse models of diabetes showed that hypoimmune islets escape the etiologic autoimmune response.[4]

The gene-editing process did not change the cell-type composition of the islets, a finding that is in keeping with the results of preclinical studies showing that gene editing and lentiviral transduction does not affect islet composition or insulin secretion.[4,5] Thus, CD47 overexpression does not seem to negatively affect insulin secretion, although it was recently suggested that genetic ablation of CD47 stimulates exocytosis of granules containing insulin.[9] The dose of 79.6 million HIP islet cells was intentionally low to meet the regulatory requirements for a first-in-human study. It seems unlikely that the small dose favorably affected cell survival, since we observed a strong immune reaction against the unmodified and partially edited islet cells. Moreover, a smaller total islet mass has not been associated with graft survival in allogeneic islet transplantation.[10] A mean (±SD) of 11,547±1604 islet equivalents per kilogram of body weight,[11] with 1560 cells per islet equivalent,[12] is capable of producing sustained insulin independence; the dose of 79.6 million HIP islet cells used in the current study was 7.1% of that dose. The observed C-peptide levels were consistent with the transplanted HIP islet-cell mass, which suggests that a full dose of HIP islet cells (i.e., approximately 18 million per kilogram) could produce insulin independence. The fact that the HIP islet-cell graft could be clearly identified on PET-MRI indicates radiotracer uptake and therefore vascularization in the forearm muscle, a finding that is similar to our previous observations with non–genetically modified islets.[13] Furthermore, the survival of allogeneic HIP islet-cell grafts for 6 months in nonhuman primate quadriceps muscle without evidence of a reduction in mass[14] or weakening of endocrine function[5] is consistent with muscle being a viable site for islet engraftment. The stability of the C-peptide levels over time in the participant in the current study also supports this hypothesis.

In most studies of allogeneic islet transplantation, patients with type 1 diabetes have received more than one islet transplant. Across different studies, patients have received a median of two islet transplants; some have received three[15] or five[10,15] allogeneic islet transplantations. HIP islet-cell grafts could, in the future, be transplanted

iteratively to adjust for insulin independence. The HLA-replete cell population in the UP421 product could theoretically induce donor-specific antibodies and leave the patient sensitized, because a single sensitization event by an allogeneic HLA can result in lifelong B-cell memory with a risk of reactivation after subsequent exposure. However, anti-HLA donor-specific antibodies do not pose a risk for subsequent HIP islet-cell transplants, and the rapid disappearance of the unmodified cells may result in a weaker antibody response or a reduced odds of such occurrence. The induction of donor-specific HLA antibodies after allogeneic islet-cell transplantation has been reported,[15] but this often occurred several months after transplantation.[16] Most patients do not have development of donor-specific antibodies even after receiving multiple islet transplants.[16]

The risks of gene editing in cellular therapeutics have been investigated most comprehensively in chimeric antigen receptor (CAR) T cells. In a recent safety analysis involving 783 patients from 38 trials of T-cell therapy with more than 2200 total patient-years of observation, only a single case of secondary T-cell lymphoma was reported.[17] There was no evidence of CAR transgene integration in lymphoma tissue and no indication that insertional mutagenesis contributed to T-cell transformation, although an isolated case of indolent CAR-positive T-cell lymphoma that occurred after CAR T-cell therapy had been reported previously.[18] Although integration of viral vectors into specific genes has been reported to cause notable clonal expansions in engineered T cells,[19,20] in neither case were those malignant. With the established safety of CAR T-cell therapy, the risk for insertional mutagenesis in engineered islet products could be lower, given that islet cells are generally nonproliferative. The availability of the CD47-targeting antibody magrolimab, which was shown to selectively and effectively eliminate HIP islet cells in allogeneic, diabetic humanized mice,[4] could add another layer of safety for clinical HIP islet-cell transplantation.

On the basis of previous preclinical studies, the hypoimmune phenotype provides protection against alloimmunity for cell types other than islet cells.[21-23] Although inducing immune tolerance of allogeneic transplants has long been viewed as a holy grail, our study, although preliminary, suggests that immune evasion is an alternative concept for the circumvention of allorejection.[24]

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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Supplementary Appendix

Supplement to: Carlsson P-O, Hu X, Scholz H, et al. Survival of transplanted allogeneic beta cells with no immunosuppression. N Engl J Med. DOI: 10.1056/NEJMoa2503822

This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

Table of Contents

Methods

Pancreas procurement and islet isolation

Pancreases from brain-dead donors were allocated through Scandiatransplant to the Nordic Network for Clinical Islet transplantation, with the same acceptance criteria as for kidney transplantation. The islet isolation process, maintenance of purified islets, packing and distribution have previously been described in detail and are available at http://nordicislets.medscinet.com[1,2].

Manufacturing of UP421

Allogeneic primary islets meeting the authorized tissue establishments release criteria were shipped to the GMP manufacturing site (Oslo University, Norway, Authorization no. 9722-1) in a qualified temperature monitored transport container. Upon receipt, the islets were stored at 37°C, 5% CO_2, and 95% humidity until the manufacturing process started. Islet clusters were dissociated into single cell suspension using AccuMax (StemCell Technologies, Vancouver, BC, Canada). Singularized islet cells were resuspended in electroporation buffer (Maxcyte, Rockville, MD) at a concentration of 200 x 10^6 cells per ml and transferred into G-1000 chambers (Maxcyte), each containing 100 x 10^6 cells and 25 µg/ml of the nuclease Cas12b (Trilink BioTechnologies, San Diego, CA) and 50 µg/ml of each single guide RNA targeting *B2M* and *CIITA* (Synthego Corporation, Redwood City, CA), respectively. The islet cells were transferred into low attachment 6-well plates in complete islet medium [CMRL1066 media (Corning, Corning, NY) supplemented with HEPES (1x, Gibco, Waltham, MA), 1% L-Glutamine (200mM; Gibco), 1% Nicotineamide-Sodium pyruvate (Swedish Pharmacy APL, Sweden), 10% human pooled pathogen inactivated (INTERCEPT, Cerus Europe BV, Amersfoort, Netherlands) AB serum (Blood Services, Uppsala University Hospital), 0.01 mg/ml Gentamicin (40mg/ml; Braun, Melsungen, Germany), and 0.01 mg/ml Ciprofloxacin (2mg/ml; Actavis, Oslo, Norway), rested for 1h at 37°C, 5% CO_2, and 95% humidity before moving the plates on the orbital shaker (IBI Scientific, Dubuque, IA) for cell re-clustering.

After 2 days, islet cell clusters were singularized again with AccuMax as described above before transduction with an EF1α-human CD47 lentiviral vector with a MOI of 7 (provided by Sana Biotechnology). Media change was performed the day after transduction with lentivirus and refreshed every 2-3 days. Throughout the process, islet cells were kept on the orbital shaker as described above, allowing them to re-cluster. The final genetically modified islet cells (UP421) were formulated for qualified transportation in a Platelet Storage Bag (Terumo, BCT, Zaventem, Belgium) as a fresh product on day 7. Final release tests and in-process controls was performed according to European Pharmacopoeia.

UP421 transplantation

The UP421 product was transferred from the transport container into 5 sterile Falcon tubes (Corning) and centrifugated at 300g for 4 minutes immediately before transplantation. The pellets were transferred to 18G Venflon® catheters (Becton Dickson Infusion Therapy, Helsingborg, Sweden) in the operating room. The islets were transplanted in aliquots between the fibers of the brachioradialis muscle of the left forearm of the recipient under general anesthesia as previously described[3]. The total UP421 product volume was 3.2 ml and the implantation procedure lasted for 90 min.

Flow cytometry
To assess HLA I, HLA II and CD47 expression by flow cytometry (BD FACS Canto II cytometer, BD Biosciences, Franklin Lakes, NJ), a few islet clusters were dissociated into single cells as described above and stained with anti-HLA-A,B,C antibody (clone G46_2.6, BD Biosciences), anti-HLA-DR,DP,DQ antibody (clone Tu39, BD Biosciences) and anti-CD47 antibody (clone B6H12, BD Biosciences). Results were shown in percentage HLA I or HLA II negative cells or CD47 overexpressing cells, compared to the unedited cells. Data were acquired and analyzed with BD FACS Diva Software version 6.0 (BD Biosciences).

Clinical Protocol
See Supplement.

Cell Sorting of patient's peripheral blood mononuclear cells (PBMC)
PBMCs were isolated from patient blood samples collected at baseline and week 1, 2, 3, 4, 6, 8, and 12 after UP421 transplantation. PBMCs were stained in PBS (Gibco) supplemented with 2% heat-inactivated FSC (Gibco) with AF488-labeled CD3 (clone UCHT1; Biolegend, San Diego, CA) or PerCP-Cy5.5-labeled CD56 (clone MEM-188, Biolegend) antibodies. Cell sorting was performed for CD3-positive T cells and CD3-negative and CD56-positive natural killer (NK) cells using BD FACSAria Fusion.

Macrophage differentiation from monocytes
Patient PBMCs were plated in 24-well plates at a concentration of 1 $x10^6$ cells per ml macrophage media (RPMI 1640 Glutamax, 10% FCS, 1% Pen/Strep, all Gibco) and 10 ng/ml human M-CSF (Gibco). Media was changed every other day until day 6. Macrophages were stimulated from day 6 with 1 µg/ml human IL-2 (Peprotech, Cranbury, NJ) for 24 hours before the cells were used.

Isolation of islet cell subpopulations for immune assays
All WT, DKO, and HIP islet cells for the immune assays were isolated through flow cytometry sorting from the UP421 product.

ELISpot Assay
Human IFN-γ ELISpot assays (BD Bioscience) were performed with WT, DKO or HIP islet cells as stimulator cells after mitomycin-treatment with 50 µg/ml for 30 min and patient T cells as responder cells. A total of 1 x 10^5 stimulator cells were incubated with 1 x 10^5 recipient responder cells for 48 hours and incubated with HRP Streptavidin (BD Bioscience). Spots were developed using AEC Substrate Solution (BD Bioscience) and IFN-γ spot frequencies were enumerated using an ELISpot plate reader (AID Diagnostika GmbH, Strassberg, Germany).

In vitro killing assays (XCelligence)
T cell, NK cell and macrophage killing assays were performed on the XCelligence MP platform (Agilent Technologies, Santa Clara, CA). Specialized 96-well E-plates (Agilent Technologies) were coated and 4 x 10^4 target islet cells were plated in 100 µl islet media. After the Cell Index reached 0.7, effector cells were added at an effector cell to target cell (E:T) ratio of 1:1. NK cells were stimulated with 1 µg /ml human IL-2 (Peprotech).

PBMC and serum killing as well as CDC and ADCC killing assays were also performed on the XCelligence MP platform. For CDC assays, 100 µl of untreated, complement-containing serum (1:1 mixed with media) was added. For PBMC and ADCC assays, 50 µl heat-inactivated serum with 4 x 10^4 human NK cells or human PBMCs were added. As killing control, target cells were treated with 2% TritonX100 (Sigma-Aldrich, St. Louis, MO). As survival control, cells were only incubated with media. Data were standardized and analyzed with the RTCA software (Agilent Technologies).

Donor specific antibodies (DSA)

Patient sera were heat-inactivated at 56°C for 30 minutes to deplete complement. Equal volumes of the sera and singularized WT, DKO or HIP islet cells (5 x 10^6 cells/ml) were incubated for 45 minutes at 4 °C. The cells were then labeled with FITC-conjugated goat anti-human IgM (Thermo Fisher, Waltham, MA) or IgG (Sigma-Aldrich), and mean fluorescence intensity was assessed using flow cytometry (Attune, Thermo Fisher).

C-peptide ELISA

Basal C-peptide levels or C-peptide levels following a Mixed Meal Tolerance Test (MMTT; 0 min, 15 min, 30 min, 60 min, 90 min, 120 min) in patient's serum were quantified using a highly sensitive Human C-Peptide ELISA Kit (Abcam, Cambridge, UK). Briefly, standards or samples were added to the 96-well ELISA plate along with the provided antibody cocktail and incubated for 1 hour. After washing, TMB Substrate was added to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. One hundred µl of Stop Solution was added to each well. Samples were analyzed in a microplate reader at 450 nm (Perkin Elmer, Waltham, MA).

Magnetic Resonance Imaging (MRI)

Subjects were scanned on a 3T MR scanner (Philips Healthcare, Achieva dStream, Best, the Netherlands). Subjects were positioned headfirst in supine position. An 8-channel small extremity phased array coil served for signal reception. Survey (Fast Field echo) images were acquired in axial, sagittal, and coronal directions to guide subsequent imaging planning for the study. T2-weighted (T2w), T1-weighted (T1w) and T2-STIR-weighted turbo spin echo (TSE) sequences (slice thickness 3 mm, spacing between slices 0.3 mm) were acquired in axial, sagittal, and coronal directions. Additionally, an axial Proton density-weighted (PDw) TSE with SPAIR were acquired. Axial diffusion-weighted imaging (slice thickness 4 mm, spacing between slices 0.4 mm) were performed using a spin-echo sequence with echo-planar imaging readout with multiple diffusion-sensitizing values (b = 0, 400, 800 s/mm^2). The total measurement time of the entire scan session was 40 to 45 min.

PET-MRI

[^{68}Ga]Ga-NODAGA-exendin-4 is a PET tracer which binds to GLP1R as has been validated for detection of beta cell derived insulinomas[4] and intrahepatic islet grafts[5]. [^{68}Ga]Ga-NODAGA-exendin-4 was produced according to full GMP as described previously[4].

Following a 3 hour fast, the patient was placed in supine position with the arms along the sides in the gantry of an integrated PET/MRI scanner (Signa 3T, GE Healthcare). The left forearm with the islet graft was in the center of field of view. A target dose of 100 MBq [^{68}Ga]Ga-NODAGA-

exendin-4 (corresponding to approximately 10 µg peptide mass) was injected intravenously in the right arm. A 60-minute dynamic PET scan was initiated at injection. Anatomical MRI scans were performed in parallel.
The PET signal in the brachioradial muscle and relevant tissues were analyzed using PMOD software (PMOD Technologies) using co-registered MRI sequences as anatomical references.

Author contributions:
P.O.C. serves as the Principle Investigator of the trial (ClinicalTrials.gov ID NCT06239636), designed the study, vouches for the data and analyses, co-wrote the paper and decided to publish the paper. X.H. performed the manufacturing, gathered and analyzed the immunobiology data. H.S. performed and supervised the GMP manufacturing, designed the study, and co-wrote the manuscript. S.I. isolated the pancreatic islets and gathered and analyzed the islet data. T.L. and T.S. performed the surgical procedure. P.L. and O.E. gathered and analyzed the MRI and PET/MRI data. D.Y. established and supervised the VCN assay. O.K. lead and supervised the islet isolation and team, designed the study, and cowrote the manuscript. T.D. analyzed the data, developed, and produced the figures and wrote the manuscript. S.S. conceptualized and designed the study and experiments, supervised the project, and co-wrote the manuscript. All authors helped edit the manuscript. There are no agreements concerning data confidentiality between the sponsor, authors, or institutions.

UP421 genome integrity: Summary and Conclusions

The UP421 manufacturing process was developed using islets isolated from five donors. Measures of risk assessed were evaluation of receptor surface expression by flow cytometry and CD47 quantification, evaluation of insulin secretion, evaluation of LVV integration via assessment of VCN, evaluation of off-target editing, evaluation of on-to-on translocation, evaluation of ISA, and evaluation of chromosomal structural variations via KaryoStat analysis. **In summary, the results demonstrated low levels of risk related to oncological and genotoxic safety for UP421 for the following reasons**:

The combined OTA and flow cytometry data (n=5) demonstrated that Cas12b mRNA with sgRNAs efficiently edited and disrupted the *B2M* and *CIITA* genes. The levels of HLA I and HLA II protein surface expression were the determining factor for whether the cells will be rejected by the transplant recipient. Thus, while OTA was used in these studies to characterize the UP421 DP, HLA I/II cell surface protein expression was used as release criteria for UP421. The combined flow cytometry and CD47 quantification data (n=5) showed that UP421 had consistent and reproducible high levels of CD47 expression observed across the five batches of UP421 manufactured from 5 different donors.

The insulin secretion assay (n=5) demonstrated that wild type islets (WT) and UP421 DP, each prepared from the same donor, had similar insulin secretion responses to 5 mM glucose (>20,000 mIU/10^6 cells for all UP421 batches), indicating that the UP421 genome engineering process did not impact glucose sensing or insulin secretion as measured *in vitro*.

In the off-target analysis experiment (n=2), UP421 DP was evaluated at 80 potential off-target cleavage sites identified for the *B2M* and *CIITA* editing reagents via Cas-OFFinder analysis, as well as GUIDE-seq in highly edited T cells (mean indel signal of > 89% across loci). Cas-OFFinder is a cell type-independent in silico analysis used to scan the human genome for sequences exhibiting similarity to the *B2M* and *CIITA* on-target sites. For this analysis, all sites with three or fewer mismatches to an on-target sequence were considered as potential off-targets, along with sites bearing up to four mismatches that were located within genome regions exhibiting a greater likelihood of functional consequence (i.e., coding exons, untranslated regions, splice sites and promoters). The GUIDE-seq analysis was performed with the same gene editing reagents used to manufacture UP421 and yielded evidence of cutting at only the on-target loci (> 20,000 tags recovered for each sgRNA), with no compelling signal of double-stranded breaks seen at any other genomic locus (maximum signal of three tags recovered). While no compelling signal of off-target cleavage was observed, 38 low confidence candidate off-target sites (2 or 3 tags recovered at each locus) were carried forward into amplicon sequencing studies of UP421 edited cells. No evidence of double stranded DNA cleavage at any of the 80 candidate off-target sites was observed in material representative of UP421 DP and no statistically significant off-target sites were identified using a False Discovery Rate threshold of < 0.3 in the amplicon sequencing studies. The off-target study in UP421 batches revealed that each of the Cas12b guides used in the manufacturing process exhibit a very high level of on-target cleavage preference. In particular, amplicon sequencing to interrogate candidate off-target sites (identified via Cas-OFFinder as well as GUIDE-seq analysis) revealed no evidence of off-target editing via standard statistical analysis (FDR< 0.3). These results are consistent with prior reports of Cas12b nuclease system as being specific[6].

In the on-to-on target translocation experiment (n=3), UP421 DP was evaluated for on-to-on target translocation species. At the aggregate levels observed, on-to-on target translocation species represented a low safety risk for the following reasons. The low frequency of on-to-on target balanced translocations (at < 0.5%, on average) represented a low safety risk for the following reasons. First, the biology associated with the *B2M* and *CIITA* genes involved in the translocations suggested no increased risk associated with their translocation products. Second, there is precedence for cells being generated with similar translocations that have been administered to patients with no reported safety consequences to date. In addition, these levels and types of chromosomal translocations are consistent with other ex vivo cell therapies (e.g., CAR T therapies) in which cells have been edited simultaneously at two or more loci (including *B2M* and *CIITA*) and have been used in clinical trials with no safety concerns to date[7-9]. Importantly, these studies used higher cell doses than UP421 per patient.

The KaryoStat+ genomic structural variant analysis demonstrated that the UP421 manufacturing process did not introduce chromosomal aberrations.

The ISA characterization (n=3) data demonstrated that the integration of LVV in UP421 appears to be semi-random based on insertion site (IS) frequency across UP421 batches. ISs were diverse in each UP421 batch and the ISs were diverse across the UP421 batches as well. The data demonstrated that each UP421 batch had a high level of polyclonality and high IS diversity, which are comparable to or greater than the diversity of lentiviral vector-modified drug products used in the clinic to date[10-12]. Overall, this supported the notion that the insertional profile of the LVV does not pose a risk of genotoxicity higher than the one reported for other clinical applications of the same platform.

The conclusions herein represent the evaluation of UP421 data (five batches made from 5 donors) using assessment of receptor surface expression by flow cytometry and CD47 quantification, insulin secretion, LVV integration via assessment of VCN, on-target and off-target editing, on-to-on translocation, ISA, and chromosomal structural variations via KaryoStat analysis. **The combined OTA and flow cytometry data (n=5) demonstrated that Cas12b mRNA with sgRNAs efficiently edit and disrupt the *B2M* and *CIITA* genes. UP421 LVV consistently produced consistent and reproducible high levels of CD47 expression. No bona fide sites of off-target editing were identified, aggregate on-to-on target translocation rates were consistent with other cellular products with multiplexed editing at two or more loci, and there was no evidence of gross chromosomal abnormalities or change in the insulin producing capacity. Finally, ISA demonstrated that LVV integration is semi-random and that UP421 is polyclonal.**

Based on these data, the oncological and genotoxic safety risk is considered consistent with that of other gene-modified products. This assessment was supported by the NHP data[13] which demonstrated that intramuscularly injected HIP islet cells were non-proliferative (as demonstrated by bioluminescence imaging for up to 9 months) and stayed localized to the site of implantation.

Supplementary Figures

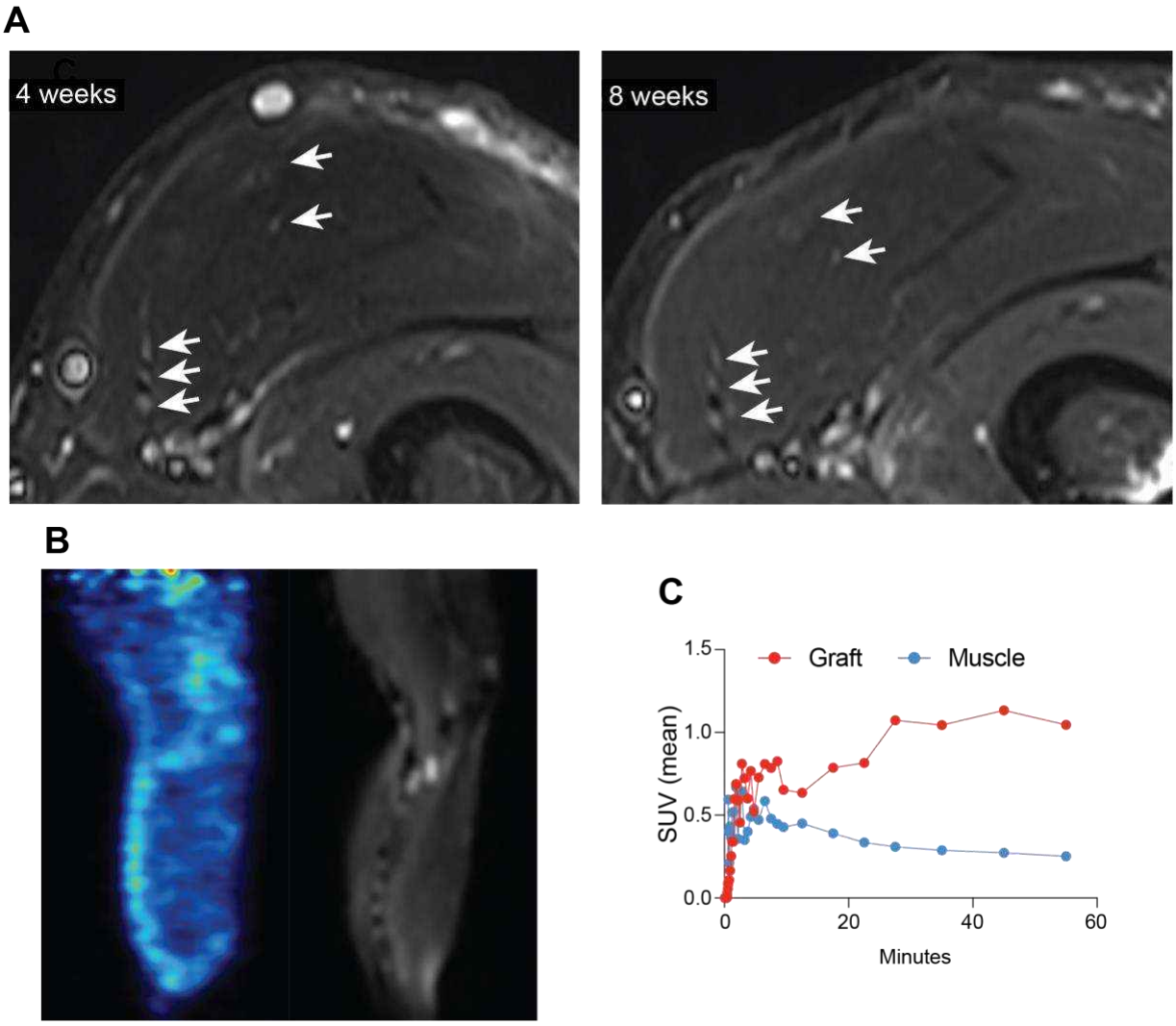

Figure S1: *Imaging of the islet graft*.
Panel A shows T2-STIR-weighted MRI images of the study subject's left forearm. White arrows point at several punctual signals at the site of graft injection in the brachioradialis muscle with no inflammation and no safety or pathological related observations. Panel B shows a PET/MRI after 12 weeks using the GLP-1R targeting tracer [^{68}Ga]Ga-NODAGA-exendin-4. GLP-1R signal co-localized with the UP421 injection sites. Panel C shows a standardized uptake value (SUV) graph for tracer uptake at graft sites vs. surrounding muscle. Tracer accumulation was only observed at graft sites, while tracer washout was observed in muscle.

Supplementary Tables

Event name	Adverse Event	AE Start Date	AE Stop Date	Serious AE
Adverse Event 1	Thrombophlebitis	2024-12-11	2025-01-27	No
Adverse Event 2	Worsening infected ulcer tip of finger	2024-12-17	2025-02-25	No
Adverse Event 3	Worsening of hyperhidrosis	2024-12-23	2025-03-24	No
Adverse Event 4	Paresthesia local left lower arm	2024-12-23		No

Relationship with study product	Relationship with study procedure	Relationship with other	Please Specify Relationship with Other	Action Taken with Study Drug:
Unlikely	Probable	Unlikely		Not Applicable
Unlikely	Unlikely	Probable	cat bite	Not Applicable
Unlikely	Unlikely	Possible	Improved metabolic control	Not Applicable
Unlikely	Possible	Unlikely		Not Applicable

Concomitant or additional treatment given	Maximum AE Intensity	AE Outcome
Yes	2 - Moderate	Recovered/Resolved
Yes	2 - Moderate	Recovered/Resolved
No	1 - Mild	Recovered/Resolved
No	1 - Mild	

Table S1: *Adverse effects*.

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Protocol

Protocol for: Carlsson P-O, Hu X, Scholz H, et al. Survival of transplanted allogeneic beta cells with no immunosuppression. N Engl J Med. DOI: 10.1056/NEJMoa2503822

This trial protocol has been provided by the authors to give readers additional information about the work.

This supplement contains the following items:

1. Final Protocol

CLINICAL STUDY PROTOCOL

"First-in-human safety study of hypoimmune pancreatic islet transplantation in adult subjects with type 1 diabetes"

Protocol version:	*3.1*
EUCT number:	*2023-507988-19-00*
Investigational Product:	*Genetically modified human pancreatic islets (UP421)*
Development phase:	*I*
Sponsor:	*Region Uppsala, Uppsala University Hospital*
Sponsor Representative:	*Per-Ola Carlsson; Professor in Medical Cell Biology,* *Senior Consultant in Endocrinology and Diabetology* *+46 70 2721167*
Authors:	*Per-Ola Carlsson, Olle Korsgren*
Principal Investigator:	*Per-Ola Carlsson*

The clinical study will be conducted, and essential documentation archived, in compliance with relevant UCR Standard Operating Procedures (SOPs) and standards, which incorporate the requirements of the ICH Guideline for Good Clinical Practice and Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014.

The following amendment(s) is/are accompanying the protocol:

1. Date: Contact Person *(initials)*:

2. Date: Contact Person *(initials)*:

SYNOPSIS

Title of study:
"First-in-human safety study of hypoimmune pancreatic islet transplantation in adult subjects with type 1 diabetes"

EU trial number: 2023-507988-19-00

Sponsor
Region Uppsala (Akademiska sjukhuset)

Name of investigational product:
UP421 (genetically modified human pancreatic islets)

List of auxiliary medicinal products:

Basiliximab, Cefuroxim, Etanercept, Mycophenolat, Omeprazol, Sulfametoxazol, Tacrolimus, Valganciclovir

Dosage and administration of investigational product:

$25 \times 10^6 – 80 \times 10^6$ islet cells transplanted to forearm muscle

Intervention:

One-time transplantation of Langerhans islet cells into forearm muscle of two subjects. Both receive treatment. Safety is monitored with frequent follow-up visits over the course of a year, including medical examinations, blood tests and MRI scans. Cell function is monitored with blood samples and continuous glucose measurement.

Investigator(s) and study center(s):
Per-Ola Carlsson, Dept of Diabetology and Endocrinology, Uppsala University Hospital

Planned study period:	**Study phase:**
Q3 2023-Q1 2025	I

Rationale

Type 1 diabetes is a chronic disease where affected individuals have lost their own insulin production. The only way to cure the disease is to replace the lost insulin-producing cells with new ones. The current study tests the hypothesis whether genetically modified Langerhans islet cells containing insulin-producing cells from a deceased organ donor can be transplanted safely and help to regain insulin production in individuals with type 1 diabetes without need in simultaneous treatment with immunosuppressive medicines.

Objectives:
Main Objective:
To investigate the safety of an intramuscular transplantation of genetically modified allogeneic human islets (study product UP421) in adult subjects diagnosed with type 1 diabetes.

Secondary objective:
Study changes in beta-cell function, metabolic control and immunological response to pancreatic islets during the first year following treatment.

Number of subjects (planned):
2

Diagnosis:
Type I Diabetes for ≥ 5 years

Inclusion criteria:

1. Signed informed consent for participation in the study.
2. Diagnosis of type 1 diabetes mellitus (T1D);
   i) for ≥ 5 years and
   ii) at least one or more HbA1c documented in the subject's medical journal or Swedish National Diabetes Registry during the last five-year period must be ≥70 mmol/mol.
3. The subject must be involved in intensive diabetes management defined as self-monitoring of subcutaneous glucose level by continuous glucose monitoring or by intermittent scanning glucose monitoring no less than a mean of three times per day averaged over each week and by the administration of three or more insulin injections per day or insulin pump therapy. This management must be under the direction of an M.D specialised in endocrinology and diabetology with support of a diabetes nurse at a specialist clinic for Endocrinology and Diabetology or Internal Medicine during the 12 months prior to study enrolment;
4. C-peptide negative (C-peptide < 0.01 nmol/L) in response to a mixed meal tolerance test (MMTT);
5. Positive for antibodies to either GAD or IA2 at screening;
6. 30-60 years of age at time of enrolment;
7. HbA1c ≥70 mmol/mol;
8. Exogenous insulin needs <1 IU/kg;
9. Body weight <80 kg;
10. Female subjects of child-bearing potential must agree to using adequate contraception until one year after the administration of UP421, as outlined in https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf
    A woman is considered of childbearing potential if she is not surgically sterile or isles than 1 year since last menstrual period.
    Adequate contraception is as follows:
    a) combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal)
    b) progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable)
    c) intrauterine device (IUD)
    d) intrauterine hormone-releasing system (IUS)
    e) bilateral tubal occlusion
    f) vasectomised partner

g) sexual abstinence

Male subjects must not intend to procreate until one year after the administration of UP241. Males must be willing to use effective measures of contraception (condoms) during the whole trial period.

Exclusion criteria:

1. Any previous organ transplantation;
2. Any systemic immunosuppressive medication for any other disease;
3. Any history of malignancy;
4. Use of any investigational agent(s) within 4 weeks of enrollment;
5. Use of any anti-diabetic medication, other than insulin, within 4 weeks of enrollment;
6. Active infections including Tuberculosis, HIV, HBV and HCV;
7. Liver function test value for AST, ALT, GGT or ALP exceeding the respective reference interval for the clinical assay at Uppsala university hospital;
8. Serological evidence of infection with HTLVI or HTLVII;
9. Pregnancy, nursing, intention for pregnancy;
10. Chronic kidney disease grade 3 or worse (eGFR<60 mL/min/1.73m^2 as estimated by creatine measurement using the revised Lund-Malmö Equation) ;
11. Medical history of cardiac disease, or symptoms at screening consistent with cardiac disease;
12. Known autoimmune disease other than type I diabetes, coeliac disease, thyroid disorder, pernicious anaemia, or vitiligo;
13. Administration of live attenuated vaccines <6 months before transplant;
14. Positive for ZnT8 auto-antibodies;
15. Untreated proliferative diabetic retinopathy;
16. Major ongoing psychiatric illness which the Principal Investigator judges increases the risk of noncompliance or does not allow safe participation in the study;
17. Ongoing substance abuse, drug or alcohol; or recent history of treatment noncompliance;
18. Known hypersensitivity to ciprofloxacin, gentamicin, or amphotericin (since these are used in the manufacturing process of UP421);
19. Any other condition that in the opinion of the Principal Investigator does not allow safe participation in the study.

Main trial endpoint: Safety. Number of treatment-related adverse events as assessed by CTCAE v5.0 (time frame: 12 months after treatment).

Secondary endpoints:

1. Immune evasion of implanted cells, as evaluated in systemic PBMC and serum at 0 (before tx), 2, 4, 8, 12, 18, 26, and 52 weeks post-transplantation.
2. Presence of peak c-peptide >0.01 nmol/L, in response to the mixed meal tolerance test at 4, 8, 12 ,18, 26, and 52 weeks post-transplantation
3. Presence of non-fasting c-peptide concentrations >0.01 nmol/L at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 52 weeks post-transplantation.
4. Presence of peak c-peptide >0.20 nmol/L, in response to the mixed meal tolerance test at 4, 8, 12 ,18, 26, and 52 weeks post-transplantation.
5. Survival of implanted cells, as evaluated by MRI, within 48h after transplantation and 2, 4, 6, 8, 12, 26, and 52 weeks post-transplantation,n, as well as by PET/MRI at one scan in between 8-12 weeks post-transplantation and one scan at 52 weeks post-transplantation.
6. C-peptide AUC in response to the mixed meal tolerance test at 4, 8, 12, 18, 26, and 52 weeks post-transplantation.
7. Delta changes in insulin requirement/kg BW at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 52 weeks post-transplantation when compared to before transplantation.
8. Delta changes in HbA1c at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 26, and 52 weeks post-transplantation when compared to before transplantation.
9. Delta changes in glucose variability (time in range, SD, CV) and hypo/hyper glycaemia duration derived from a continuous glucose monitoring system performed at 4, 8, 12, 18, 26, and 52 weeks post-transplantation when compared to before transplantation.
10. Score in diabetes treatment satisfaction questionnaire in transplanted patients at 4, 8, 12, 18, 26, and 52 weeks post-transplantation when compared to before transplantation.

Study design: One-armed, open, single centre study with one-year follow-up after treatment.

Ethical considerations:

There is no guarantee that the subjects will benefit from participation in the study but if the treatment proves to be effective the ability to form their own insulin can be a great advantage for them. This can lead to easier diabetes treatment, it becomes easier to maintain a good blood sugar balance which in its turn can reduce the risk of diabetes complications, both in the short and long term. If it turns out that hypoimmune beta cells can recreate the body's own insulin production without the need for immunosuppression it would be of great benefit to individuals with diabetes. In this trial a low cell dose has been chosen for safety reasons but if it proves safe and effective more benefit can be given by increasing the cell dose and offering curative treatment. The cell product is genetically modified and being tested for the first time in humans which is a risk in itself. Islet cells, however, have a very low ability to divide and form new cells, i.e. the risk that the cells would start to grow uncontrolled and form a tumour is very low. Based on animal studies, the treatment has been deemed safe and no specific risks have been demonstrated. We therefore assess that the possible benefit outweighs the risks.

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LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADA	American Diabetes Association
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
ARO	Academic Research Organisation
AST	Aspartate aminotransferase
ATMP	Advanced Therapeutic Medicinal Product
AUC	Area Under the Curve
BP	Blood Pressure
BW	Body Weight
CD	Cluster of Differentiation
CGM	Continuous Glucose Monitoring
CM	Centimeters
CRF	Case Report/Record Form
CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR Associated Protein
CRP	C-Reactive Protein
CV	Coefficient of Variation
DSA	Donor Specific HLA Antibodies
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
DTSQ	Diabetes Treatment Satisfaction Questionnaire
ERA	Ethical Review Authority
FAS	Full Analysis SET
FUP	Follow Up
GAD	Glutamic Acid Decarboxylase
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GGT	ɣ-glutamyl transferase
GLP-1 RA	Glucagon Receptor Like Peptide-1 Receptor Analogue
GMP	Good Manufacturing Practice
HbA1c	Haemoglobin A1c
HBV	Hepatitis B Virus
HCG	Human Choriogonadotropin
HCV	Hepatitis C Virus
HIP	Hypoimmune
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
IA2	Insulinoma Antigen 2
ICH	International Conference on Harmonisation
IMP	Investigational medicinal product
IP	Investigational product
LVV	LentiViral Vector
MedDRA	Medical Dictionary for Regulatory Activities
MIC	MHC class I chain-related
MMF	Mycophenolate mofetil
MMTT	Mixed Meal Tolerance Test
MPA	Medical Products Agency
MRI	Magnetic Resonance Imaging

NK	Natural Killer
NNCIT	Nordic Network for Clinical Islet Transplantation
NYHA	New York Heart Association
PBMC	Peripheral Blood Mononuclear Cells
PET	Positron emission tomography
PK	Protrombin complex
PPS	Per Protocol Set
PTT	Partial Thromboplastin Time
RSI	Reference Safety Information
SAE	Serious Adverse Event
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Event
TPO	Thyroid Peroxidase
T1D	Type 1 Diabetes
UCR	Uppsala Clinical Research Center
ZnT8	Zinc Transporter 8

1 GENERAL INFORMATION/STUDY ADMINISTRATIVE STRUCTURE

	Name, title, address, and e-mail address	**Telephone numbers**
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Laboratory	All clinical laboratory samples will be measured at Uppsala University Hospital Laboratory where biobank samples also will be stored.	

	Name, title, address, and e-mail address	Telephone numbers
Data Safety Monitoring Board	DSMB Chair: **1**. Ulf Smith, MD, PhD Professor of Internal Medicine Department of Internal Medicine SU/Sahlgrenska, Blå Stråket 3, 413 45 Göteborg, Sweden E-mail: ulf.smith@medic.gu.se DSMB Members: **2.** Mikael Rydén, MD, PhD Professor of Clinical and Experimental Adipose Research H7 Endocrinology and diabetes, Department of Medicine Karolinska Institutet 17177 Stockholm, Sweden E-mail: mikael.ryden@ki.se **3.** Anders Fasth, MD, PhD Professor of Pediatric Immunology, Dept of Pediatrics, University of Gothenburg, The Queen Silvia Children's Hospital, SE-416 85 Göteborg, Sweden E-mail: anders.fasth@gu.se	
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2 SIGNATURE PAGE

SPONSOR/ PRINCIPAL INVESTIGATOR/PROTOCOL AUTHOR

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Signature and date:

Please see electronic
signature_____

3 BACKGROUND INFORMATION

3.1 Pathophysiology of Diabetes

At the onset of type 1 diabetes (T1D), the beta-cell mass has regularly decreased to 20-40% of normal levels (1) and continues to drop after a brief honeymoon period. The inherently skewed glucose homeostasis of type 1 diabetes entails a life-long balancing act for patients. On the one hand, chronic hyperglycaemia goes with several severe complications, on the other hand, hypoglycemic episodes are potentially lethal. The life-saving discovery of insulin came in 1922, and while refined, exogenous insulin delivery still cannot mimic the endogenous glucose regulation of intact pancreatic islets. Insulin secretion also in the level of 10 % of normal is associated with both lower HbA1c and blood glucose variability, as well as a lower risk of ketoacidosis (2, 3). Additionally, some insulin production reduces the risks of severe hypoglycaemia and late diabetes complications substantially.

In Sweden there are currently about 50 000 subjects over the age of 18 that are affected by T1D. Almost all these subjects are included in the National Diabetes Registry (NDR). Almost all clinically relevant parameters are regularly updated in the NDR, offering a unique national database for clinical management and research in T1D. Available treatment modalities for subjects with T1D have improved dramatically over the last decade and are readily available without cost for the patients. Today, most subjects are offered the use of devices for Continuous Glucose Measurements (CGM and isCGM) and many have pumps for subcutaneous administration of insulin.

The board for welfare in Sweden (Socialstyrelsen) has defined the target level for glucose metabolism to be HbA1c $\leq$ 52 mmol/mol. In spite of the technical improvements only 27% of all subjects ($\geq$ 18 years) with T1D have a HbA1c $\leq$ 52 mmol/mol. Notably, the mean age of these subjects are only 49 years, i.e., persons in the middle of their lives with many years until retirement. Further, NDR reveals that these subjects only a few years after diagnosis of T1D are unable to achieve an acceptable glucose control. These individuals with unacceptable control of their glucose metabolism over several years have a high risk for developing serious late complications of T1D, e.g., stroke, heart infarction, nephropathy, retinopathy and blindness, amputations and premature death, and can therefore be considered as medical emergencies.

We know via the large DCCT and EDIC studies (4, 5) that better glucose control due to intensive T1D care (HbA1c in the range of 55 mmol/mol) for a period of 7-8 years constitutes pronounced protection from T1D late complications even though participating subjects within only 3 months after the DCCT trial had HbA1c levels equal to that of the control group. This offered a unique opportunity to study the long-term benefits of a period of acceptable glucose metabolism on late complications. Now, almost 25 years later the EDIC study clearly shows a pronounced and maintained protective effect on all late complications of T1D (6). The DCCT study also showed that the risk for being affected with severe hypoglycaemia was related to better glucose control (7).

Despite great medical advances, people with T1D in Sweden still have a shortened lifespan of 10-18 years depending on age of onset and sex (8). Notably, this reduction in lifespan is mainly caused by those with high HbA1c as discussed above. An ideal intervention would restore some or all endogenous insulin-production and halt the disease from progressing. To date, no intervention strategies to restore endogenous beta-cell function are available.

3.2 Pancreas and Islet Transplantation

In principle, the cure for type 1 diabetes lies in the possibility of replacing destroyed or exhausted beta cell mass in order to restore two essential functions: sensing blood sugar levels

and secreting appropriate amounts of insulin and glucagon in the vascular bed. Currently, the only available clinical approach of restoring beta cell mass in patients with diabetes is the allogenic transplantation of beta cells (i.e., pancreas or islet transplantation). Clinical trials performed in the last three decades have shown that restoration of beta-cell function via transplantation of isolated islet cells or vascularised pancreas reproducibly allow achievement of a more physiological release of endocrine hormones than exogenous insulin in diabetic patients (9).

Current immunosuppressive regimens can prevent islet failure for months to years, but chronic immunosuppression often leads to toxicity in multiple organ systems and subsequently often new symptoms, or disorders develop post-transplant as a consequence of the side-effects of immunosuppressive drugs, such as opportunistic infections, malignancies, renal dysfunction, hypertension and others (10). In addition, the most commonly used agents (like calcineurin inhibitors and rapamycin) are also known to impair normal islet function and/or insulin action. Therefore, strategies to overcome the need of long-term immunosuppression are needed.

The primary advantage of the UP421 hypoimmune platform compared to other allogeneic pancreatic islet cell transplants is the potential of long-term normoglycaemia without the need of immunosuppression.

3.3 UP421

UP421 is composed of human pancreatic islets obtained from regular deceased organ donors, isolated using the same protocol as for clinical islet transplantation, but in addition genetically modified to evade allogeneic immune cell recognition. UP421 is transduced via a lentiviral vector (LVV) to incorporate the gene for overexpression of CD47. UP421 is also gene-edited with a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated protein (Cas) 12b system to abrogate the function of beta-2-microglobulin (B2M) and the class II major histocompatibility complex transactivator (CIITA) genes. The overexpression of CD47 is key for evasion from cells of the innate immune system. The impairment of HLA I/II function through knockout of B2M and CIITA genes via CRISPR/Cas12b system serves the evasion from cells of the adaptive immune system. Together, the aim is to enhance survival and persistence of the transplanted genetically modified islets.

The UP421 drug product is a suspension of fresh (non-cryopreserved) UP421 pancreatic islets, which are formulated for intramuscular injection into a patient. UP421 is intended for the treatment of type 1 diabetes as a novel approach for clinical islet transplantation without the need of long-term systemic immunosuppression in the intramuscular location (forearm). The production of the Advanced Therapy Medicinal Product (ATMP) will be performed in the GMP facility at Oslo University Hospital, Radiumhospitalet.

3.4 Non-Clinical Studies

Allogeneic immune recognition and rejection remains an obstacle in transplant and cell therapy development; recently, researchers have turned to the field of fetal maternal tolerance. During pregnancy, the maternal immune system is tolerant of allogeneic paternal antigens through a series of multiple complex changes that have evolved over time and occur during placental implantation and early embryonic development (11). The Schrepfer lab examined syncytiotrophoblast cells which form the interface between maternal blood and fetal tissue and attempted to identify pathways and factors that are critical for immune tolerance. Over the course of a decade and multiple experiments, the lab uncovered that elimination of HLA class I and II expression and overexpression of CD47 were important genetic modifications to evade immune recognition. CD47, a membrane protein that interacts with signal regulatory

protein-alpha (SIRPalpha) on innate immune cells, is key for evasion of innate immune cells, including NK cells and macrophages which can immediately kill implanted cells (12, 13). It was recognised that this combination of genetic modifications might support allogeneic transplantation without the need for immunosuppression and enable a new class of therapies. This concept was illustrated in several publications involving hypoimmune mouse-induced pluripotent stem cells which were differentiated into multiple cell types for in vivo transplantation studies (14, 15). During development, NK cells undergo an educational "licensing" process to ensure that only those that express a cognate inhibitory receptor for self-HLA class I molecules become functionally mature. This central self-tolerance mechanism sets the triggering threshold, and the integration of all transmitted activating and inhibitory signals determines the outcome and magnitude of interactions with target cells. In-depth analysis of NK cell interactions with immune-edited cells revealed a new immune checkpoint, SIRPalpha, which delivers a strong inhibitory signal when engaging with its ligand CD47. An immune editing strategy including CD47 thus provides protection not only against macrophages but also NK cell killing (13). Recent data has clarified that this genetic engineering approach does not induce tolerance, which requires immune recognition and host immune education and acceptance. Rather, data indicate a complete lack of immune recognition of the transplanted cells.

3.5 Dose Selection Strategy

Completed exploratory in vitro and in vivo studies (see IB) using research grade HIP islet cells have demonstrated the intended pharmacological activity of glucose sensing and insulin secretion that is similar to unedited islet cells. HIP islet cells uniquely evade innate and adaptive host immune system responses when compared to unedited or HLA-I/II KO islet cells. Additionally, administration of research grade HIP islet cells was well-tolerated in mice and in non-human primates. Taken together, the exploratory pharmacology data suggest that HIP islet cells have similar biological activity as unedited islet cell transplantation with clinical experience and similar findings are anticipated with UP421. Therefore, non-clinical pharmacology studies will not be used to identify the minimum effective dose intended to inform the clinical dose for intramuscular islet cell transplantation. The proposed clinical dose and dose range will be based upon clinical experience with allogeneic and autologous islet cell therapies. Since this is a first in human study, a low dose, about or less than 1/10 of the dose required to cure a T1D patient (16), will be selected for first treatment in the two participants, in order to focus on safety, while still providing the potential to evaluate graft survival by C-peptide measurements and to facilitate improved metabolic control. Forthcoming clinical studies will aim to provide a full islet cell dose comparable with that currently used in clinical islet transplantation.

The Phase 1 clinical study design follows principle of EMA Guideline" Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products". The overall objective of this clinical study is to evaluate the safety of hypoimmune edited cadaveric pancreatic islet cells as a novel approach for clinical islet transplantation without the need of long-term systemic immunosuppression in the intramuscular location (forearm).

3.6 Administration of IP

Study product UP421, with genetically modified allogeneic human primary islets, will be transported by courier from the manufacturing site in Oslo (Norway) to the operating theatre in Uppsala (Sweden) in a temperature monitored transportation box qualified for temperature control for the stability for shipping of fresh suspension cells and solid organs for clinical transplantation. Upon arrival, UP421 will be kept in a controlled dedicated place until administration is scheduled. Administration will be performed by a trained surgeon familiar with the transplantation techniques and handling of modified biological materials.

Once the UP421 arrives at the clinical site in the shipping container, the infusion bag (Accessory Platelet Storage Set, Terumo BCT) containing UP421 is controlled for identity by checking the Certificate of Analysis and identification of the product is confirmed. Also, the correct UP421 strength will be confirmed upon arrival at the administration site.

The container and closure should be carefully visually inspected for any damage. If the UP421 primary container is damaged, the visual appearance of the product is affected, and/or visual particulates are identified, the product must not be used and discarded. If the temperature monitored in the insulated box deviates from the storage conditions the manufacturer should be contacted. Administration will be performed within 8 h after release of the IP from the manufacturing site.

The consented subject will receive sedation and allowed to enter the operating room. An ultrasound-guided Supraclavicular Brachial Plexus Nerve Block of the non-dominant arm will be applied by an experienced anesthesiologist according to established routines. Alternatively, and if preferred for medical reasons, general anesthesia could be used. The operating area will be sterilised and dressed for surgery according to hospital routines.

In parallel, the UP421 primary container will be placed in the surgical room and preparation of cells for administration will take place. The primary container will be disinfected prior to preparation for administration. UP421 cells will be transferred to sterile 50 cc tubes to allow sedimentation for 10 min. As next about 10 µl of packed cell suspension will be gently aspirated in the peripheral end of a venous catheter of 16-18g dimension (e.g., Venflon 18g), connected to a single use, sterile syringe, which is then used to distribute the IP among brachioradialis muscle fibers of the recipient. This procedure will be repeated until all hypoimmune islets have been transplanted. All manipulations will be performed within the surgical room according to a protocol protocol developed for the Nordic Network for Clinical Islet Transplantation (NNCIT; (17, 18). Finally, the insertion in the skin will be closured by surgical sutures according to hospital routines. The implantation procedure is expected to last less than 60 min. The subjects will then remain in hospital overnight and be discharged the following day.

In clinical islet cell transplantation intraportally to the liver anticoagulant protocols are included in order to counteract an instant blood mediated inflammatory reaction (IBMIR) in the blood stream and promote survival of the cells. For intramuscularly transplanted islets those are not exposed to blood and therefore any anticoagulants are not needed. We have confirmed experimentally that at the intramuscular site an IBMIR response is not evoked (27). In our previous clinical transplantations of islets to the intramuscular site we have not used anticoagulant protocols with safety, as well as excellent function and revascularisation of the implanted islets (17, 18).

3.7 Study Rationale

Type 1 diabetes most often debuts in childhood or adolescence and is a chronic disease with

life-long needs for exogenous insulin treatment. Pancreatic islet transplantation has the possibility to restore glucose homeostasis and alleviate the individual from exogenous insulin injections. However, presently substantial immunosuppressive treatment is necessary, which increases the risk of severe infections, renal failure and cancer development. For most people with type 1 diabetes, besides those with severe hypoglycemic attacks with recurrent insulin coma episodes, it is preferable to remain on insulin treatment than become insulin free require life-long immunosuppression. However, if islet cell replacement could be performed safely without the need of immunosuppression, subjects with type 1 diabetes could benefit.

The purpose of this study (phase I first in human) is to treat adult subjects with type 1 diabetes with genetically modified allogeneic pancreatic islets in order to evaluate safety and the possibility to restore at least part of the insulin production without the need of simultaneous immunosuppression. The primary endpoint is overall participant safety. In order to investigate if the pancreatic islet cells survive even in a fully immune competent individual, C-peptide as a measure of transplant insulin production will be evaluated in response to mixed meal tolerance tests and also components of metabolic control (HbA1c, Time in Range of continuous glucose measurements and exogenous insulin needs) will be evaluated as secondary endpoints. Any immunological response to the islet graft will be evaluated by immunological studies of whole blood and plasma. The study population will consist of two adult subjects 30-60 years of age (inclusive at both ends), having type 1 diabetes diagnosed for more than five years.

Any risks associated with the procedure [including associated morbidity] are more problematic at a younger age. Therefore 30-60 years of age, , is deemed the most reasonable age for inclusion in this study, avoiding the risks for any procedure-related events with age, for example.

Since the study aims to investigate whether insulin production at least partly can be restored, all subjects eligible for study must have a mixed meal tolerance test stimulated C-peptide concentration <0.01 nmol/L, i.e., not measurable on the clinical assay. Most subjects diagnosed with type 1 diabetes for more than five years fulfill this criterion.

There are approximately 2000 newly diagnosed patients with type 1 diabetes each year in Sweden (19). There are no regional differences in Sweden.

4 STUDY OBJECTIVES

4.1 Primary Objectives

To investigate the safety profile of an intramuscular transplantation of genetically modified allogeneic human islets (study product UP421) in adult subjects that have been diagnosed with type 1 diagnosis for 5 years or more.

4.2 Secondary Objectives

Study changes in islet-cell function, metabolic control, Diabetes Treatment Satisfaction and immunological response to pancreatic islets during the first year following treatment.

4.3 Primary Safety Endpoint

Safety parameters will be evaluated in the intervention trial at each study visit and recorded as adverse events (AEs). Any grade 3 event (or higher) will be evaluated by DSMB. The primary outcome measure will be:

> Number of treatment-related adverse events as assessed by CTCAE v5.0 [Time Frame: Twelve months after treatment]. This includes assessment of any adverse events during the procedure, and any postsurgical complication e.g., muscle pain, local hemorrhage, local infection or fever. Assessment will also include e.g. any assisted hypoglycaemia and tumour formation. Adverse events will all be classified with regard to seriousness, expectedness and relatedness to the investigational product. Any occurring adverse event will be handled according to clinical routine.

4.4 Secondary Efficacy Endpoints

1. Immune evasion of implanted cells, as evaluated in systemic PBMC and serum at 0 (before tx), 2, 4, 8, 12, 18, 26, and 52 weeks post-transplantation.
2. Presence of peak c-peptide >0.01 nmol/L, in response to the mixed meal tolerance test at 4, 8, 12 ,18,26, and 52 weeks post-transplantation.
3. Presence of non-fasting c-peptide concentrations >0.01 nmol/l at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 52 weeks post-transplantation; i.e. Kaplan-Meier analysis of survival time for islet grafts.
4. Presence of peak c-peptide >0.20 nmol/L, in response to the mixed meal tolerance test at 4, 8, 12 ,18,26, and 52 weeks post-transplantation.
5. Survival of implanted cells, as evaluated by MRI, within 48h after transplant and 2, 4, 6, 8, 12, 26, and 52 weeks post-transplantation, as well as by PET/MRI at one scan in between 8-12 weeks post-transplantation and one scan at 52 weeks post-transplantation.
6. C-peptide AUC in response to the mixed meal tolerance test at 4, 8, 12, 18,26, and 52 weeks post-transplantation.
7. Delta changes in insulin requirement/kg BW at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 52 weeks post-transplantation when compared to before transplantation.
8. Delta changes in HbA1c at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 26, and 52 weeks post-transplantation when compared to before transplantation.
9. Delta changes in glucose variability (time in range, SD, CV) and hypo/hyper glycaemia duration derived from a continuous glucose monitoring system performed at 4, 8, 12, 18, 26, and 52 weeks post-transplantation when compared to before transplantation.

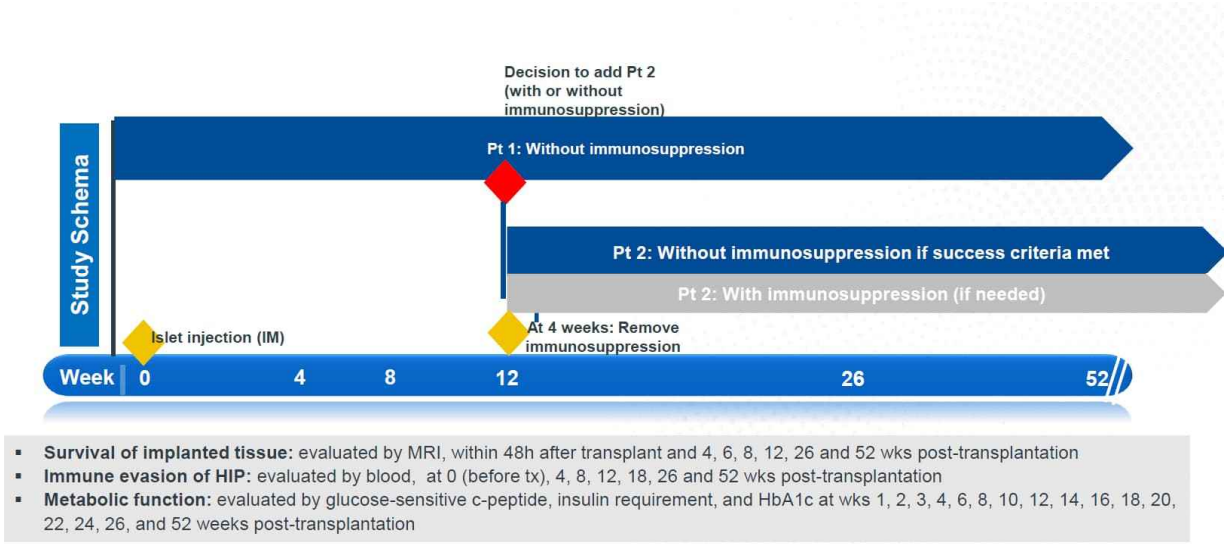
10. Score in diabetes treatment satisfaction questionnaire in transplanted patients at 4, 8, 12, 18, 26, and 52 weeks post-transplantation when compared to before transplantation.

5 STUDY DESIGN

5.1 Study Outline

The study will be a first in human trial with a single arm study design. The first participant (Pt. 1; see Figure 1) is planned to receive an intramuscular hypoimmune islet transplantation dose of 25-80E+6 (25 000 000-80 000 000) UP421 islet cells (forearm) without immunosuppression and be evaluated after four weeks. The cadaveric hypoimmune islets will be implanted in the intramuscular space (forearm). If satisfactory graft function (see below for criterion), a second participant will be dosed in a similar manner (Pt. 2; see Figure 1). If the first participant's graft function does not fulfil the predefined criterion, the second participant will be provided with the standard protocol for immunosuppression applied for clinical islet transplantation for 4 weeks, followed by withdrawal. This will ascertain that no false negative result has occurred due to nonspecific inflammation at the implantation site.

Figure 1: Schematic Outline of Study



Criteria for success of first patient after 12 weeks:

1) No AEs/SAEs related to treatment or study procedure (CTCAE grade 3 or higher)
2) Mixed meal tolerance test shows measurable C-peptide >0.01 nmol/L (as measured by the clinical chemistry lab at Uppsala University Hospital) at any time between 4-12 weeks.

If criterion 2 is met, a second participant is to be transplanted without immunosuppression.

In case of criterion 1) shows CTCAE grade 3 or higher, trial will be discontinued and DSMB advice needed.

The rationale for the selection of a minimum of 12 weeks between dosing subject 1 and 2 is the following:

a. The observation period of first subject's graft must be at least one-two weeks in order to allow evaluation of whether any acute total loss of the graft occurs due to non-specific inflammation. Moreover, acute rejection of any non-gene edited cells is simultaneously to be expected within these two first weeks. A minimum four-week

period will therefore be sufficient to guide on graft function and whether to include transient use of immunosuppressives for subject 2.

b. For pancreatic islets implanted to forearm muscle, both experimental and clinical studies suggest a revascularisation (engraftment) period of 2 weeks (Christoffersson et al 59:2569-2578, 2010). Before full revascularisation, the islet graft is expected to be vulnerable to metabolic stress, and therefore an additional 14 days prior to performing the first mixed meal tolerance test is a minimum for readout of metabolic function. A minimum four-week period has therefore been selected to be on the safe side and not harm the islet graft.

c. For safety follow up, MRI investigations of implant in forearm muscle will be a key component. In order to allow the necessary time to assess any trend change in the morphology of the graft, an extended 12-week sentinel dosing has been selected which will allow six MRI investigations post transplantation prior to decision. The only exception is if there is no visible islet graft detectable by MRI already at four weeks post transplantation, in combination with a mixed meal tolerance test at four weeks that shows no measurable C-peptide (<0.01 nmol/L), if so a four-week period is considered sufficient to allow treatment of second subject under the umbrella of transient immunosuppressive treatment."

Any immunosuppression will follow the existing SOP for islet transplantation in the Nordic Network for Clinical Islet Transplantation (NNCIT) that Uppsala is a central core of by having the human islet isolation centre for the Network, as well as being one of the most active hospitals in performing clinical islet transplants. The immunosuppression regimen will in line with this consist of induction therapy with Basiliximab (2 x 20 mg iv) followed by Tacrolimus (start dose 0.1 mg/kg/24h; with target concentration of 10-12) and MMF immunosuppression (500 mg 2x2, dose adjusted thereafter based on AUC), as well as CMV prophylaxis with Valganciclovir 450 mg 2x1, ulcer prophylaxis with omeprazole 20 mg 1x1, TNF-alpha inhibition with etanercept (50 mg iv, followed by 25 mg sc on day 3, 7 and 10) and standard antibiotics.

5.2 Study Assessments and Procedures

The study starts with a screening period to obtain informed consent, screening, and inclusion in the study (*see* Section 5.3). Throughout the study, all participants will continue their insulin treatment, with insulin doses adjusted to maintain optimal blood glucose control as per clinical practice. All participants will follow the visit schedule shown in Section 5.3.

Visit 1 (screening visit): A screening visit will take place before treatment starts. At the screening visit, the subject will be informed of the study by the study doctor, have a discussion regarding the participant information/consent form, including objectives, procedures, and possible risks and benefits of the study. If the subject decides to be a part of the study, informed consent is signed by the subject and study doctor before any study activities are started, e.g., listing of concomitant medications, baseline symptoms, medical history, demography, physical examination incl. cardiovascular examination, electrocardiography, echocardiography, clinical chemistry tests incl. pregnancy test (HCG) (females), HbA1c, fasting plasma C-peptide concentration, HLA-, MIC-, GAD-, IA2-, Znt8- and TPO-antibodies, and other laboratory tests (HIV, HBV, HCV and blood grouping). Samples for immunology tests will be taken. The investigator will confirm all eligibility criteria. This includes documentation of demography, medical history, concomitant medications, baseline symptoms, clinical chemistry incl. C-peptide in response to an MMTT, HbA1c, pregnancy test, and vital signs. Diabetes care will be optimised. Diary cards will be dispensed.

If a patient meets at least one exclusion criterion, the patient will be considered a screening failure.

Visit 2 (day 0-1): Before treatment, physical examination incl. cardiovascular examination, body temperature, electrocardiography, and check of concomitant medications, insulin requirements, baseline symptoms, DTSQ, clinical chemistry, forward and reverse blood grouping and vital signs will be performed. CGM will be conducted for 14 days and diary card collected. Diabetes care will be optimised. IP will be implanted intramuscularly and followed by an MRI examination within 48h. Vital sign and body temperature will be checked, as well implantation site, and need to be normal before the patient is discharged as planned the following day. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visits 3-4 (day 7, 14): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp, clinical chemistry and samples for immunology tests (day 14) will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided. MRI of graft will be performed on day 14 (visit 4).

Visit 5 (day 21): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 6 (day 28): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry and samples for immunology tests will be taken. DTSQ evaluated, mixed meal tolerance test performed, CGM will be conducted for 14 days prior to visit. MRI of graft will be performed. Concomitant medications, insulin requirements, and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 7 (day 42). Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp, clinical chemistry and samples for immunology tests will be taken will be taken. MRI of graft will be performed. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 8 (day 56): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry and samples for immunology tests will be taken. DTSQ evaluated, mixed meal tolerance test performed, CGM will be conducted for 14 days prior to visit. MRI of graft will be performed. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided. [68Ga]Ga-NODAGA-exendin-4 (68Ga-exendin)-PET/MRI scan will be conducted at either of the visits 8-10.

Visit 9 (day 70): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided. [68Ga]Ga-NODAGA-exendin-4 (68Ga-exendin)-PET/MRI scan will be conducted at either of the visits 8-10.

Visit 10 (day 84): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry and samples for immunology tests and autoantibodies will be taken. DTSQ evaluated, mixed meal tolerance test performed,

CGM will be conducted for 14 days prior to visit. MRI of graft will be performed. [68Ga]Ga-NODAGA-exendin-4 (68Ga-exendin)-PET/MRI scan will be conducted at either of the visits 8-10.

Concomitant medications, insulin requirements, and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 11 (day 98): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 12 (day 112): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 13 (day 126): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry and samples for immunology tests will be taken. DTSQ evaluated, mixed meal tolerance test performed, CGM will be conducted for 14 days prior to visit. Concomitant medications, insulin requirements, and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 14-16 (days 140, 154 and 168): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 17 (day 180): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry and samples for immunology tests and autoantibodies will be taken. DTSQ evaluated, mixed meal tolerance test performed, CGM will be conducted for 14 days prior to visit. MRI of graft will be performed. Concomitant medications, insulin requirements, and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 18 (day 270): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry will be taken. Concomitant medications and adverse events checked. Diary cards will be collected and diabetes care optimised. New diary cards will be provided.

Visit 19 (day 365): Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp and clinical chemistry and samples for immunology tests will be taken. MRI of graft will be performed. DTSQ evaluated, mixed meal tolerance test performed, CGM will be conducted for 14 days prior to visit. Concomitant medications, insulin requirements, and adverse events checked. Diary cards will be collected and diabetes care optimised. [68Ga]Ga-NODAGA-exendin-4 (68Ga-exendin)-PET/MRI scan will be conducted.

After this visit the subject will be followed in a separate LTFU-study for 14 years following the completion of this one-year study after last dosed study subject.

Physical examination incl. of the implantation site and cardiovascular examination, vital signs, body temp, and clinical chemistry (haemotocrit, haemoglobin, red blood cells, platelets, white blood cells, sodium, potassium, P-creatinine, total bilirubin, ALT, AST, P-glucose,

CRP, C-peptide, HbA1c) will be included in these biannual visits.

5.3 Schedule of Events

Visit	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Day[1] / app. week		**0-1**	**7 W1**	**14 W2**	**21 W3**	**28 W4**	**42 W6**	**56 W8**	**70 W10**	**84 W12**	**98 W14**	**112 W16**	**126 W18**	**140 W20**	**154 W22**	**168 W24**	**180 W26**	**270 W39**	**365 W52**
Obtain informed consent	X																		
Initial lab screen[2]	X																		
Demography	X																		
Medical history	X																		
Baseline symptoms	X																		
Concomitant meds	X																		
UP421 administration		X																	
Physical exam	X	X[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body temp		X[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of inclusion/exclusion criteria	X	X[3]																	
Electrocardiography	X	X[3]																	
Echocardiography	X																		
DTSQ, QoL		X[3]				X		X		X			X				X		X
Height[4], Weight, Vital signs	X	X[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mixed meal tolerance test	X					X		X		X			X				X		X
Laboratory tests[5, 6, 7]		X[3]	X[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CGM		X[3]				X		X		X			X				X		X
Magnetic Resonance Imaging[9]		X[10]		X		X	X	X		X							X		X
AE/SAE reporting		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diabetes/insulin diary		X[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PET/MRI								X[11]	X[11]	X[11]									X

1: Visits should be performed ± three days of the designated day.

2: C-peptide, P-glucose, HbA1c, P-creatinine, Hepatitis B& C, tuberculosis, HIV, HBV, HCV, HTLVI, HTLVIII, PK, PTT, D-dimer, GAD-ab, IA2-ab, ZnT8-ab, TPO-ab, CRP, haemotocrit, haemoglobin, red blood cells, platelets, white blood cells, liver status (AST, ALT, ALP, GGT, bilirubin), sodium, potassium, B-HCG (females before menarche), blood grouping, HLA, MIC, and ANA antibodies

3: To be performed before UP421 administration

4: Only at visit 1

5: Includes CRP, P-glucose, C-peptide, HbA1c at all marked visits and haemotocrit, haemoglobin, red blood cells, platelets, white blood cells, sodium, potassium, P-creatinine, total bilirubin, ALT, AST at visits 2, 3, 4, 6, 8, 10, 12, 14, 17, and 19. PK, APTT and D-dimer at visits 2 (the day after cell therapy) and 3, and forward and reverse blood grouping at visit 2.

6: Serum (4mL/patient/visit) and whole EDTA blood (15mL/patient/visit results in app. 20M isolated PBMCs) for immunological tests at visits 2, 4, 6, 8, 10, 13, 17, and 19.

7: Blood sample for measuring of auto-antibodies to GAD, IA-2, ZnT8, ANA, HLA, and MIC antibodies will be obtained at visits 1, 10, 17.

8: Includes if use of immunosuppressives measurements of tacrolimus concentration and MMF AUC.

9: Magnetic resonance imaging will be performed to evaluate cellular mass, signs of fibrosis or inflammation, and degree of blood vessel formation.

10. Within 48 h of implantation
11. PET/MRI scan at either of the visits 8-10

6 SELECTION AND WITHDRAWAL OF SUBJECTS

6.1 Subject Inclusion Criteria

Subjects eligible for inclusion in this study must fulfil the following criteria:

1. Signed informed consent for participation of the study.
2. Diagnosis of type 1 diabetes mellitus (T1D);
   i) for ≥ 5 years and
   ii) at least one or more HbA1c documented in the subject's medical journal or Swedish National Diabetes Registry during the last five-year period must be ≥70 mmol/mol.
3. The subject must be involved in intensive diabetes management defined as self-monitoring of subcutaneous glucose level by continuous glucose monitoring or by intermittent scanning glucose monitoring no less than a mean of three times per day averaged over each week and by the administration of three or more insulin injections per day or insulin pump therapy. This management must be under the direction of an M.D specialised in endocrinology and diabetology with support of a diabetes nurse at a specialist clinic for Endocrinology and Diabetology or Internal Medicine during the 12 months prior to study enrolment.
4. C-peptide negative (C-peptide < 0.01 nmol/L) in response to a mixed meal tolerance test MMTT).
5. Positive for antibodies to either GAD or IA2 at screening.
6. 20-45 years of age at time of enrolment.
7. HbA1c ≥70 mmol/mol.
8. Exogenous insulin needs < 1 IU/kg.
9. Body weight <80 kg.
10. Female subjects of child-bearing potential must agree to using adequate contraception until one year after the administration of UP421, as outlined in https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf.

    A woman is considered of child-bearing potential if she is not surgically sterile or is less than 1 year since last menstrual period. Adequate contraception is as follows:

    a) combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal)
    b) progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable)
    c) intrauterine device (IUD)
    d) intrauterine hormone-releasing system (IUS)
    e) bilateral tubal occlusion
    f) vasectomised partner
    g) sexual abstinence

    Male subjects must not intent to procreate until one year after the administration of UP241. Males must be willing to use effective measures of contraception (condoms) during the one-year trial period.

6.2 Subject Exclusion Criteria

1. Any previous organ transplantation.

2. Any systemic immunosuppressive medication for any other disease.
3. Any history of malignancy.
4. Use of any investigational agent(s) within 4 weeks of enrollment.
5. Use of any anti-diabetic medication, other than insulin, within 4 weeks of enrollment.
6. Active infections including Tuberculosis, HIV, HBV, and HCV.
7. Liver function test value for AST, ALT, GGT, or ALP exceeding the respective reference interval for the clinical assay at Uppsala university hospital.

   Increased plasma concentrations of alanine aminotransferase (>0.75 µkatl/L for females or >1.1 µkat/L for males) and/or aspartate aminotransferase (>0.60 µkat/L for females or >0.75µkat/L for males), and/or γ-glutamyl transferase (>0.75 µkat/L for females 18-40 years of age or >1.2 µkat/L for females >40 years or >1.3 µkat/L for males >40 years or >1.9 µkat/L for males >40 years) and/or alkaline phosphatase (>1.8 µkat/L).
8. Serological evidence of infection with HTLVI or HTLVII.
9. Pregnancy, nursing, intention for pregnancy.
10. Chronic kidney disease grade 3 or worse (eGFR<60 mL/min/1.73m2 as estimated by creatine measurement using the revised Lund-Malmö Equation)
11. Medical history of cardiac disease, or symptoms at screening consistent with cardiac disease.
12. Known autoimmune disease other than type I diabetes, coeliac disease, thyroid disorder, pernicious anaemia, or vitiligo.
13. Administration of live attenuated vaccines <6 months before transplant.
14. Positive for ZnT8 auto-antibodies.
15. Untreated proliferative diabetic retinopathy.
16. Major ongoing psychiatric illness which the Principal Investigator judge increases the risk of noncompliance or does not allow safe participation in the study.
17. Ongoing substance abuse, drug or alcohol; or recent history of noncompliance.
18. Known hypersensitivity to ciprofloxacin, gentamicin, or amphotericin (since these are used in the manufacturing process of UP421 and trace amounts may be present in final product).
19. Any other condition that in the opinion of the Principal Investigator does not allow safe participation in the study.

Subjects fulfilling **any** of the following criteria at screening are not eligible for inclusion in this study. No additional exclusions may be applied by the investigator to ensure that the study population will be representative of all eligible subjects.

6.3 Withdrawal of Subjects

Participants are free to discontinue their participation in the study at any time for any reason without affecting their right to appropriate follow-up investigation and future treatment.

A discontinuing participant should be asked to come to the clinic for a final follow-up visit if accepted by the participant. Safety assessments should be performed, if possible.

The reasons for a subject having discontinued the study will be documented in the Case Report Form (CRF).

Any study subject that are included in trial, but withdraw prior to treatment, may be replaced by new study subject. Replacement is not allowed following study treatment.

6.3.1 Withdrawal of Immunosuppressives

In order to minimise the risks of side effects of any used immunosuppressives, any adverse outcome considered related to treatment with immunosuppressives will cause the immunosuppressive treatment to be immediately withdrawn, as per standard clinical practice. Frequent study visits (during this period once weekly) will enable early identification of any side effect for safety intervention.

6.4 Subject Log and Screening of Subjects

This First in Human trial will recruit two study participants. Investigators must keep a record, a screening log, of all participants that were considered for enrolment even if they were not subsequently enrolled. This information is necessary to verify that the participant population was selected without bias. The reasons for non-eligibility are to be defined in terms of one or more of the eligibility criteria. A screening number will be allocated to each subject considered for enrolment. The Investigator must file a Participant log of all subjects enrolled, which includes sufficient information to link records, i.e., the CRF and clinical records. This list should be preserved for possible future inspections/audits. Given there are only two trial participants (both of whom receive the same dose range UP421 islet cells) and the possibility of investigating the effects of no immunosuppression for the second trial participant is incorporated in the trial design, there is no randomisation or blinding in this trial therefore efforts to minimise bias are focused primarily on selection bias as described. The trial will be conducted in compliance with the regulation (17a in Annex I).

7 TREATMENT OF SUBJECTS

7.1 Treatment Administration

Subjects 1-2 will receive 25-80 x 10^6 UP421 islet cells into forearm muscle in the study.

7.2 Description of Investigational Products

7.2.1 UP421

The IP is a cell suspension with genetically modified human pancreatic islet cells (UP421). The treatment is allogenic and consists of pancreatic islet cells isolated from the pancreas of one organ donor (organ donors are the same as for conventional pancreas and pancreas islet transplantation, i.e., brain-dead deceased organ donor).

UP421 is an investigational medicinal product and it not authorised in any country. In this study protocol, the expression "Investigational Product" is synonymous with the expression "Advanced Therapy Investigational Medicinal Product" (ATMP) used in the "detailed guideline on good clinical practice specific to advanced therapy medicinal products" (European Commission DG Enterprise & Industry, Unit F2. Detailed guideline on Good Clinical Practice specific to advanced therapy medicinal products. Doc. Ref. ENTR/F/2/SF/dn D (2009) 35810.).

7.3 Description of Auxiliary Products

The immunosuppressive agents (as described in Section 5.1) are auxiliary medicinal products. All are authorised products and utilised as per their licensed indications.

7.4 Packaging and Labelling of Investigational Products

7.4.1 Manufacturer

The Section of Cell Therapy at Oslo University Hospital (OUS) at Ullernchausseen 70, N-0310 Oslo, Norway will serve as manufacturer of the IP. The UP421 product will be released by the Qualified Person (QP) – Section of Cell Therapy, OUS.

Further details of the manufacturing process are described in the Investigator's Brochure and Investigational Medicinal Product Dossier.

7.4.2 Packaging, Labeling and Traceability

All packaging and labeling operations at the Manufacturer are performed according to GMP and Good Clinical Practice (GCP) guidelines (International Conference on Harmonsation). Efficacy guideline E6; Good Clinical Practice. www.ich.org. May 1996), as well as applicable national regulatory requirements.

The material is coded and traceable to the donor.

The bags with IP are labelled (see Content of Product label below). The slip will describe the total number of cells and donor/patients' unique subject number. The number of cells transplanted must be documented in the Drug Accountability Log and in the CRF.

The bags with cells will be labeled in Swedish and comply with the legal requirements. The investigator will maintain an accurate record of the shipment and dispensing of the study drug in a drug accountability log. Monitoring of drug accountability will be performed by the field monitor during site visits and at the completion of the trial. At the end of the study, a copy of the completed drug accountability log will be sent to the sponsor.

7.5 Storage and handling

After preparation by the Manufacturer, the IP is transported in a validated, temperature

controlled (8-25 degrees C) container to the study site. Administration will be performed within 8 h after release of the IP from the manufacturing site.

Study treatment will be handled by a designated person (study nurse) at the study site, stored safely and properly, and kept in a secured location accessible only to the investigator and delegated staff members. Clinical supplies will only be dispensed in accordance with the protocol.

7.6 Concomitant Therapy

All subjects will receive standard insulin treatment subcutaneously. Insulin treatment will be prescribed and collected by the subjects from the pharmacy per routine clinical practice. Study participants will not, if not having a clinical indication for such specific treatment, receive concomitant medication that may interfere with glucose regulation other than insulin treatments, e.g. oral anti-diabetic therapies, GLP-1 RA´s, or any systemic immunosuppressive treatments. Subjects are allowed to take other medications that do not interfere with glucose regulation. The investigator should instruct the subject to notify the study site about any new medications he/she takes after the start of the study drug. All medications and significant non-drug therapies (including physical therapy and blood transfusions) administered after the subject starts treatment with study drug must be listed in the CRF and medical records.

All concomitant medications will be listed in the subjects CRF which will be developed and managed by the site.

7.7 Compliance with the Treatment

Subjects will not handle study treatment themselves. IP will be handled at the visit by study staff at Uppsala University Hospital. Background insulin therapy will be handled by the subjects themselves according to the normal routine.

7.8 Accountability of Investigational Products

The study nurse(s) will maintain a Drug Accountability Log detailing the dates and quantities of IP received from the Manufacturer and the administration to subjects. The used vials and bags are disposed of at the investigator site according to the hospital routines for infectious material.

7.9 Continuation of Treatment

The study primary endpoint (safety) is completed at 12 months follow up after transplantation of UP421.

The study subjects will be followed in a separate LTFU-study for 14 years following the completion of this one-year study after last dosed study subject. Therefore, in accordance with the EMA/CAT guideline document, safety and efficacy data will be collected for 15 years for all patients who have been included in clinical studies with the investigational drug UP421 (26).

The subjects will continue standard insulin treatment during this time.

7.10 End of Study

Last subject, last visit will be conducted one year (day 365; which is denoted visit 19) after last treatment in study. The latter equates to day 365 after treatment of subject 2 in the present protocol.

8 CLINICAL ASSESSMENTS

8.1 Demographic Data and Screening Assessments

At screening (Visit 1), demographic data, including initials and age, will be collected and documented in the CRF. Full information about the subjects' Social Security Number (personnummer) and the name is to remain confidential in the records of the Investigator. An extended physical examination will be performed.

Parameters will include:

- Gender
- Age
- Weight
- Length
- Pulse
- Blood pressure
- Examinations of mouth, throat, thyroid gland, palpable lymphatic nodes, heart, lung, skin, and abdomen
- Electrocardiography

8.2 Medical History

A complete medical history will be recorded during the screening visit (Visit 1). The medical history will include a review of all past and current relevant diseases and surgeries prior to screening, as judged by the Investigator. The medical history will be recorded and classified based on the Medical Dictionary for Regulatory Activities, MedDRA.

Assessments:

- Concomitant medication
- Disease history/duration:
    - Previous medical history
        - Duration of diabetes
        - Glycemic control
        - Hypoglycaemia frequency
        - Malignancy
        - Chronic infections, e.g., tuberculosis, hepatitis, or HIV
        - Any autoimmune disease besides type 1 diabetes
        - Other relevant diseases or performed surgery
    - Medical history
        - Fasting plasma C-peptide level
        - HbA1c level
        - Insulin treatment
        - Cardiovascular disease
        - Kidney disease (creatinine clearance GFR)
        - Contraceptive use of female subjects
        - Alcohol and drug consumption
        - Drug allergies
        - Concomitant medications
        - Active infections

8.3 Concomitant Medication

Information about prior and concomitant medications will be collected at screening (Visit 1). The Investigator or designee will assess changes in concomitant medications throughout the study by asking the subject at each visit. Any changes reported by the subject will be recorded in the CRF. Subjects should report all concomitant medications. Subjects are to be instructed to report any changes in concomitant medications; they are to be questioned by study personnel regarding concomitant medications at each visit.

8.4 Physical Examination

A physical exam will be performed several times during the study and prior to UP421 treatment. The physical exam will include the examination of general appearance, heart, and lung auscultation. After implantation, the implantation site will be examined at all study visits. Information for all physical examinations will be included in the source documentation (medical record) at the study site. Significant findings that are present prior to the start (baseline symptoms) of treatment will be included in the subject's CRF. Worsening of baseline symptoms during the course of the study will be recorded as Adverse events. Significant findings made after the start of study treatment that meet the definition of an Adverse Event will be recorded in the subject's CRF.

8.5 Vital Signs

Vital signs include BP and pulse measurements. After the subject has been sitting for five minutes with back supported and both feet placed on the floor, systolic and diastolic blood pressure will be measured with an appropriately sized cuff.

8.6 Height and Weight

Height in centimeters (cm) is measured at visit 1 (screening) and body weight (to the nearest 0.1 kg in indoor clothing, but without shoes) will be measured at visits 1-19.

9 SUBJECT'S ASSESSMENTS

9.1 Measurement of Plasma Glucose

Study participants will record plasma glucose concentrations by their own measurement of plasma glucose by glucose reagent strips as 7-point profiles for three consecutive days prior to visits in the study. These measurements will be used to optimise diabetes treatment regimens and modify insulin doses as per clinical practice. Alternatively, intermittent scanning glucose monitoring, or continuous glucose monitoring systems may be used.

Insulin doses and measured plasma glucose values are written down by subject in the diary for three days prior to each visit. The amount of insulin recorded plasma glucose values, time, and date will be included. This will be used to calculate:

- The number of subjects insulin-independent (ADA criteria).
- The number of subjects with daily insulin needs <0.25U/kg.
- Insulin requirement/kg body weight.

Exogenous insulin requirements will be assessed at study visits based on subject recorded doses during the three consecutive days prior to the visit. Mean daily insulin requirements are calculated based on these recordings.

9.2 Diabetes Treatment Satisfaction Questionnaire

Diabetes treatment satisfaction changes in the study will be measured by a specific form, a diabetes treatment satisfaction questionnaire, to be filled in by participants. Efficacy of treatment is measured as change in this parameter when compared to before the start of treatment.

10 LABORATORY ASSESSMENTS

Peripheral blood will be taken from the subject. Blood sample volumes covering clinical efficacy chemistry and laboratory safety assessments will be <90 mL for all visits, and less than 15 mL for most visits (750 mL in total during the one-year study).

10.1 Haematology

Haemoglobin, haematocrit, red blood cell count, white blood cell count, neutrophil count, lymphocyte count, monocyte count, and platelet count will be measured prior to and during the study. The selected analysis will also be made at FUP-visits after premature discontinuation.

10.2 Clinical Chemistry

Routine lab parameters will be analysed at the central clinical chemistry laboratory at the local site.

CRP, haematocrit, haemoglobin, red blood cells, platelets, white blood cells, liver status (AST, ALT, ALP, GGT, bilirubin), PK, PTT, D-dimer, B-HCG, sodium, potassium, P-glucose, C-peptide, HbA1c will be measured during the study. Screening for chronic infections will be performed by tests for HIV, tuberculosis, HTLVI, HTLVIII, Hepatitis B, and C. Blood grouping,forward and reverse blood grouping will be performed prior to surgery.

In order to avoid false negative results, C-peptide concentrations will also be analysed at Uppsala University with an ultrasensitive C-peptide ELISA (Mercodia©; Cat. No. 10-1141-01). The assay has been calibrated against the International Reference Reagent for C-peptide (C-peptide 84/510; a WHO standard). The detection limit of the ultrasensitive C-peptide from Mercodia is 1.167 – 130 pmol/L, with inter- and intra-assay CVs at 5.5 and 3.8% at 37 pmol/L. All plasma samples investigated for C-peptide will be analysed as duplicates. Samples with a CV-value >15% will be excluded.

10.3 Immunology Tests

Antibodies to GAD, IA-2, ZnT8, TPO, ANA, HLA, and MIC will be measured.

Antibodies will be analysed at Uppsala University Hospital.

Blood samples for immunology testing will be separated into serum and PBMCs. Serum samples will be stored at -70°C. Whole blood with PBMCs will be stored at -150°C. The PBMCs will be further studied for determining the proportion of different immune cells, such as antigen-presenting cells, T-cells, regulatory T-cells, regulatory B-cells, and other innate immune cells by using flow cytometry. Also, PBMCs will be used for determining the immune evasion by measuring T cell activation/DSA.

10.4 Mixed Meal Tolerance Test

Measurement of endogenous insulin production as C-peptide concentrations in response to a mixed meal tolerance test is an important efficacy measure of the present study. Efficacy of treatment is measured as change in this parameter when compared to before the start of treatment. After an overnight fast, the subject will perform a standardised MMTT.
The subjects drink a solution of resource protein (Novartis, 6 mL/kg, maximal dose 360 mL).
Blood samples will be taken for analysis at time-point 0, 15, 30, 60, 90, and 120 minutes.
In peripheral venous blood plasma from every time-point, C-peptide values will be measured, and AUC C-peptide concentration calculated for the test.

10.5 Monitoring of Glucose Variability

Continuous glucose monitoring (CGM) will be performed. For individuals with no ongoing CGM by their own device a subject-blinded system will be used. For those that have CGM already, these values can be used. For those that have CGM, an open CGM will be used, since their own CGM already provides them with continuous data. CGM will, at each evaluation, be used for 14 days and used for calculation of time-in-range 3.9-10 mmol/L. The recordings will at the end of the 14-day period be reviewed by study nurse/study doctor and used for the optimisation of insulin treatment.

10.6 Measurements of HbA1c

Measurements of HbA1c will be performed to assess metabolic control during the study.

11 IMAGING ASSESSMENTS

11.1 Magnetic Resonance Imaging

Magnetic resonance imaging will be performed repeatedly during the study in order to assess safety and viability, including size, of implanted IP.

11.2 Echocardiography

Echocardiography will be performed at screening to ascertain acceptable cardiac functionality and allow surgical procedure.

11.3 Positron Emission Tomography

Positron emission tomography/magnetic resonance imaging with the tracer [68Ga]Ga-NODAGA-exendin-4 (68Ga-exendin) will be conducted to assess the survival of insulin-producing cells in implanted IP.

12 SAFETY ASSESSMENTS

12.1 Definition of Adverse Events

An Adverse Event (AE) is any untoward medical occurrence in a subject administered IMP and does not necessarily have a causal relationship with this product. An AE can be any unfavourable and unintended sign, abnormal laboratory finding, symptom or disease temporally associated with the use of IP, whether or not related to the product.

12.2 Methods for Eliciting, Recording and Follow-up of Adverse Events

AEs occurring during the study will be collected at Visits 2-19. All information about the AEs, whether spontaneously reported by the subject, documented in diaries, discovered by the Investigator questioning or detected through physical examination, laboratory test, or other means, will be documented.

All AEs occurring during the cause of the study will be recorded on a separate AE form in the CRF. The following information will be recorded:

- Description of the event.
- Date of onset.
- Date of resolution (or that the event is ongoing).
- Action taken as a result of the event.
- The seriousness of the event.
- The severity of the event.
- The outcome of the event.
- Investigator´s assessment of relationship to study medication.

AE will be coded in MedDRA.

12.3 Assessment of Intensity

Each AE is to be classified according to CTCAE version 5.0 by the investigator as mild, moderate, or severe.

Mild: Acceptable. The subject is aware of symptoms or signs, but these are easily tolerated.

Moderate: Disturbing. The AE is discomforting enough to interfere with usual daily activities.

Severe: Unacceptable. The subject is incapable of working or performing usual daily activities.

12.4 Assessment of Causality

Following definitions will be used to assess causality:

- Probable – the adverse event has a strong temporal relationship to the investigational product or recurs on rechallenge, and another etiology is unlikely or significantly less likely
- Possible – the adverse event has a suggestive temporal relationship to the investigational product, and an alternative etiology is equally or less likely
- Unlikely – the adverse event has no temporal relationship to the investigational product or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the investigational product and the adverse event)

An adverse event is considered causally related to the use of the investigational product when the causality assessment is probable or possible.

12.5 Definition of Serious Adverse Events

Each AE is to be classified by the investigator as serious or non-serious. Seriousness is not defined by a medical term; it is a result or an outcome. An AE is defined as a Serious Adverse Event (SAE) if it:

- results in death
- is life-threatening
- requires in subject hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- results in a congenital anomaly
- other medically important events

Planned hospitalisations (e.g. administration of study investigational product) should not be considered an SAE unless the planned hospitalisation is prolonged.

12.6 Definition of Suspected Unexpected Serious Adverse Reactions

Each SAE that is at least possibly related to an IMP is to be classified by the investigator as expected or unexpected. A SAE that is at least possibly related to an IMP, and ***unexpected***, is defined as a Suspected Unexpected Serious Adverse Reaction (SUSAR). It is expected if it is already known from earlier trials or is mentioned in relevant documents (Investigator's Brochure).

12.7 Reporting of Serious Adverse Events

In addition, SAEs will also be reported by the Investigator to the Sponsor on a separate SAE form within 24 hours after the SAE has been communicated to the investigator. Follow-up information describing the outcome of the SAE and action taken will be reported as soon as it is available. The original SAE form must be filed in the investigator's binder. SAEs which are still ongoing at the end of the study period must be followed up to determine the outcome.

12.8 Reporting of Suspected Unexpected Serious Adverse Reactions

The sponsor must report all SUSARs that resulted in death or was life-threatening to the EU Common Eudravigilance database within 7 days, and if necessary, complete the report within the following 8 days. Other SUSAR should be reported within 15 days. SUSARs will be sent on CIOMs forms to the MPA (registrator@mpa.se) for registration in the EudraVigilance database. Since the study is only conducted at one site (Uppsala University Hospital), which is the same as the Sponsor, Principal Investigator will already be informed, but if needed the sponsor will report all SUSARs to any Site Principal Investigator involved in the trial with the IP.

12.9 Follow-up of Adverse Events and Serious Adverse Events

For all AEs, the subject will be followed until either the AE has ceased or stabilised, and a potential causality between the study treatment and the AE has been assessed.

12.10 Annual Development Safety Update Report (DSUR)

As long as the study is ongoing, the Sponsor will annually send a summary of all SAEs to the MPA. The report will also summarise the safety and the risk for the subjects in the trial.

12.11 Reference Safety Information

The reference safety information (RSI) is used for determining the expectedness of a Serious Adverse Reaction (SAR). If the serious event is considered related to the IMP and the serious reaction is not included in the RSI, then this becomes a SUSAR and must be reported to the regulatory authority as per statutory timelines. The RSI is located in the latest version of the product's Investigator's Brochure (IB), section 8.0.

12.12 Pregnancy and Assessment of Fertility

All females who are not surgically sterile will have a serum pregnancy test (B-HCG) before inclusion in the study. If positive, the subject must be discontinued from the trial. To ensure subject safety, each pregnancy occurring while the subject is in the study will be reported to the Sponsor within 24 hours of learning of its occurrence. The pregnancy will be followed up to determine the outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any congenital abnormalities, maternal or newborn complications.

12.13 Data Safety Monitoring Board

The data safety monitoring board (DSMB) is constituted of physicians with appropriate medical and scientific expertise in diabetology and cellular therapy, appointed by the Sponsor to give advice during the study and to recommend whether to modify or stop the clinical investigation. The DSMB will be consulted on safety issues at any time during study when judged applicable by the sponsor medical expert and/or P.I. The DSMB will always be assembled if an AE of grade 3 or higher, assessed as related to the study product or the study procedure would occur. The sponsor will assure that the DSMB has access to available safety data and test results. The DSMB may, for any safety concerns, recommend stopping the trial. It is the responsibility of the Sponsor to decide whether a premature end of the study will be made based on the advice provided by the DSMB.

13 DATA MANAGEMENT AND STATISTICS

13.1 Data Management

Data management based on Good Clinical Practice (GCP) refers to the activities defined to achieve safe routines to enter participant information into a database, avoiding errors efficiently. Data management and handling of data will be conducted according to the study specific Data Management Plan. The investigator will maintain source documents for each subject in the study. All information in the CRF will be traceable to these source documents (e.g., the medical record), with the exemption of data where CRF is used as a source. The data will be verified for missing data, inconsistencies, and for any necessary medical clarifications by the monitor and the data manager.

13.2 Study Database

An Electronic Data Capture (EDC) system will be used to capture data for the study database (CRF).

Clinical data will be entered from the source documents, which are to be defined at the site before the inclusion of the first subject. Authorised study site personnel designated by the Investigator will complete data collection. Appropriate training in the EDC system will be completed by study site personnel prior to any data being entered into the system.

The CRF should be completed for each study subject that has signed the informed consent and has been performing any screening visit procedures. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data recorded in the CRFs. The subject's identity must always remain confidential. All subjects will get a screening number, which will be used on the CRF. If some assessments are not done, or if certain information is not available, not applicable or unknown, the Investigator should indicate this in the CRF. The Investigator will be required to sign off the clinical data by electronic signature.

13.3 Statistical Analysis

13.3.1 Determination of Sample Size

For this first in-human trial, a sample size of two participants has been selected. Two individuals are considered sufficient to evaluate whether the study product can survive and evade allogeneic and/or autoimmune attack, while still minimising the number of individuals exposed in the trial. Safety in these individuals will be closely monitored. If this first trial is successful, a follow up study will be designed for safety with an increased number of subjects, while also evaluating possibilities for improvement of metabolic control in treated subjects.

13.3.2 Analysis Population

The following analysis populations will be defined for analysis:

Safety analysis set – includes all subjects who receive study medication.

Full analysis set (FAS) – includes all screened subjects.

Per protocol set (PPS) – a subset of the FAS consists of all subjects in FAS who take a full dose of study medication and have no major protocol deviations affecting the primary endpoint analyses. Major protocol deviations leading to exclusion from PPS will be specified prior to database lock on a blinded basis and documented in a separate document.

A CONSORT diagram (Consolidated Standards of Reporting Trials) will be used to describe the participant flow during the study, including all screened subjects. This procedure will

follow the CONSORT guidelines (http://www.consort-statement.org/).

13.3.3 Demographics and other Baseline Characteristics

Demographics and baseline characteristics and symptoms will be summarised for the FAS and Safety set. Medical history will be presented in per-patient data listings.

Missing values

Missing data (also withdrawals) for a particular endpoint will discard that subject only from that specific analysis.

13.3.4 Analysis of Safety

AEs will be coded and tabulated by MedDRA System Organ Class (SOC) and by preferred term. A summary table will be presented with total number and number of patients with:

- AEs
- serious AEs
- related AEs
- AE leading to discontinuation

The safety analyses will be based on the Safety analysis set.

14 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The operations manager ("verksamhetschefen") will permit study-related monitoring and regulatory inspection(s), providing direct access to source data/hospital records. The Investigator verifies that each subject has consented in writing to direct access to the original source data/hospital records by the signed Informed Consent. During monitoring, the data recorded in the CRFs will be controlled for consistency with the source data/hospital records by the study monitor (source data verification). The monitor will sign a secrecy agreement.

15 QUALITY CONTROL AND QUALITY ASSURANCE

15.1 Source Data

The requirements regarding information in the medical records follow "Patientdatalagen" (SFS 2008:355) and "The Medical Product Agency's regulations on clinical trials of medicinal products for human use" (LVFS 2011:19:), which means that except information that is of importance for the wellbeing and care of the subject, the following minimum study-specific information must be recorded:

- Study title and a brief description of the study in terms of treatment, dosing, and assessments
- Date when participant information was given and when signed Informed Consent was obtained
- Participant study number
- Medically responsible study doctor, with contact details
- Study visits
- Adverse Events (serious and non-serious)

For details and information that is study-specific and of no interest for the medical care of the subject, CRF and other documents may be considered as source data. Prior to the study start the expected source location of source data (e.g., medical record notes, laboratory reports, and the CRF itself) must be identified and documented. This will be done by completing a site-specific Source Data List.

15.2 Disclosure of Data

Data generated by this study will be available for inspection by local regulatory authorities, Uppsala or their designee, applicable foreign health authorities, and the IRB/IEC as appropriate. Patients or their legal representatives may request their medical information be given to their personal physician or other appropriate medical personnel responsible for their welfare.

Patient medical information obtained during the study is confidential and disclosure to third parties other than those noted above is prohibited. The Sponsor policy requires all notified breaches to be investigated, categorised, and remediation activities implemented. When the rights and freedoms of the data subject are affected then data breaches are notified to the relevant authorities and, when there is a high risk, to the data subject.

15.3 Study Monitoring

The Sponsor will appoint an independent monitor for quality control of the study. Monitoring will be performed before, during, and after study completion in accordance with the ICH GCP guidelines. The extent of monitoring will be described in a monitoring plan, which will be approved by the Sponsor. Study conductance, source data, drug accountability, adherence to GCP, and regulatory requirements will be monitored.

A monitor from UCR will be responsible for coordinating the activities of the study and ensuring adherence to ICH GCP guidelines. Before study initiation, the protocol and CRFs will be reviewed with the investigator and staff. The monitor will visit the sites during the course of study in order to verify adherence to the clinical study protocol, completeness, and accuracy of data entered to the CRF by comparing them with the source documents.
The monitor will review the progress of the clinical investigation, and periodically facilities used in the clinical investigation (e.g., local laboratory) may be reviewed. Visits will be documented in a monitoring log in the Investigators file, a monitor report for each visit will be sent to and signed by the Sponsor, and follow-up letters are sent to the site after each visit.

Monitoring visits will be arranged in advance with clinical investigational personnel at a mutually acceptable time. Sufficient time will be allowed by the clinical investigational personnel for the monitor to review CRFs and relevant source documents. The investigator will be available to answer questions or resolve data clarifications. The investigator and other responsible study personnel must be available during the monitor visits and should prepare appropriate material as source data verification documents and CRFs for the monitor. The monitoring will be described in a monitoring plan based on the UCR SOP for monitoring, protocol and regulatory requirements. The monitoring plan will be approved by the Sponsor. The investigator will ensure that the clinical investigation participants are aware of and consent that personal information will be scrutinised during the data verification process as part of clinical investigation-related monitoring and possibly auditing by quality assurance personnel hired by the Sponsor or inspections by personnel from the regulatory authority(-ies). However, personal information should be treated as strictly confidential.

15.4 Training of Study Personnel

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed. All investigators and other responsible staff should be listed together with their function in the study on a delegation and signature list. This log should be continuously updated, signed by the investigator, and stored in the Investigators file.

15.5 Changes to the Study Protocol

No changes in the study procedures shall be affected without the mutual agreement of the Principal Investigator and the Sponsor. Non-significant changes may be documented as signed protocol amendments, but substantial changes to the assessments or study design should be notified for review and approval from the MPA and ERA.

16 ETHICS

This study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP) and the ethical principles that have their origin in the Declaration of Helsinki.

16.1 Ethical Review Authority (ERA)

It is the responsibility of the Sponsor to obtain approval of the study protocol/ protocol amendments, the patient information and the Informed Consent from the ERA before enrolment of any subject into the study.

The investigational products of the study will not be delivered to the study centre until approval from the ERA has been obtained.

16.2 Ethical Conduct of the Study

This study is designed and shall be implemented, executed and reported in accordance with the principles of the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH GCP) (CPMP/ICH/135/95), applicable local regulations (i.e., European Directive 2001/20/EC) and the ethical principles of the Guidelines of the World Medical Association (WMA) Declaration of Helsinki (as amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013). This study will not commence until a Clinical Trial Authorisation, CTA is obtained from the MPA. A favorable ethical opinion will be obtained from the ERA prior to the commencement of this study. The study will be conducted in accordance with this study protocol, deviations are to be made in the form of amendments, which must be approved by the Principal Investigator, Sponsor, MPA, and ERA.

16.3 Risk – Benefit Assessment

16.3.1 Risks of Surgery

Study product with human pancreatic islets will be implanted in forearm muscle during ultrasound-guided supraclavicular brachial plexus nerve block or, if needed, general anaesthesia. The subjects will then remain in hospital overnight and be discharged the following day. The islets will be dispersed in aliquots between the fibres of the brachioradialis muscle of a forearm. The implantation procedure is expected to last less than 60 min. The risks associated with the procedure are few and mainly associated with the inevitable risk of general anaesthesia if such is needed. The most common ones postoperatively are general discomfort, dizziness, headache, itchiness, sore throat, difficult to urinate, nausea and vomiting. For young, more or less healthy people the mortality of general anaesthesia is very low, approximately 1:100 000. Local implantation reactions (pain, itching, swelling or redness around the site of implantation) may also occur.

16.3.2 Risks of Immunosuppressives if Used

Immunosuppression with basiliximab, tacrolimus, MMF and etanercept will be included in the protocol for second subject, only if there is failure of first graft directly after implantation. The rationale will then be to exclude the possibility of false negative outcome of IP implantation due to non-specific inflammatory effects. The used doses of immunosuppressives are identical to those used in the SOP for ordinary islet transplantation in the Nordic Countries, but with the exception that treatment will only occur for the first 4 weeks after IP implant. Side effects are those listed in FASS for each of the drugs, but include risk of opportunistic infections, anaemia, leucopoenia, thrombocytopenia, leucocytosis, hypertension, diarrhoea, and nausea. Long term side effects such as increased risk of malignancies and renal failure are less likely with the limited treatment period as bridging therapy. In order to minimise the risks of side effects of any used

immunosuppressives, any SAE considered related to treatment with immunosuppressives will cause the immunosuppressive treatment to be immediately withdrawn. Frequent study visits (during this period once weekly) will enable early identification of any side effect for safety intervention.

16.3.3 Risks of Gene-editing Procedure

There will not be any systemic administration of gene-edited cells. Instead, ex vivo gene-editing will occur of human pancreatic islet cells during the manufacture of the IP UP421. Given that the genetic modifications of UP421 are performed ex vivo and that no vector material is present in the final product the risk for transduction efficient vector/GMO shedding to the environment after local intramuscular administration of UP421 is considered highly unlikely. Human pancreatic islet cells have an extremely low proliferation capacity comparable to neural cells (Perl et al JCEM 2010). They also rarely form tumours (incidence 1-2 per million people) with only 10-20 % being malignant (20). During the last 20 years more than 4000 islet allotransplants have been performed worldwide with no reports of any tumour formation (21). While CRISPR/Cas technique allows a more specific gene-editing, lentiviral vectors are known to be less specific (22). The target for a lentiviral vector is however transcriptional units of the DNA, which means that there is only an increased chance of insertion into a transcriptional unit involved in cell division when transducing a dividing cell (23). Targeting non-dividing/very rarely dividing cells such as islet cells are expected to substantially reduce any oncogenic potential. Clinical data of insertional mutagenesis were only obtained in genetically modified proliferative haematopoietic stem cells rather than in quiescent cell types (24).

The IP will be inserted into the brachioradialis musculature and frequently monitored during the study both clinically and by imaging techniques (PET and MRI). If tumour formation occurs, the selection of the brachioradialis muscle, instead of the more commonly used liver implantation site for pancreatic islets, will enable surgical removal of the implant. The technique for implantation to allow function but still possibilities of removal in case of tumour formation was originally developed for auto transplanted parathyroid glands (25), but later adapted by the Nordic Network for Clinical Islet Transplantation (NNCIT) for pancreatic islets (17, 18).

In the protocol, gene edited islet cells only will be used for the IP UP421. If there would be any non-gene edited cells present in the IP, those cells would be expected to be rapidly rejected by the allogeneic recipient. Due to the small graft, no symptoms in the subject are to be expected even if the whole IP is rejected. There could however be a need of higher exogenous insulin doses to maintain euglycaemia if the transplanted cells exerted significant metabolic effect due to their insulin production. Since the subject nevertheless regulates insulin doses based on their own measurements of glucose, this is considered to be no risk for the subject.

Since gene editing will only be performed of IP ex vivo without systemic administration, there is no expected risks of reprotoxicity.

Given the lack of quantifiable risk long term in humans in this First in Man trial, as a risk mitigation measure, participants will be followed up for safety and efficacy for a total of 15 years.

16.3.4 Specific Risk-benefit Analysis During the Current COVID-19 Pandemic

The safety of the study subjects, as well as healthcare professionals and researchers dealing with them and sample processing, has been fully evaluated and risk assessed (see table). In line with EMAs guidelines on points to consider in implications of COVID-19 on methodological aspects of ongoing and new clinical trials (EMA/158330/2020 Rev. 1) this

trial has been designed with patient safety as primary end point and of paramount importance. Additionally, adequate measures will be taken to adhere to any local or governmental recommendations. The hospitals and the national recommendations will be followed regarding the possibility of conducting monitoring visits to the hospitals due to COVID-19.

16.3.5 Table. Risks Associated with Performing the Clinical Trial Under the Pandemic Virus Situation

Risk no	Identification of risk / benefit	Risk to occur	Consequence of occurrence	Handling
1	Potentially additional CoVid-19 exposure for participants and staff due to study visits	Low	Medium	Strict enforcement of general CoVid-19 guidelines. All staff involved in study are vaccinated prior to study start (requirement for study staff). Previsit written and oral participant CoVid-information are provided. Research visits are kept separate from routine care visits. Reimbursement is offered for separate transportation modes (such as taxi) to minimise risk of exposure.
2	Type 1 diabetes increases vulnerability to CoVid-19	Low	Medium	Severe CoVid-19 pathology is highly unusual in young adults, regardless of T1D. The impact of IMP is potentially beneficial through reducing the diabetic burden.
3	Sampling environment is a potential risk of exposure to personnel and participants	Low	Medium	Sampling is performed by trained professionals in accordance to applicable guidelines for hygiene, handling material of potential biohazard, as well as using adequate protective equipment as a barrier between personnel and participants.

16.3.6 Risk of PET/MRI

PET/MRI scans with the tracer [68Ga]Ga-NODAGA-exendin-4 (68Ga-exendin) will be conducted at two occasions during the one year study period. 68Ga-exendin bind to Glucagon

Like Peptide-1 receptors (GLP1R), and PET signal in tissue is indicative of viable GLP1R-expressing islet cells. 68Ga-exendin PET examinations is used both in Uppsala and worldwide since 10 years for diagnosis of insulinoma (pancreatic beta cell derived tumours) and research studies. 68Ga-exendin is administered as a bolus solution in an intravenous catheter. 68Ga-exendin is labelled with a weakly radioactive nuclide (Gallium-68) that decays with a half-life of 68 minutes. The effective dose of 68Ga-exendin is 0.0071 mSv/MBq (Boss M, Buitinga M, Jansen TJP, Brom M, Visser EP, Gotthardt M. PET-Based Human Dosimetry of 68Ga-NODAGA-Exendin-4, a Tracer for β-Cell Imaging. J Nucl Med. 2020;61(1):112-116), and the participant will be dosed up to 300 MBq per PET/MRI examination. The effective dose per examination is thus 2.13 mSv and the individual tissue with highest dose is kidney. The effective dose of 2 PET/MRI examination is therefore 4.26 mSv, which is below the limit of 10mSv for category IIb studies aimed at the "diagnosis, cure or prevention of disease". The administered radiation is gone from the body after around 6 hours. The MRI scan does not yield any additional radiation dose. Females in fertile age is administered a pregnancy test before each PET examination to rule out pregnancy.

16.3.7 Benefits

A successful intervention would be highly beneficial for treated subjects as it is likely to provide them with a lower HbA1c, less blood glucose fluctuations, and diminished risk of ketoacidosis. It would also substantially decrease the risks of severe hypoglycemic events and late complications. Sufficient islet mass implanted is expected to fully reverse diabetes with no exogenous insulin dependence.

The fatal role of a consistently high HbA1c for long term complications and premature death is well documented in the literature. Recent Swedish data indicate that the risk of ischemic heart disease is higher in subjects with type 1 than type 2 diabetes, where a relative risk increase of 30 % has been reported in the Swedish National Diabetes Registry (Herrington and Bragg. Heart 103(21):1656-1657, 2017). In subjects with type 1 diabetes with a mean HbA1c of 8.2 % (66 mmol/mol), risk of fatal or non-fatal ischemic heart disease was quadrupled during an eight-year study period when compared to age- and sex-matched healthy controls. For those with the worst metabolic control (HbA1c ≥9.7 %; ≥83 mmol/mol) the relative hazard for ischaemic heart disease was 11 times higher and for women even 18 times higher when compared to controls (Matuleviciene-Anängen et al. Heart 103 (21):1687-1695, 2017). The onset of type 1 diabetes at a regularly much younger age than type 2 diabetes, with an increased HbA1c for many more years, is likely to explain why subjects with type 1 diabetes are much more susceptible to coronary disease than subjects with type 2 diabetes despite that the other classical risk factors obesity, high blood pressure, dyslipidemia and smoking are much more common in subjects with type 2 diabetes (Herrington and Bragg 2017). To ensure a high-risk type 1 diabetes population is targeted by the inclusion criteria, type 1 diabetes must have been diagnosed more than five years, the HbA1c at screening must be ≥70 mmol/mol and at least one more HbA1c (documented in the subject's medical journal or Swedish National Diabetes Registry during the last five-year period) be ≥70 mmol/mol. The patient must also be involved in intensive diabetes management; defined as self-monitoring of subcutaneous glucose level by continuous glucose monitoring or by intermittent scanning glucose monitoring no less than a mean of three times per day averaged over each week and by the administration of three or more insulin injections per day or insulin pump therapy. Diabetes management must be under the direction of an M.D specialised in endocrinology and diabetology with support of a diabetes nurse at a specialist clinic for Endocrinology and Diabetology or Internal Medicine during the 12 months prior to

study enrolment.

16.4 Participant Information and Informed Consent

The ICF and any changes to the ICF made during the course of the study must be agreed to by Uppsala or their designee, and the IRB/IEC prior to its use and must be in compliance with ICH GCP guidelines, local regulatory requirements, and legal requirements.

The informed consent procedure will be performed according to the local regulatory authorities and GCP/ICH guidelines. It is the responsibility of the Investigator to provide each subject with full and adequate verbal and written information about the objectives, procedures, and possible risks and benefits of the study. All subjects should be given the opportunity to ask questions about the study and should be given sufficient time to decide whether or not to participate in the study. The written participant information must not be changed without prior discussion with the Sponsor.

The subjects will be notified of their voluntary participation and of their freedom to withdraw from the study at any time and without giving any particular reason. Subjects must also be informed that withdrawing from the study will not affect their future medical care, treatment, or benefits to which the subject is otherwise entitled.

The Investigator is responsible for obtaining written Informed Consent from all subjects prior to enrolment in the study before any study-specific procedures are performed, including screening procedures. The Investigator should file the signed Informed Consent Forms in the Investigator's File. The ICF is subject to inspection by a representative of Uppsala, their representatives, auditors, the IRB/IEC, and/or regulatory agencies. If substantial changes are made to the ICF, patients may be asked to re-consent as soon as possible.

A copy of the participant information and the Informed Consent Form should be given to the subject.

16.4.1 Subject Confidentiality

Personal information is protected by GDPR. Documents connected to the subject will be kept at the clinic. The investigator will permit study-related monitoring providing direct access to source data and hospital records by a secrecy agreement.

16.5 Biobank

Research samples will be collected and stored according to Swedish biobank law (2023:38) and regulations.

16.6 Record Keeping

To enable audits and evaluations by the Sponsor and inspections by regulatory authorities, the Investigator shall keep records (essential documents) of the study for at least 30 years after the end of the study. This includes any original source data related to the study, the subject Identification log (with subject numbers, full names, and addresses), the original signed Informed Consent Forms, and detailed records of investigational product disposition.
The Sponsor is also, as per ICH GCP -requirements, responsible for archiving their part of the study documentation.

16.6.1 Retention of Records

To enable evaluations and/or audits from regulatory authorities or Uppsala, the Investigator will keep records, including the identity of all participating patients (sufficient information to link records [e.g., eCRFs and hospital records]), all original signed ICFs, copies of all eCRFs, SAE forms, source documents, and detailed records of administered U421. The records will

be retained by the Investigator according to specifications in the ICH GCP guidelines, local law regulations, or as specified in the clinical study agreement, whichever is longer. The Investigator must obtain written permission from Uppsala before disposing of any records, even if retention requirements have been met.

16.7 Archiving

The investigator and Sponsor shall keep records of essential documents of the study for 30 years or according to national guidelines. The documents must be stored in a safe area with restricted access. It is the Investigators obligation to store the documents at a safe place where it cannot be stolen or otherwise fall into the wrong hands.

16.8 Audits and Inspections

Authorised representatives of Uppsala, a regulatory authority, or an IRB/IEC may visit the site to perform audits or inspections, including source data verification. The Investigator and other responsible study personnel must be available during these visits and should devote sufficient time to these processes.

The purpose of an audit or inspection is to independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements.

16.9 Study Discontinuation

The sponsor reserves the right to discontinue the study at any time point in the trial in the following cases:

- An unexpectedly high proportion of AE:s that are possibly or probably related to the investigational product.
- Problems with manufacturing or stability of the investigational product.
- New findings concerning the investigational product that change the benefit/risk ratio.

After such a decision, all CRFs must be completed as far as possible.

The appointed DSMB may also recommend discontinuing the study for the above listed safety reasons.

In all cases, however, subjects having received study IP will be followed for 15 years for safety as per the '*EMA Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells"* (26).

17 FINANCING AND INSURANCE

The sponsor is responsible for ensuring proper provision has been made for insurance and indemnity to cover their liability and the liability of the Principal Investigator and staff. Subjects participating in the study are covered by the Swedish Patient Insurance ("Patientskadeförsäkringen") and the Swedish Pharmaceutical Insurance ("Läkemedelsförsäkringen).

18 PUBLICATION POLICY

The results of the present clinical investigation will be registered at www.clinicaltrials.gov and published in a peer-reviewed scientific journal, e.g., New England Journal of Medicine, Lancet, Diabetes, Diabetes Care, or Diabetologia. A manuscript for submission will be generated within 6 months of study completion. After completion of the study, the results will be summarised in an ICH-E3 clinical study report, submitted to the CTIS within 12 months after last patient's last visit according to regulatory requirements. After the report is signed by both the Sponsor and Investigators, the contents may be published or presented at conferences by either party. Each Investigator is obligated to keep data pertaining to the study confidential. No one can prevent publication of the ICH-E3 contents, but the not publishing party has 30 days to comment on the manuscript.

19 SUPPLEMENTS

19.1 Changes of the Study Protocol

No change in the study procedures will be affected without the mutual agreement of the Investigator and the Sponsor (except where necessary to eliminate an immediate hazard to subjects). All changes of the final study protocol must be documented by signed protocol amendments. If substantial changes to the design of the study are made, the MPA and the IEC will be notified for review and approval.

19.2 Application to Regulatory Authorities

Prior to initiating the clinical study, the Sponsor will submit an application for authorisation to conduct the study, including all required documents, to the MPA. Additional documentation may be demanded for investigational products with special characteristics. The application fee will be paid to the MPA after receiving the invoice.

A concise benefit/risk assessment for the clinical study, including a description of the analysis behind the construction of the study protocol, will be presented. The analysis will describe why this particular study is necessary, why this design was chosen and how the length of the study was determined. The primary end-point(s) and the inclusion and exclusion criteria will be well defined and justified.

19.3 Staff Information

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

19.4 Study Timetable

Q3 2023-Q1 2025

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21 SIGNED AGREEMENT OF THE STUDY PROTOCOL

EUCT number: 2023-507988-19-00

Title of the study: *"First-in-human safety study of hypoimmune pancreatic islet transplantation in adult subjects with type 1 diabetes*"

I, the undersigned, have read and understand the protocol specified above and agree on the contents. The study protocol, the Clinical Study Agreement and the additional information given in the Investigator's Brochure will serve as a basis for co-operation in this study.

I agree to conduct the study according to this protocol and according to the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice and the applicable national laws and regulations.

Principal Investigator	Per-Ola Carlsson, Professor of Medical Cell Biology, Senior Physician in Endocrinology and Diabetology Dept. of Endocrinology and Diabetology Uppsala University Hospital SE-75185 Uppsala, Sweden
Signature and date:	

_____

Signature page

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Data Sharing Statement

Carlsson P-O, Hu X, Scholz H, et al. Survival of Transplanted Allogeneic Beta Cells with No Immunosuppression. N Engl J Med. DOI: 10.1056/NEJMoa2503822.

Question	Authors' Response
Will the data collected for your study be made available to others?	No
Would you like to offer context for your decision?	—
Which data?	—
Additional information about data	—
How or where can the data be obtained?	—
When will data availability begin?	—
When will data availability end?	—
Will any supporting documents be available?	—
Which supporting documents?	—
Additional information about supporting documents	—
How or where can supporting documents be obtained?	—
When will supporting documents availability begin?	—
When will supporting documents availability end?	—
To whom will data be available?	—
For what type of analysis or purpose?	—
By what mechanism?	—
Any other restrictions?	—
Additional information	—

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Apr 18, 2025 09:29:18 EDT
New England Journal of Medicine

Per-Ola Carlsson

Discloser Identifier: 948281

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   Yes.

   a. **Please list the other authors' names here.**

      Xiaomeng Hu, Hanne Scholz, Sofie Ingvast, Torbjörn Lundgren, Tim Scholz, Olof Eriksson, Per Liss, Di Yu, Tobias Deuse, Olle Korsgren, Sonja Schrepfer.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Tobias Deuse

Discloser Identifier: 744919

Disclosure Purpose: 25-03822

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
Sana Biotechnology	Consultant	Self

Category: Consultant
Description: Consultant for hypoimmune and clinical
Additional Information: equity

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Olof Eriksson

Discloser Identifier: 1056226

Disclosure Purpose: 25-03822

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
Antaros Tracer	Employment	Self
Title: Chief Scientific Officer **Additional Information:**	**Position Description:** Chief Scientific Officer	
Antaros Tracer	Stock	Self
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-cells with no Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Xiaomeng Hu

Discloser Identifier: 1239013

Disclosure Purpose: 25-03822

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
Sana Biotechnology	Employment	Self
Title: Senior Principle Scientist **Position Description:** **Additional Information:**		
Sana Biotechnology	Stock	Self
Additional Information:		
Sana Biotechnology	Stock Option	Self
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Apr 28, 2025 03:12:46 EDT
New England Journal of Medicine

Sofie Ingvast

Discloser Identifier: 1239015

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Olov Korsgren

Discloser Identifier: 592426

Disclosure Purpose: 25-03822

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
sana bio	Stock	Self
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

May 01, 2025 12:20:46 EDT
New England Journal of Medicine

Per Liss

Discloser Identifier: 1239018

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Apr 20, 2025 02:37:03 EDT
New England Journal of Medicine

Torbjörn Lundgren

Discloser Identifier: 1239016

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Apr 19, 2025 08:13:25 EDT
New England Journal of Medicine

Hanne Scholz

Discloser Identifier: 321785

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

May 01, 2025 11:52:00 EDT
New England Journal of Medicine

Tim Scholz

Discloser Identifier: 1239017

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

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sonja schrepfer

Discloser Identifier: 1239019

Disclosure Purpose: 25-03822

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
Sana Biotechnology	Employment	Self
Title: Senior Vice President **Position Description:** Head of Hypoimmune Platform **Additional Information:** Scientific Founder and Employee of Sana Biotechnology		
Sana Biotechnology	Stock	Self
Additional Information: Equity		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-cells with no Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Apr 20, 2025 04:00:10 EDT
New England Journal of Medicine

Di Yu

Discloser Identifier: 1239020

Disclosure Purpose: 25-03822

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Survival of Functional Transplanted Allogeneic Beta-Cells with No Immunosuppression

3. **Are you the corresponding author?**

   No.

Certification

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