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**First report on the effective intraperitoneal therapy of insulin-dependent Diabetes mellitus in pet dogs using “Neo-Islets,” aggregates of adipose stem and pancreatic islet cells**

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## 21 **Abstract**

22 We previously reported that allogeneic, intraperitoneally administered “Neo-Islets,” composed  
23 of cultured pancreatic islet cells co-aggregated with high numbers of immunoprotective and  
24 cytoprotective Adipose-derived Stem Cells, reestablished, through omental engraftment,  
25 redifferentiation and splenic and omental up-regulation of Regulatory T-cells, normoglycemia in  
26 autoimmune Type-1 Diabetic Non-Obese Diabetic (NOD) mice without the use of  
27 immunosuppressive agents or encapsulation devices. Based on these observations, we are  
28 currently testing this Neo-Islet technology in an FDA guided Pilot Study (INAD 012-776) in  
29 insulin-dependent, spontaneously diabetic pet dogs by the intraperitoneal administration of  
30  $2 \times 10^5$  Neo-Islets/kilogram body weight to metabolically controlled (blood glucose,  
31 triglycerides, thyroid and adrenal functions) animals under sedation and local anesthesia and  
32 ultrasound guidance. We report here initial observations on the first 4 Neo-Islet-treated, insulin  
33 dependent pet dogs that are now in the intermediate-term follow-up phase of the study (> 6  
34 months post treatment). Current results indicate that in dogs, Neo-Islets appear to engraft,  
35 redifferentiate and physiologically produce insulin, and are neither rejected by auto- or allo-  
36 immune attacks, as evidenced by (a) an absent IgG response to the allogeneic cells contained in  
37 the administered Neo-Islets, and (b) progressively improved glycemic control that achieves up to  
38 a 50% reduction in daily insulin needs paralleled by a significant fall in serum glucose levels.  
39 This is accomplished without the use of anti-rejection drugs or encapsulation devices. No  
40 adverse or serious adverse events related to the Neo-Islet administration have been observed to  
41 date. We conclude that this minimally invasive therapy has significant translational relevance to  
42 veterinary and clinical Type 1 Diabetes Mellitus by achieving complete and at this point partial  
43 glycemic control in two species, i.e., diabetic mice and dogs, respectively.

## 44 **Introduction**

45 Diabetes Mellitus is a common endocrine disorder in dogs, and it is estimated that there are  
46 currently 700,000 insulin-dependent pet dogs in the US [1–4]. Their care is burdensome and  
47 expensive for their owners. As in humans, Type 1 Diabetes Mellitus (T1DM) in dogs is caused  
48 by lack of insulin secretion in response to glucose, resulting in hyperglycemia, acid-base and  
49 electrolyte disorders, polydipsia, polyuria and weight loss, and is accompanied by a broad  
50 spectrum of diabetes-induced end organ and other complications, including blindness due to  
51 retinopathy and cataracts, opportunistic infections, neurological and other serious micro- and  
52 macro-vascular complications [4–6]. Although dogs were the model in which insulin was  
53 originally discovered, and remain a major large animal model for the refinement of diabetic  
54 treatments such as pancreas and islet cell transplants, almost no advances in the treatment for  
55 diabetic dogs have been made in the last 50 years [7]. A few studies have examined xeno- or  
56 allogeneic islet transplantation to reverse or ameliorate diabetes in dogs and have had varying  
57 degrees of success. Yet, insulin replacement therapy and blood glucose monitoring remain the  
58 only currently available therapy for these animals [7–9]. Due to the challenges of medically  
59 managing a diabetic dog, up to 40% of owners regrettably opt to euthanize their dogs within a  
60 day of diagnosis rather than treat them [1,10].

61 While the pathogenic mechanisms of canine T1DM are still incompletely understood, there  
62 is evidence that autoimmunity plays a role in approximately 1/3 of cases [3,4,7,10–12]. T1DM  
63 occurs with equal frequency in male and female neutered dogs, but as with Non-Obese Diabetic  
64 (NOD) mice, at higher frequency in intact females vs. males, suggesting a role for female  
65 hormones in the development of the disease in dogs [1,3,13–15]. While T1DM affects both  
66 juvenile and adult dogs [1,3,7], it is more commonly seen in adults, generally diagnosed between

67 the ages of 3 and 15 years [3,10]. Some groups have reported isolation of auto-antibodies to  
68 proinsulin, GAD65 and IA-2 from the sera of diabetic animals [16,17]. Others, however, have  
69 been unable to confirm the presence of such auto-antibodies in the same, previously tested sera,  
70 nor in sera from other diabetic dogs [2,14]. On the other hand, several studies have found a  
71 genetic association between certain dog leukocyte antigen alleles (DLA) and the development of  
72 DM in dogs, similar to that found between HLA alleles and the development of DM in humans  
73 [12,18,19]. Despite some controversy as to the immune-mediated destruction of beta cells, all  
74 pioneering work on islet and pancreas transplantation for humans was carried out in dogs and  
75 clearly demonstrated the need for immunosuppression or immune-isolation, as well as sufficient  
76 nutrition/oxygenation and vascularization for islet allo-graft survival [7,20].

77 Dog survival time post the diagnosis of diabetes is short [1,10]. In one study, median post-  
78 diagnosis survival time was found to be only 57 days, due either to pet owners' unwillingness to  
79 care for a diabetic animal, or to the dog suffering from advanced stages of diabetic complications  
80 at the time of diagnosis. For dogs surviving beyond the first day after diagnosis, the median  
81 survival time was 2 years [1]. These low survival rates, and clear unwillingness of some owners  
82 to care for diabetic animals, underscore the need for novel and effective therapeutics that remove  
83 much of the burden of diabetes treatment and maintenance from pet owners, and to facilitate the  
84 survival of affected dogs.

85 We previously demonstrated that allogeneic, intraperitoneally administered "Neo-Islets"  
86 (NIs), composed of culture expanded islet cells (ICs) co-aggregated with high numbers of  
87 immunoprotective and cytoprotective Adipose-derived Stem Cells (ASCs), could reestablish  
88 normoglycemia in NOD mice with autoimmune T1DM without the use of encapsulation devices  
89 or immunosuppressive agents [21]. Glycemic control was similarly achieved using dog-derived

90 ASCs and ICs in a Streptozotocin (STZ) model of diabetes in NOD/SCID mice [21]. Dose  
91 finding studies indicated that a dose of  $2 \times 10^5$  canine Neo-Islets (cNIs) per kg b.wt. would be  
92 sufficient to control blood glucose levels [21].

93 Based on these studies, we initiated an FDA-CVM guided Pilot Study (INAD 012-776) to  
94 assess the (i) safety, (ii) feasibility and (iii) efficacy of allogeneic cNIs in significantly reducing  
95 or eliminating the need for exogenous insulin in spontaneously diabetic, autoimmune or insulin  
96 resistant, insulin-dependent pet dogs. We further assessed whether the administered NIs elicited  
97 an allo-immune response. This Pilot study is currently ongoing at Veterinary Specialty Hospital  
98 in San Diego, CA, and at Washington State University in Pullman, WA. Six dogs have been  
99 treated and four followed for more than 6 months. We report here on the course of the first four  
100 NI treated dogs that have been followed for more than 6 months. The overall rationale of  
101 demonstrating that this NI therapy is also effective in a second, larger diabetic mammal, the dog,  
102 is the fact that this will further strengthen the justification for the currently planned conduct of a  
103 clinical trial in study subjects with T1DM.

## 104 **Materials and Methods**

### 105 **Reagents**

106 Reagents used and their manufacturers are listed as indicated below, except for PCR reagents  
107 and primers which are listed in S1 Table.

### 108 **Study Design**

109 An FDA guided pilot study (INAD 012-776) conducted with IACUC approval at (a) Washington  
110 State University in Pullman, WA (WSU) and (b) the Veterinary Specialty Hospital in San Diego,

111 CA (VSH). Ten Insulin dependent pet dogs are included. Eight dogs have been enrolled  
112 according to the criteria in Table 1. Informed consent was obtained from all dog owners prior to  
113 enrollment. One owner withdrew her dog prior to treatment, 6 dogs have been treated, and 4 of  
114 those (VSH-01, VSH-02, WSU-01 and WSU-02) have been followed for 6 months or longer.  
115 Enrolled dogs' demographics are shown in Table 2 and comorbidities in Table 3. Dogs are  
116 screened and followed as shown in S2 Table. Pre-treatment serum samples from the treated dogs  
117 were tested for the presence of islet autoantibodies (see below for details), and all dogs were  
118 examined for comorbidities. After blood glucose and triglyceride levels were optimally  
119 controlled,  $2 \times 10^5$  allogeneic NIs per kg b.wt. were given i.p.. In all animals, blood Glucose and  
120 Fructosamine levels, insulin requirements, body weights, food intake, formation of antibodies to  
121 allogeneic NIs, animal activity and the development of adverse events were closely monitored by  
122 the PIs and the primary veterinarians for each dog.

123 **Table 1. Enrollment Criteria.**

Enrollment Criteria	<ul style="list-style-type: none"><li>• Insulin dependent, diabetic dog on established insulin and diet regimen</li><li>• Weight between 5 and 12 kg</li><li>• Spayed, if female</li></ul>
Exclusion Criteria	<ul style="list-style-type: none"><li>• History of malignancy</li><li>• Significant illness unrelated to the diabetic state such that the PI believes the dog to be a poor candidate</li><li>• Significantly advanced age such that the PI believes the dog to be a poor candidate</li><li>• Contraindication to general anesthesia</li><li>• Participation in another, ongoing clinical trial</li></ul>
Specific Enrollment Criteria Related to Diabetes Mellitus in Dogs	Presence of <ul style="list-style-type: none"><li>• End-organ damage</li><li>• Cataracts</li><li>• Neuropathy</li><li>• Renal disease</li><li>• History of pancreatitis</li></ul>

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126 **Table 2: Study Subject Demographics**

Subject #	Treated	Gender	Breed	Weight at enrollment (kg)	Approx. Age at enrollment (yrs)	Duration of DM (yrs)	insulin dose/day, regimen at enrollment or treatment (later)
VSH-01	yes	M	French Bulldog	12.3	9	2.5	4.5-5.5 U Novalin BID
VSH-02	yes	M	Bichon Mix	6.9	7	0.5	5 U Vetsulin BID
VSH-03	yes	F	Bichon Poodle Mix	5.8	11	2	7.5 U Vetsulin BID
VSH-04	yes	M	Chihuahua Mix	7.3	12	1	9 U Vetsulin BID
VSH-05	stabilizing	M	Poodle	5.3	7	0.2	7 U Vetsulin BID
VSH-06	withdrawn	M	Chihuahua Mix	7	7	1	6 U Vetsulin BID
WSU-01	yes	F	American Eskimo	11	1.8	0.8	2.5 U Vetsulin BID
WSU-02	yes	M	Chihuahua Mix	7	1	0.5	4 U Vetsulin BID

127 Abbreviations: U = Units, WSU = Washington State University, VSH = Veterinary Specialty  
 128 Hospital, BID = twice per day. Insulin is listed as per dose. If given BID, double for the daily  
 129 dose.

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139 **Table 3: Clinical data on treated dogs**

<b>VSH-01</b> <ul style="list-style-type: none"><li>• Male, neutered (DOB 8/8/08)</li><li>• No history of pancreatitis, but evidence on screening US of suspected chronic or previous pancreatitis</li><li>• Cataracts; moderate adrenomegaly, mild renal degenerative changes</li></ul>	<b>VSH-02</b> <ul style="list-style-type: none"><li>• Male, neutered (DOB 3/28/11)</li><li>• No history of pancreatitis, but evidence on screening US of suspected chronic or previous pancreatitis</li><li>• Hypothyroid (0.1 mg Synthroid BID), diagnosed through screening; mild renal degenerative changes</li></ul>
<b>VSH-03</b> <ul style="list-style-type: none"><li>• Female, spayed (DOB 6/19/06)</li><li>• No history or evidence of pancreatitis</li><li>• Cataracts, hepatomegaly, elevated ALP, hyperlipidemia (150 mg Gemfibrozil BID)</li></ul>	<b>VSH-04</b> <ul style="list-style-type: none"><li>• Male, neutered (DOB 10/10/05)</li><li>• No history or evidence of pancreatitis</li><li>• Cataracts, hepatomegaly, elevated ALP + ALT, hyperlipidemia (150 mg Gemfibrozil BID)</li></ul>
<b>WSU-01</b> <ul style="list-style-type: none"><li>• Female, spayed (DOB 6/15/16)</li><li>• No history nor evidence of pancreatitis</li><li>• Hypercholesterolemia</li><li>• History of liver disease</li></ul>	<b>WSU-02</b> <ul style="list-style-type: none"><li>• Male, neutered (DOB 4/15/17)</li><li>• No history nor evidence of pancreatitis</li></ul>

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## 142 **Blood glucose levels**

143 Blood glucose levels were assessed by the owner at least twice daily (morning and evening)

144 using either an *AlphaTrak* or *FreeStyle Libre* glucometer prior to insulin administration. Insulin

145 (Vetsulin or Novalin) was administered BID based on blood glucose readings, and the doses

146 given are recorded.

## 147 **Cells**

### 148 **Cell donor information**



149 All cells, Adipose-derived Stem Cells (ASCs) and Islet Cells (ICs), for the study were obtained  
150 through an NIH sharing agreement from 27 dogs being euthanized under a University of Utah  
151 IACUC approved protocol. This study involved no use of test agents, but did involve the use of a  
152 surgically implanted pacemaker in some dogs to induce heart failure, as well as the use of  
153 resynchronization therapy via the pacemaker as an experimental treatment. Dogs were up-to-date  
154 on vaccinations at the time of euthanasia.

### 155 **Islets and ASCs**

156 Islets and ASCs were isolated and cultured from dogs as previously reported [21–26], and as  
157 described in detail in the Supporting Information section of our previous publication [21]. Prior  
158 to NI formation, cultured ASCs were characterized for their ability to undergo trilineage (adipo-,  
159 osteo-, chondrogenic) differentiation as described [21] and for surface marker expression of  
160 CD90, CD44, CD34, CD45 and DLA-DR as in our previous publication [27], and using the  
161 following antibodies: Phycoerythrin (PE)-labeled, monoclonal, rat anti-dog CD90 IgG2b, and  
162 Isotype (Invitrogen, 12-5900 and 12-4031-83); Allophycocyanin (APC)-labeled, monoclonal,  
163 mouse anti-dog CD44 IgG1; and Isotype (R&D, FAB5449A and IC002A); PE-labeled,  
164 monoclonal, mouse anti-dog CD34 IgG1, and Isotype (BD Biosciences, 559369 and 554680); R-  
165 PE-labeled, monoclonal, rat anti-dog CD45 IgG2b, and Isotype (BioRad MCA1042PE and PA5-  
166 33195), PE-labeled, monoclonal, mouse anti-human HLA-DR IgG2a (cross-reacts with dog), and  
167 Isotype (BD Biosciences 555812 and 555574). All antibodies were used at the concentrations  
168 recommended by their respective manufacturers.

## 169 **Cell banking**

170 Passage 0 (P0) cultured islet cells and P2 ASCs were suspended in CryoStor CS10 (BioLife  
171 Solutions, 210102) and banked, frozen in liquid nitrogen (-140°C) until ready for final expansion  
172 and NI formation. Prior to freezing, cells were release tested for viability, sterility, endotoxin,  
173 mycoplasma, expression of various genes involved in immune modulation, cell survival and  
174 angiogenesis, and dog-specific adventitious agents.

## 175 **rtPCR**

176 rtPCR was carried out as described in our previous publication [21] using the reagents and  
177 primers listed in S1 Table. In brief, RQ was calculated through normalization to internal  
178 (deltaCT; beta actin and beta 2 microglobulin) and external controls (delta-deltaCT; parent cells),  
179 both accomplished using the ABS 7500 Real Time PCR System and software. Results are  
180 presented as  $\log_{10}(\text{RQ}) \pm \log_{10}(\text{RQ}_{\text{min}} \text{ and } \text{RQ}_{\text{max}})$ . Differences between expression levels  
181 greater than  $\log_{10}(\text{RQ}) 2$  or  $\log_{10}(\text{RQ}) -2$  were considered significant.

## 182 **Final Product (NI) formation and storage**

183 NIs were formed in ultra-low adherent 10-layer Cell Stacks (Corning, custom made product)  
184 from freshly cultured banked ICs and ASCs using  $70 \times 10^6$  cells per layer and 140 ml of DMEM  
185 (Gibco 11885-084) + 10% dog serum (Golden West) as described in our previous publication  
186 [21]. NIs were harvested and resuspended in 50 to 100 ml of sterile Plasmalyte A (Baxter,  
187 2B2543) + 2% HEPES (Gibco), pH 7.4 at a concentration of  $2 \times 10^7$  clustered cells/ml, and  
188 placed in a sterile 100 ml syringe (Wilburn Medical, WUSA/100). The final product was release  
189 tested (viability, sterility, endotoxin, mycoplasma, gram stain, gene expression of insulin (INS),  
190 glucagon (GCG), somatostatin (SST), pancreatic polypeptide (PPY), pancreatic and duodenal

191 homeobox 1 (PDX1), vascular endothelial growth factor A (VEGF $\alpha$ ), stromal cell derived factor  
192 1 (CXCL12), and stored and transported to the study site at 4°C for administration within 48 hrs  
193 of packaging.

## 194 **Cell bank and final product release testing**

195 Cell viability was assessed using Fluorescein diacetate (Sigma, F7378) and Propidium Iodide  
196 (Life Technologies P3566) as per the manufacturers' instructions. Sterility was assessed as  
197 described in 21 *CFR 610.12*, using Tryptic Soy Broth (Sigma, 22092) and Fluid Thioglycollate  
198 Medium (Sigma, T9032) and following the manufacturer's instructions. Endotoxin levels were  
199 determined using the Charles River Endosafe Nexgen PTS system and reagents (Charles River)  
200 following the manufacturer's instructions. Possible Mycoplasma contamination was assessed  
201 using the MycoAlert PLUS Mycoplasma Detection Kit (Lonza, LT07-701 and LT07-518) per  
202 the manufacturer's instructions. Samples of banked cells from each donor dog were sent to  
203 Zoologix for relevant adventitious agent testing. Prior to NI formation, ASCs were assessed for  
204 expression of genes involved in immune modulation, cell survival and angiogenesis. ICs were  
205 analyzed for expression of islet hormone associated genes. Gram Staining was conducted by  
206 standard methods using a kit (Sigma, 77730-1KT-F). Cell and NI release criteria are listed in  
207 Table 4.

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215 **Table 4: Release criteria for cells and Neo-Islets**

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	<b>Viability</b>	<b>Sterility</b>	<b>Endotoxin</b>	<b>Mycoplasma</b>	<b>Adventitious Agents</b>	<b>Gene expression (rtPCR)</b>	<b>extracellular marker (FACS)</b>
<b>ASCs</b>	≥ 70%	No growth after 14 days	< 5.0 EU/ml	B/A <1.2	negative for all tested	Significant increase in Ido 1 expression Upon overnight culture with INF- $\gamma$	≥ 90% CD90, CD44; ≤ 4% CD34, CD45, DLA-DR
<b>Islet Cells</b>	≥ 70%	No growth after 14 days	< 5.0 EU/ml	B/A <1.2	negative for all tested	+ Ins	NA
<b>Neo-Islets</b>	≥ 70%	negative gram stain; no growth after 14 days	< 5.0 EU/ml	B/A <1.2	NA	+ Ins	NA

217 Adventitious Agents tested:

218 *Neorickettsia risticii*, *Mycoplasma haemocanis*, *Mycoplasma canis*, *Bartonella henselae*, *B.*  
 219 *bacilliformis*, *B. clarridgeiae*, *B. elizabethae*, *B. quintana* and *B. vinsonii* subsp. *Berkhoffii*,  
 220 *Brucella abortus*, *B. microti*, *B. melitensis*, *B. pinnipedialis*, *B. suis*, *B. canis*, *B. ovis* and *B.*  
 221 *neotomae*, *Neorickettsia helmintheca*, *Cryptococcus neoformans*, Influenza A H5N1, H5N2,  
 222 H1N1, H2N2, H3N8, H4N6, H7N7, H8N4 and H9N2, canine herpesvirus, *Toxoplasma gondii*

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## 225 **Testing of treated dogs' sera for antibodies to Islet Cells and ASCs**

226 Prior to and at ~6 weeks post NI treatment, 1 ml of serum was collected from each study dog.

227 5x10<sup>4</sup> banked allogeneic ASCs, and (separately) 5x10<sup>4</sup> banked allogeneic ICs were incubated

228 for 30 min at room temperature with 100 uL serum each. Cells were then centrifuged at 600 x g

229 for 5 min; the supernatant was removed and the cell pellets resuspended in 200 uL Phosphate

230 Buffered Saline (PBS, Sigma, 11666789001) +1% Fetal Bovine Serum (FBS, GEHealthcare,  
231 SH30910.03). Cells were then incubated at room temperature for 30 min with a 1:200 dilution of  
232 FITC-labeled, polyclonal rabbit anti-dog IgG or Isotype control (Jackson ImmunoResearch; 304-  
233 095-003, lot #13971 and 011-090-003, lot #127025 respectively). They were then centrifuged,  
234 washed 1x with PBS + 1% FBS; resuspended in 400 uL PBS + 1% FBS, and analyzed by FACS  
235 as previously described [21].

## 236 **Enzyme Linked Immunosorbent Assays (ELISAs)**

237 ELISAs for GAD65 and IA2 autoantibodies were kindly carried out by Dr. Boris Fehse,  
238 University of Hamburg, Germany, using GAD65 and IA2 ELISA kits (both from Euroimmun,  
239 Luebeck, Germany, EA 1022-9601 G and EA 1023-9601 G, respectively) and the same serum  
240 samples were analyzed for allo-antibody levels. The GAD65 kit has previously been shown to  
241 cross-react with dog antibodies [28].

## 242 **NI Administration**

243 NIs were administered to dogs by the site's veterinary ultrasonographer as follows: an i.v.  
244 catheter was placed, and the dog was sedated with dexmedetomidine (5 mcg/kg) and butorphanol  
245 (0.1-0.2 mg/kg). Abdominal fur was shaved, and skin was prepared for aseptic injection. NIs  
246 were warmed to room temperature, and an 18 gauge, sterile cannula was placed, after local  
247 anesthesia was administered, through the linea alba, ~ 3-4 cm cranial to the umbilicus, under  
248 ultrasound guidance to intraperitoneally infuse normal saline test solution, then the suspended  
249 NIs over a 2-3 minute period. Once the administrations were completed, the cannula was  
250 withdrawn. Ultrasound imaging was carried out to check for abdominal bleeding. Sedation was  
251 reversed with Antisedan, and the dog was monitored until determined stable by the PI and

252 veterinary staff. Dogs returned home with their owners the same day, once the PI determined that  
253 they were stable and ready. Owners were advised that the dogs' serum glucose levels should be  
254 kept  $\leq 210$  mg/dL to protect the graft cells and facilitate redifferentiation into insulin producing  
255 cells.

## 256 **Follow-up Schedule**

### 257 **Month 1**

258 Dog owners check and record their dog's blood glucose levels every 12 hours; record food and  
259 water intakes and weights once per week on supplied forms. Dog owners administer insulin to  
260 dogs as needed and as instructed by the PI. Dogs are brought in for a physical examination and  
261 laboratory studies as indicated in S2 Table.

### 262 **Months 2 - 12**

263 Dog owners continue to check and record their dog's blood glucose levels twice per day; record  
264 dogs' daily food and water intakes and weights every other week on supplied forms and  
265 electronically through month 6, and then as deemed necessary by the PI. Dog owners are  
266 responsible for the continued administration of insulin to dogs as needed and as instructed by the  
267 PI. Dogs are brought in for a physical examination once per month post treatment through month  
268 6, and once per quarter in months 6 - 12. Fructosamine levels, a chemistry panel and urinalysis  
269 are obtained at the 3rd and 6th month visits. At the 6 months visit, a CBC, Chemistry and  
270 electrolyte panel, HbA1c, and urine are collected. At each visit, potential changes in the degree  
271 of preexisting end-organ damage are carefully documented.

### 272 **Years 1-3**

273 Dog owners are responsible for continued checking of blood glucose levels and administering  
274 insulin as deemed necessary by the PI. Dogs are brought in for a physical examination once per  
275 quarter through the 36th month post treatment, to assess changes in the degree of documented  
276 end-organ damage. Fructosamine levels, HbA1c and chemistry panels are checked at each visit.  
277 Once per year, a CBC and a urinalysis are obtained.

## 278 **Statistical Analysis**

279 Unless otherwise indicated, Data are expressed as Mean  $\pm$  SEM or Mean  $\pm$  95% confidence  
280 interval, as indicated. Primary data were collected using Excel (Microsoft, Redmond, WA), and  
281 statistical analyses were carried out using Prism (GraphPad, San Diego, CA). Two tailed T-tests  
282 were used to assess differences between data means. A *P* value of  $< 0.05$  was considered  
283 significant.

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## 286 **Results**

### 287 **Study Design**

288 This FDA-CVM guided Pilot study to determine the safety, feasibility and preliminary efficacy  
289 of cNIs in eliminating or significantly reducing a diabetic dog's need for insulin will be carried  
290 out in 10, stable, insulin-dependent, diabetic pet dogs of either gender as described in Methods.

291 Accordingly, the primary endpoints are safety and feasibility, as well as a demonstrated lack of  
292 immune response to the administered cells as evidenced by either sustained euglycemia or  
293 improvement in glucose control and/or reduced need for insulin after NI administration; and the  
294 absence of IgG antibodies directed at the cells that compose the allogeneic NIs in sera of treated  
295 dogs as assessed by FACS at  $\geq 1$  month post transplantation. The secondary endpoint is  
296 reduction or elimination of need for insulin in treated dogs. The tertiary endpoint is lack of  
297 development or progression of end-organ damage in treated dogs. Adverse and Severe Adverse  
298 Events are recorded and reported over the duration of the study.

299 Dogs are enrolled according to the criteria in Table 1, treated with NIs when serum glucose and  
300 serum lipids are stable and controlled to within normal ranges, and they are monitored for  
301 adverse events, and serum glucose levels and insulin needs are recorded according to the  
302 schedule in S2 Table, and as indicated in Methods. Additionally, owners check, record and report  
303 their dogs' blood glucose levels and insulin needs twice per day, and monitor their dogs' food  
304 and water and food intake, activity and weight.



305 At this point, 8 eligible dogs have been enrolled for treatment with allogeneic NIs. The dogs’  
306 pretreatment demographics and comorbidities are summarized in Tables 2 and 3. Of these dogs,  
307 one dog’s owner withdrew him from the study prior to dosing, two are being treated for  
308 hypertriglyceridemia (Gemfibrozil, 150 mg BID, and dietary restriction) and have been treated  
309 but are not yet in the intermediate term follow-up phase of the study, while four have been  
310 treated with NIs and (VSH-01, VSH-02, WSU-01 and WSU-02) have been followed for more  
311 than 6 months. VSH-01 was a 9 year old (at dosing), male, 12 kg French bulldog who had been  
312 on insulin for approximately 2.5 years at the time of dosing. VSH-02 was a 7 year old,  
313 hypothyroid, male, 7 kg Bichon mix who had been diabetic for approximately 6 months at the  
314 time of dosing. WSU-01 was a 2 year old, 11 kg, female American Eskimo dog who had been on  
315 insulin for approximately 9 months prior to treatment. WSU-02 was a 1 year old, 7 kg Chihuahua  
316 mix who had been on insulin for approximately 6 months prior to dosing.

317 Therapeutic Doses of NIs for all treated dogs were prepared, release-tested, packaged and  
318 administered as described in Methods. All cells and final products met the release criteria listed  
319 in Table 4. Prior to administration, NIs were characterized by rtPCR for gene expression of INS,  
320 GCG, SST, PPY, PDX-1, NKX6-1, VEGFA, and CXCL12. As shown in Fig 1, while PDX-1  
321 was no longer expressed in NIs given to any dog, NIs used for treatment were shown to  
322 transcribe islet hormone genes for INS and GCG, albeit at significantly reduced levels compared  
323 to P0 cultured islet cells. NIs also expressed genes associated with ASCs cytoprotective,  
324 angiogenic and immune modulatory activities [29–34] (Fig1).

325 **Figure 1. Gene expression profiles (Log<sub>10</sub>RQ) of the NIs administered to study subjects.**

326 All gene expression levels were normalized to those of the P0 IC banks from which the IC  
327 portion of the NIs were derived. Data are expressed as mean with 95% Confidence Interval, and

328 reactions were carried out in duplicate. INS, GCG, SST, PPY, NKX6-1, VEGF, and CXCL12  
329 are all expressed in the NIs administered to each dog. PDX-1 is no longer expressed.

330

### 331 **Intermediate term blood glucose and insulin requirements**

332 As shown in Fig 2, blood glucose levels and insulin requirements for VSH-01, VSH-02, and  
333 WSU-02 were significantly reduced ( $P < 0.05$ )  $\geq$  6 months post treatment compared to baseline.  
334 Although WSU-01's mean monthly blood glucose levels 12 months post treatment were also  
335 significantly reduced compared to those at baseline ( $P = 0.0015$ ), her daily insulin needs  
336 remained unchanged (Fig 2).

337 **Figure 2. Serum glucose levels and insulin needs over time.** (A) Serum glucose levels of study  
338 dogs, as assessed and reported by owners, prior to treatment (0 months) and over the study  
339 period. As dogs were treated at different times, they are not all currently at the same post-  
340 treatment time, thus the reported follow-up period for each is different. (B) Insulin needs for the  
341 same time frame. Glucose and insulin are reported at least 2x per day. All glucose level values  
342 are averaged for the pretreatment period and for each month post-treatment. Units of insulin  
343 administered per day are calculated and averaged for each month. (C) Percent reduction in daily  
344 insulin dose at the current follow-up point from baseline and average mg/dL reduction in serum  
345 glucose from baseline along with statistical significance ( $P$  values) for each dog are shown. With  
346 the exception of WSU-01, all treated dogs currently show both a sustained reduction in serum  
347 glucose and need for insulin. WSU-01 showed sustained reduction in serum glucose for her  
348 entire 12 month follow-up period, but only sustained a 20% reduction in her need for insulin

349 over 6 months post-treatment. After 7 months, her need for insulin rebounded to her pretreatment  
350 levels and remains there now.

## 351 **Type of diabetes**

352 As part of the study, serum from dogs was obtained prior to and after NI dosing, and tested by  
353 FACS for antibodies to the administered cells, using pre-dosing serum and isotypes as negative  
354 controls. Pre-treatment testing of sera served not only the purpose of giving a baseline level of  
355 anti-ASC or anti-IC antibodies to compare to post-treatment levels, but also as an indication of  
356 whether the dogs' diabetes was auto-immune in nature, as indicated by the presence of anti-Islet  
357 Cell antibodies prior to treatment. By this criterion, three of the four tested dogs, VSH-02, WSU-  
358 01 and WSU-02, appear to have autoimmune diabetes (see Fig 3A and B), while VSH-01 may  
359 have an "insulin resistance" form of Insulin-dependent DM.

360 Sera were similarly tested for the presence of antibodies to two common human autoimmune  
361 antigens that have been associated with canine T1DM, IA2 and GAD 65 [16,17]. Only  
362 pretreatment serum from WSU-01 showed reactivity to IA2. No other sera showed the presence  
363 of antibodies to either (Fig 3C).

364 **Figure 3. Antigenic responses to NI treatment, and pre-existing presence of auto-islet**  
365 **antibodies.** Anti ASC and anti-IC IgG responses as assessed by FACS in sera of the study dogs  
366 before (A) and after (B) treatment with allogeneic NIs. Shown are percentages of FITC-labeled  
367 anti-dog IgG antibody. Sera were collected from dogs before (A) and 1.5 to 3 months after (B)  
368 NI administration. The percent of positive cells is indicated above each column. Dogs do not  
369 show increased IgG responses to either ASCs or ICs after NI administration, indicating there is  
370 no additional allo-immune response by the recipients to either cell type. Three dogs show pre-

371 existing antibodies to ICs, prior to treatment with NIs, suggesting they have an autoimmune form  
372 of diabetes. (C) Results of ELISA testing of sera from the treated dogs for specific anti-islet  
373 antigens, IA2 and GAD65, indicate that none of the dogs have antibodies to GAD65 antigen, but  
374 that WSU-01's serum contained antibodies to IA2 prior to, but not after treatment. Samples were  
375 run in duplicate. Note: a pre-treatment serum sample was not available for VSH-01.

376

### 377 **Neither auto- nor allo-immune rejection is observed**

378 Even though at least 3 of the treated dogs (VSH-02, WSU-01 and WSU-02) appear to have  
379 autoimmune diabetes (Type 1) as indicated by the presence of anti-islet cell IgG in their sera (see  
380 Fig 3), and even though the NIs used to treat them came from unrelated donors, none of the dogs  
381 appear to have rejected the NIs as indicated by the following. First, responsive dogs show  
382 continued improved blood sugars and lowered insulin requirements (see Fig 2). Second, no allo-  
383 rejection antibodies to the NIs are found in the treated dogs' sera after implantation (Fig 3).

### 384 **NI therapy appears to be safe and well tolerated in dogs**

385 In addition to regular blood glucose and weight monitoring, dogs enrolled in this study are being  
386 followed closely over the entire 3 year follow-up period with physical examinations and  
387 laboratory tests in order to detect signs of adverse events or changes in end organ damage in  
388 association with Neo-Islet therapy (see S2 Table).

389 Despite the fact that several of the currently studied dogs are of advanced age and have multiple  
390 comorbidities (see Tables 2 and 3), no adverse events attributable to the NI therapy have been  
391 observed to date. Specifically, none of the 6 treated dogs have developed adverse events such as

392 oncogenic transformation of transplanted cells, hematological changes, deterioration in organ  
393 function, lack to thrive, etc. While at this point in the study we cannot rule out with certainty the  
394 possibility that adverse events can eventually occur, data in the nearly 2 years since the first dogs  
395 were treated would indicate that Neo-Islets are safe and well tolerated.

## 396 **Discussion**

397 Intermediate term results from the current study thus far demonstrate that allogeneic NI therapy,  
398 as currently dosed, (i) is effective in improving glycemic control while durably reducing the need  
399 for insulin; (ii) it does so without eliciting an immune response, even in dogs with autoimmune  
400 diabetes; and (iii) is feasible and safe. The observed decrease in post-treatment compared to pre-  
401 treatment levels of anti-Islet Cell antibodies in two dogs (Fig 3 A) may reflect the known  
402 inhibitory actions of ASCs on B cells [35]. However, this potentially significant effect must be  
403 confirmed in additional studies. While the documented reduction in total insulin requirement  
404 occurs only gradually as transplanted ICs re-differentiate into insulin producing cells, this  
405 response does taper off subsequently, most importantly, it does not, in most cases, increase again  
406 as is seen in failing traditional intrahepatic islet cell transplants (Fig 2) [36]. The data so far  
407 indicate that the allogeneic NI grafts are stable and functioning long term, and are not being  
408 rejected, which directly demonstrates that this novel form of therapy does not require the life-  
409 long use of potentially toxic antirejection drugs. In other words, the allogeneic ASC component  
410 of the administered NIs appears to provide through its immune-modulating activity [35] both  
411 robust auto- and allo-immune isolation of the cells that make up NIs, and this without the need  
412 for often failing encapsulation devices. For example, the use of such a subcutaneously implanted  
413 encapsulation device in a clinical trial has proved problematic, as it elicited an inflammatory

414 fibrotic, foreign body type response that resulted in the death of the encapsulated insulin  
415 producing cells and thus failure so far of this mode of T1DM therapy [37].

416 Since the treated dogs are pets and the study is ongoing, the exact engraftment site of the i.p.  
417 administered NIs has not been histologically confirmed, although we believe that the main  
418 engraftment site is the omentum as we clearly demonstrated in our mouse studies (21). The fact  
419 that none of the NI treated dogs developed hypoglycemic episodes furthermore illustrates that  
420 insulin secretion by administered NIs remains physiological and occurs into the portal system of  
421 the liver, which is physiological. Late post treatment glucose tolerance tests with simultaneous  
422 monitoring of canine insulin and C-Peptide release will be conducted in all study dogs.

423 There are several possible explanations for the incomplete normalization of blood glucose  
424 levels and failure to achieve complete insulin independence. These incomplete responses may be  
425 related to an inadequate NI dose, the potential need for a second dose, as is routinely done in  
426 human islet transplants [36] and as we demonstrated to be effective in diabetic mice, and  
427 potentially suboptimal omental uptake and engraftment of NIs. In addition, the need to keep the  
428 dog post NI infusion for at least 24 hrs either in a prone or supine position is important since  
429 both of these positions facilitate the omental engraftment of administered NIs, while the  
430 assumption of an upright position will lead to the translocation of the transplants to the dog's  
431 pelvis, a location that prevents their engraftment in the omentum and failure to function as  
432 intended. The current technology exploits the omentum's ability to both release cells and to take  
433 up cell aggregates such as NIs via its milky spots, combined with its excellent arterial blood  
434 supply for oxygenation of and glucose sensing by engrafted NIs. And importantly, the  
435 omentum's venous drainage facilitates the physiological delivery of secreted insulin and other  
436 islet hormones directly into the portal system of the liver, i.e., a route that is identical to that of

437 the pancreatic veins. Since the liver inactivates up to 50% of received insulin, the post hepatic  
438 concentrations of insulin that other insulin-sensitive tissues are exposed to is significantly lower  
439 than those insulin levels that are generated by the s.c. injection of insulin that, particularly in  
440 higher doses may have adverse systemic effects [38,39].

441 Finally, the ability to generate more than 50 therapeutic NI doses from a single cadaveric  
442 pancreas and MSC donor will significantly improve the availability of this therapy for diabetic  
443 dogs and assist their owners with the care of their pets. The cost savings over time, once the NI  
444 therapy has been further optimized to durably make diabetic dogs insulin independent are  
445 predicted to be significant.

446 In conclusion, completion of the current study with the remaining dogs will include permitted  
447 protocol modifications that we expect to augment the therapeutic efficacy of the so far utilized  
448 NI treatment protocol. Nevertheless, we posit that the here presented observations not only  
449 provide evidence in support of our hypothesis that NIs when given i.p. engraft in the omentum  
450 where they redifferentiate and create a new endocrine pancreas that leads to the establishment of  
451 euglycemia and insulin-independence. The proof of principle, i.e., the demonstration that this NI  
452 therapy is also effective in a second, larger diabetic mammal, the dog, is definitively significant  
453 as this will further strengthen the justification for the currently planned conduct of a optimally  
454 designed clinical trial in study subjects with T1DM.

455

456

457

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## 462 **References**

- 463 1. Fall T, Hamlin HH, Hedhammar A, Kämpe O, Egenvall A. Diabetes mellitus in a  
464 population of 180,000 insured dogs: incidence, survival, and breed distribution. *J Vet*  
465 *Intern Med.* 2007;21: 1209–1216. doi:10.1111/j.1939-1676.2007.tb01940.x
- 466 2. Ahlgren KM, Fall T, Landegren N, Grimelius L, von Euler H, Sundberg K, et al. Lack of  
467 evidence for a role of islet autoimmunity in the aetiology of canine diabetes mellitus.  
468 *PLoS One.* 2014;9: e105473. doi:10.1371/journal.pone.0105473
- 469 3. Guptill L, Glickman L, Glickman N. Time trends and risk factors for diabetes mellitus in  
470 dogs: Analysis of Veterinary Medical Data Base records (1970-1999). *Vet J.* 2003;165:  
471 240–247. doi:10.1016/S1090-0233(02)00242-3
- 472 4. Adin CA, Gilor C. The Diabetic Dog as a Translational Model for Human Islet  
473 Transplantation. *Yale J Biol Med.* 2017;90: 509–515.
- 474 5. Gilor C, Niessen SJM, Furrow E, DiBartola SP. What’s in a Name? Classification of  
475 Diabetes Mellitus in Veterinary Medicine and Why It Matters. *J Vet Intern Med.* 2016;30:  
476 927–40. doi:10.1111/jvim.14357
- 477 6. Morgan MJ, Vite CH, Radhakrishnan A, Hess RS. Clinical peripheral neuropathy



- 478 associated with diabetes mellitus in 3 dogs. *Can Vet J = La Rev Vet Can.* 2008;49: 583–6.
- 479 7. Vrabelova D, Adin C, Gilor C, Rajab A. Pancreatic Islet Transplantation: From Dogs to  
480 Humans and Back Again. *Vet Surg.* 2014;43: 631–641. doi:10.1111/j.1532-  
481 950X.2014.12224.x
- 482 8. Abalovich AG, Bacqué MC, Grana D, Milei J. Pig Pancreatic Islet Transplantation Into  
483 Spontaneously Diabetic Dogs. *Transplant Proc.* 2009;41: 328–330.  
484 doi:10.1016/j.transproceed.2008.08.159
- 485 9. Alejandro R, Latif Z, Polonsky KS, Shienvold FL, Civantos F, Mint DH. Natural history  
486 of multiple intrahepatic canine islet allografts during and following administration of  
487 cyclosporine. *Transplantation.* 1988;45: 1036–1044. doi:10.1097/00007890-198806000-  
488 00008
- 489 10. Shields EJ, Lam CJ, Cox AR, Rankin MM, Van Winkle TJ, Hess RS, et al. Extreme Beta-  
490 Cell Deficiency in Pancreata of Dogs with Canine Diabetes. *PLoS One.* 2015;10:  
491 e0129809. doi:10.1371/journal.pone.0129809
- 492 11. O’Kell AL, Wasserfall C, Catchpole B, Davison LJ, Hess RS, Kushner JA, et al.  
493 Comparative Pathogenesis of Autoimmune Diabetes in Humans, NOD Mice, and Canines:  
494 Has a Valuable Animal Model of Type 1 Diabetes Been Overlooked? *Diabetes.* 2017;66:  
495 1443–1452. doi:10.2337/db16-1551
- 496 12. Short AD, Catchpole B, Kennedy LJ, Barnes A, Fretwell N, Jones C, et al. Analysis of  
497 candidate susceptibility genes in canine diabetes. *J Hered.* 2007;98: 518–25.  
498 doi:10.1093/jhered/esm048

- 499 13. Fall T, Johansson Kreuger S, Juberget Å, Bergström A, Hedhammar Å. Gestational  
500 diabetes mellitus in 13 dogs. *J Vet Intern Med.* 2008;22: 1296–1300. doi:10.1111/j.1939-  
501 1676.2008.0199.x
- 502 14. Fall T, Hedhammar A, Wallberg A, Fall N, Ahlgren KM, Hamlin HH, et al. Diabetes  
503 Mellitus in Elkhounds Is Associated with Diestrus and Pregnancy. *J Vet Intern Med.*  
504 2010;24: 1322–1328. doi:10.1111/j.1939-1676.2010.0630.x
- 505 15. Inverardi L, Lanzoni G, Dominguez-Bendala J, Ricordi C. MSCs for Diabetes.  
506 Mesenchymal Stromal Cells. New York, NY: Springer New York; 2013. pp. 571–597.  
507 doi:10.1007/978-1-4614-5711-4\_33
- 508 16. Davison LJ, Weenink SM, Christie MR, Herrtage ME, Catchpole B. Autoantibodies to  
509 GAD65 and IA-2 in canine diabetes mellitus. *Vet Immunol Immunopathol.* 2008;126: 83–  
510 90. doi:10.1016/j.vetimm.2008.06.016
- 511 17. Davison LJ, Herrtage ME, Catchpole B. Autoantibodies to recombinant canine proinsulin  
512 in canine diabetic patients. *Res Vet Sci.* 2011;91: 58–63. doi:10.1016/j.rvsc.2010.08.007
- 513 18. Holder AL, Kennedy LJ, Ollier WER, Catchpole B. Breed differences in development of  
514 anti-insulin antibodies in diabetic dogs and investigation of the role of dog leukocyte  
515 antigen (DLA) genes. *Vet Immunol Immunopathol.* 2015;167: 130–138.  
516 doi:10.1016/j.vetimm.2015.07.014
- 517 19. Catchpole B, Adams JP, Holder AL, Short AD, Ollier WER, Kennedy LJ. Genetics of  
518 canine diabetes mellitus: are the diabetes susceptibility genes identified in humans  
519 involved in breed susceptibility to diabetes mellitus in dogs? *Vet J.* 2013;195: 139–47.  
520 doi:10.1016/j.tvjl.2012.11.013

- 521 20. Warnock GL, Dabbs KD, Cattral MS, Rajotte R V. Improved survival of in vitro cultured  
522 canine islet allografts. *Transplantation*. 1994;57: 17–22. doi:10.1097/00007890-  
523 199401000-00004
- 524 21. Westenfelder C, Gooch A, Hu Z, Ahlstrom J, Zhang P. Durable Control of Autoimmune  
525 Diabetes in Mice Achieved by Intraperitoneal Transplantation of “Neo-Islets,” Three-  
526 Dimensional Aggregates of Allogeneic Islet and “Mesenchymal Stem Cells”. *Stem Cells*  
527 *Transl Med*. 2017;6: 1631–1643. doi:10.1002/sctm.17-0005
- 528 22. Vrabelova D, Adin CA, Kenzig A, Gilor C, Xu F, Buss JL, et al. Evaluation of a high-  
529 yield technique for pancreatic islet isolation from deceased canine donors. *Domest Anim*  
530 *Endocrinol*. 2014;47: 119–126. doi:10.1016/j.domaniend.2013.01.006
- 531 23. Woolcott OO, Bergman RN, Richey JM, Kirkman EL, Harrison LN, Ionut V, et al.  
532 Simplified Method to Isolate Highly Pure Canine Pancreatic Islets. *Pancreas*. 2012;41:  
533 31–38. doi:10.1097/MPA.0b013e318221fd0e
- 534 24. Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, et al. A  
535 population of multipotent CD34-positive adipose stromal cells share pericyte and  
536 mesenchymal surface markers, reside in a periendothelial location, and stabilize  
537 endothelial networks. *Circ Res*. 2008;102: 77–85.  
538 doi:10.1161/CIRCRESAHA.107.159475
- 539 25. Tögel F, Isaac J, Hu Z, Weiss K, Westenfelder C. Renal SDF-1 signals mobilization and  
540 homing of CXCR4-positive cells to the kidney after ischemic injury. *Kidney Int*. 2005;67:  
541 1772–1784. doi:10.1111/j.1523-1755.2005.00275.x
- 542 26. Togel F, Weiss K, Yang Y, Hu Z, Zhang P, Westenfelder C. Vasculotropic, paracrine

- 543 actions of infused mesenchymal stem cells are important to the recovery from acute  
544 kidney injury. *AJP Ren Physiol.* 2007;292: F1626–F1635.  
545 doi:10.1152/ajprenal.00339.2006
- 546 27. Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C. Administered mesenchymal  
547 stem cells protect against ischemic acute renal failure through differentiation-independent  
548 mechanisms. *Am J Physiol Renal Physiol.* 2005;289: F31-42.  
549 doi:10.1152/ajprenal.00007.2005
- 550 28. Pancotto TE, Rossmeis JH. A case of stiff dog syndrome associated with anti-glutamic  
551 acid decarboxylase antibodies. *J Clin Mov Disord.* 2017;4: 5. doi:10.1186/s40734-017-  
552 0053-3
- 553 29. Chen T, Yuan J, Duncanson S, Hibert ML, Kodish BC, Mylavaganam G, et al. Alginate  
554 encapsulant incorporating CXCL12 supports long-term allo- and xenoislet transplantation  
555 without systemic immune suppression. *Am J Transplant.* 2015;15: 618–27.  
556 doi:10.1111/ajt.13049
- 557 30. McCandless EE, Wang Q, Woerner BM, Harper JM, Klein RS. CXCL12 limits  
558 inflammation by localizing mononuclear infiltrates to the perivascular space during  
559 experimental autoimmune encephalomyelitis. *J Immunol.* 2006;177: 8053–64.
- 560 31. Tonne JM, Sakuma T, Deeds MC, Munoz-Gomez M, Barry M a, Kudva YC, et al. Global  
561 gene expression profiling of pancreatic islets in mice during streptozotocin-induced  $\beta$ -cell  
562 damage and pancreatic Glp-1 gene therapy. *Dis Model Mech.* 2013;  
563 doi:10.1242/dmm.012591
- 564 32. Yan X, Cai S, Xiong X, Sun W, Dai X, Chen S, et al. Chemokine receptor CXCR7

- 565 mediates human endothelial progenitor cells survival, angiogenesis, but not proliferation. J  
566 Cell Biochem. 2012;113: 1437–1446. doi:10.1002/jcb.24015
- 567 33. Tögel F, Zhang P, Hu Z, Westenfelder C. VEGF is a mediator of the renoprotective  
568 effects of multipotent marrow stromal cells in acute kidney injury. J Cell Mol Med.  
569 2009;13: 2109–2114. doi:10.1111/j.1582-4934.2008.00641.x
- 570 34. Sakata N, Chan NK, Chrisler J, Obenaus A, Hathout E. Bone marrow cells produce nerve  
571 growth factor and promote angiogenesis around transplanted islets. Bone. 2010;16: 1215–  
572 1220. doi:10.3748/wjg.v16.i10.1215
- 573 35. Gebler A, Zabel O, Seliger B. The immunomodulatory capacity of mesenchymal stem  
574 cells. Trends Mol Med. Elsevier Ltd; 2012;18: 128–34.  
575 doi:10.1016/j.molmed.2011.10.004
- 576 36. Lablanche S, Vantyghem M-C, Kessler L, Wojtuszczyz A, Borot S, Thivolet C, et al.  
577 Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe  
578 hypoglycaemia or poorly controlled glycaemia after kidney transplantation (TRIMECO):  
579 a multicentre, randomised controlled trial. lancet Diabetes Endocrinol. 2018;6: 527–537.  
580 doi:10.1016/S2213-8587(18)30078-0
- 581 37. Dolgin E. Encapsulate this. Nat Publ Gr. Nature Publishing Group; 2014;20: 9–11.  
582 doi:10.1038/nm0114-9
- 583 38. Gregory JM, Kraft G, Scott MF, Neal DW, Farmer B, Smith MS, et al. Insulin Delivery  
584 Into the Peripheral Circulation: A Key Contributor to Hypoglycemia in Type 1 Diabetes.  
585 Diabetes. 2015;64: 3439–3451. doi:10.2337/db15-0071

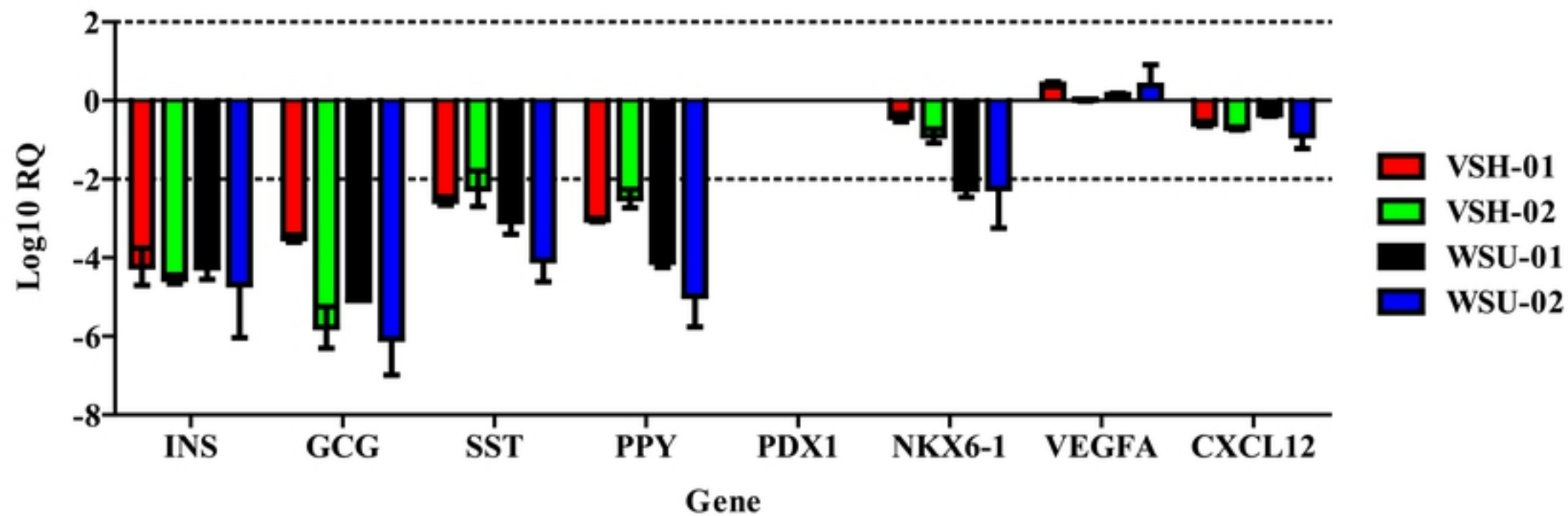
586 39. Pørksen N, Grøfte T, Greisen J, Mengel A, Juhl C, Veldhuis JD, et al. Human insulin  
587 release processes measured by intraportal sampling. *Am J Physiol Endocrinol Metab.*  
588 2002;282: E695-702. doi:10.1152/ajpendo.00516.2000

589

## 590 **Supporting information**

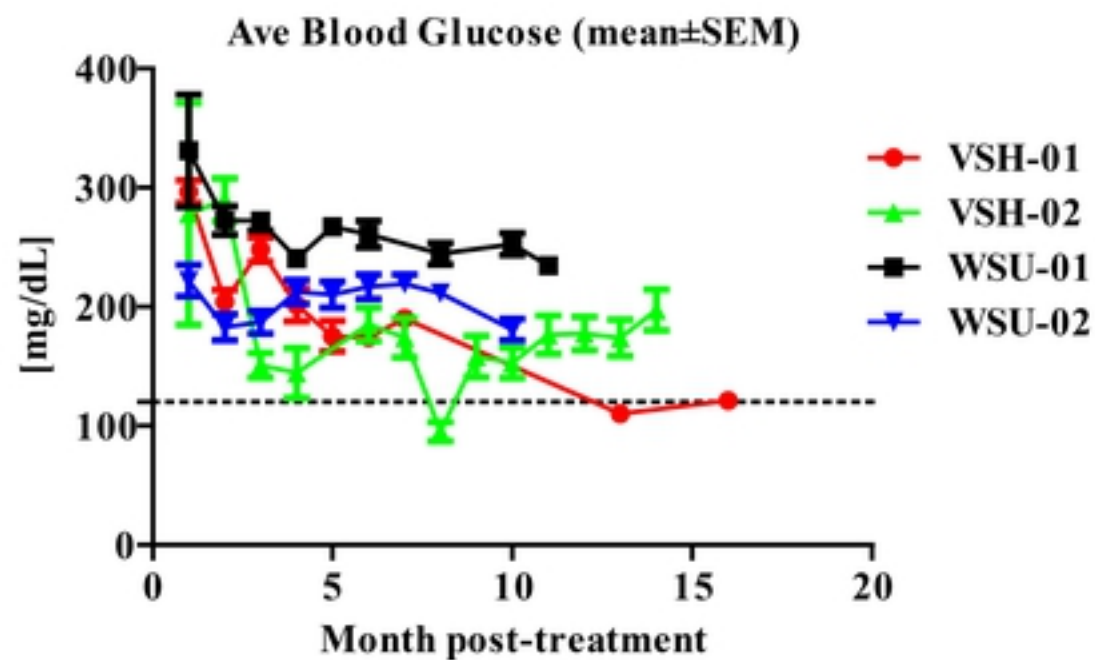
591 **S1 Table. PCR reagents used and their sources.**

592 **S2 Table. Follow-up testing schedule.**

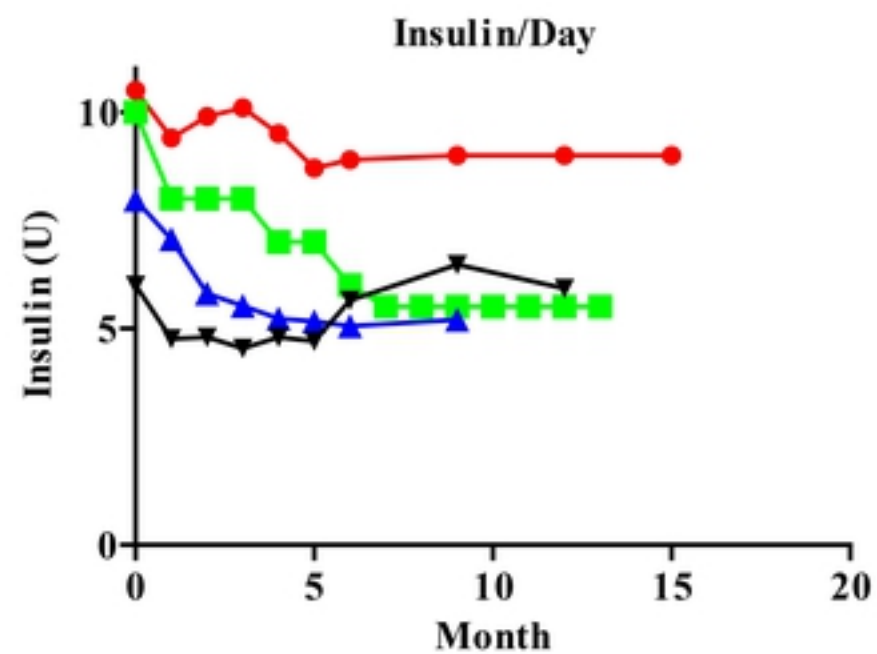


Figure\_1

A



B

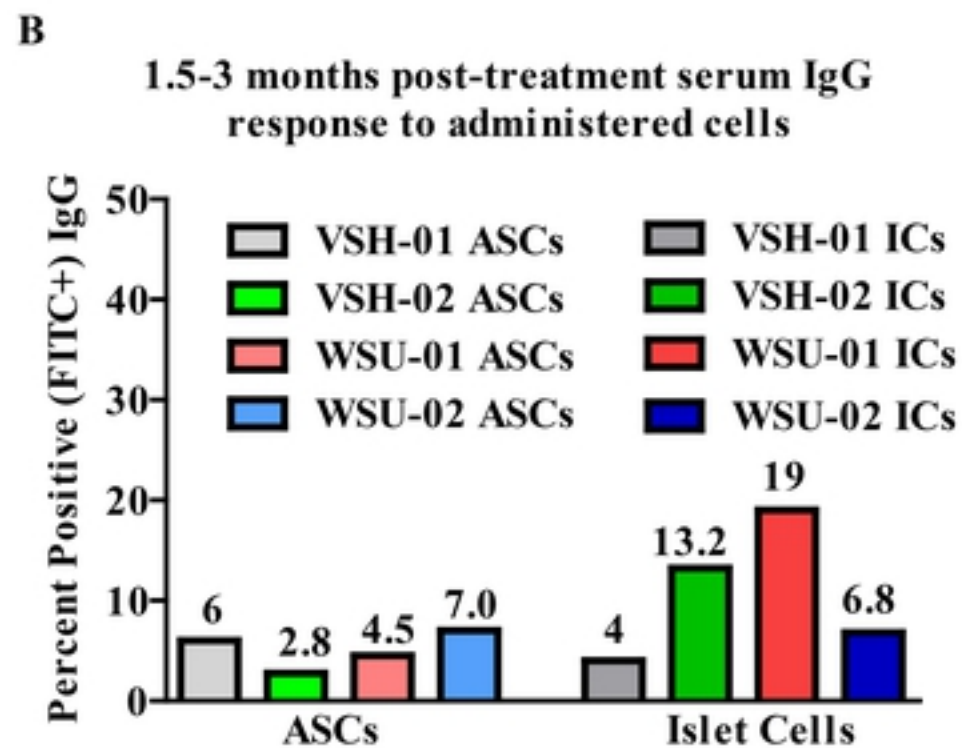
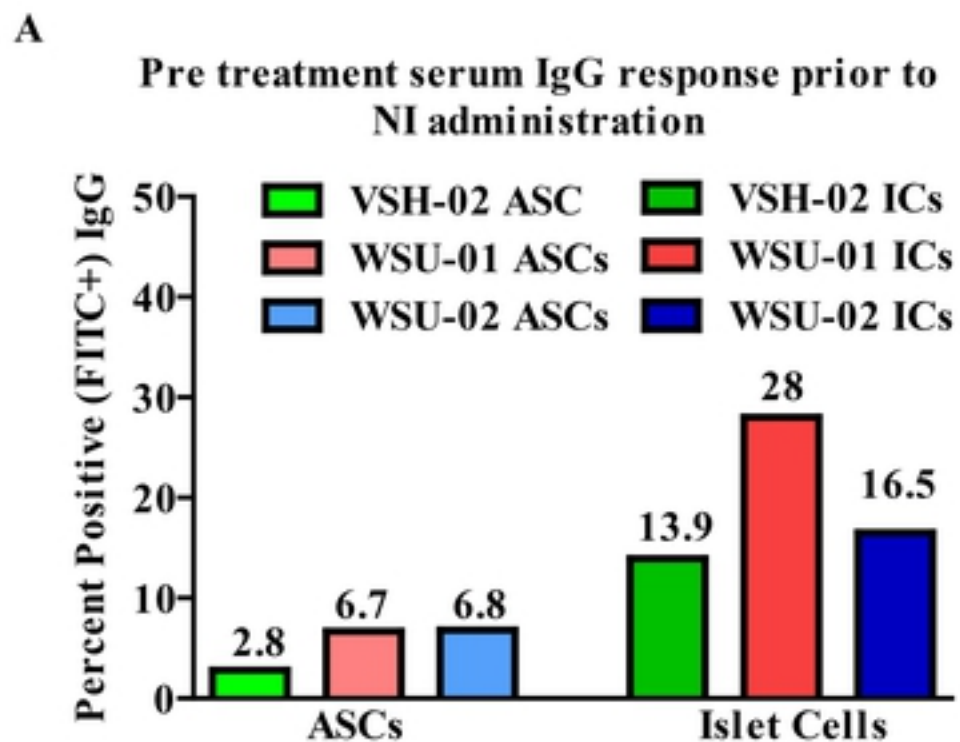


C

Dog	Reduction in daily insulin dose	Reduction in serum glucose
<b>VSH-01</b>	14% (P<0.0001) baseline to 15 months post Rx	106 mg/dL (P<0.0001) to 15 mos.
<b>VSH-02</b>	50% (P<0.0001) baseline to 13 months post Rx	92.5 mg/dL (P=0.03) 13 mos.
<b>WSU-01</b>	20% (P<0.0001) baseline to 6 months, but not after 7 months	96.6 mg/dL (P=0.0015) 12 mos.
<b>WSU-02</b>	37% (P<0.0001) baseline to 9 months post Rx	41 mg/dL (P=0.009) 9 mos.

Figure\_2





**C**

Serum Sample	Antibodies to IA-2 [IU/ml]	Antibodies to GAD65 [IU/ml]
VSH-01 post-treatment	negative	negative
VSH-02 pre-treatment	negative	negative
VSH-02 post-treatment	negative	negative
WSU-01 pre-treatment	12.966	negative
WSU-01 post-treatment	negative	negative
WSU-02 pre-treatment	negative	negative
WSU-02 post-treatment	negative	negative

Figure\_3