Direct reprogramming of non-human primate bile ducts *in vivo* and human pancreactic ductal cells *in vitro* towards a β-cell state

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. James Dutton

December 2014

Acknowledgements

I would like to thank Dr. James Dutton for his time and effort in being my advisor and providing his scientific knowledge to me.

I would also like extend my gratitude to collaborators associated with my research:

Work with non-human primates involved collaboration with Dr. Melanie Graham, Lucas Mutch, and Elizabeth Zolondek.

Work with human pancreatic tissue was in coordination with Dr. Melena Bellin, Josh Wilhelm, and Thomas Gilmore.

Dedication

This thesis is dedicated to those living with the challenge of diabetes everyday.

Abstract

Direct reprogramming of one cell type into another without passing through a pluripotent state can be promoted by expressing transcription factors necessary during embryonic development for the specification of the new cell-type. The transcription factors Pdx1, Ngn3, and MafA when expressed together by an adenoviral vector (Ad-PNM) are able to directly reprogram Sox9+ cells lining the bile ducts of mice into insulin expressing cells that restore glucose homeostasis in diabetic mice. As an extension of this work, in this research project Ad-PNM was administered *in vivo* to the liver of a non-human primate and also to human pancreatic ductal cells *in vitro*.

Table of Contents

List of Tables

List of Figures

Introduction

Studies of the direct reprogramming of somatic cells to an insulin-secreting cell-type will aid preclinical studies and future clinical translation of both *in vivo* and *ex vivo* gene therapy approaches for humans with type 1 diabetes. In 2011, approximately 25.8 million people or 8.3% of the population within the United States, had diabetes[1]. The prevalence of diabetes in the United States continues to grow every year effecting both children and adults. Diabetes is a disease that affects insulin-producing cells, β-cells, which are found in the islets of the pancreas. Insulin is needed for the transport of ingested sugars into cells to be broken down into usable energy for cellular processes. In type 1 diabetes the immune system destroys pancreatic β-cells resulting in a loss of endogenous insulin production and consequently loss of glucose homeostasis. The current standard of care for diabetes is to provide exogenous insulin through injection or an insulin-pump. Close medical care is also needed to successfully control diabetes. In the US depending on insurance coverage, these services and prescriptions come with varying out of pocket costs for patients. In 2012 the total cost for those diagnosed with diabetes in the United States was roughly $245 billion[1].

Adequate medical care and supplies are available in industrialized countries for the proper care and treatment of diabetes. In less developed countries where diabetic care is sparse, a relatively simple and safe gene therapy approach to rescue or improve blood glucose control would make a significant difference for patients. Without proper care, a person with diabetes will develop complications. These include but are not limited to high blood pressure, blindness, kidney disease, neuropathy, amputation, heart disease and stroke[1]. When complications arise the healthcare burden and risk of premature death also increases.

Currently, the only option to reach a semi-curative state is islet transplantation. Most of these are allogeneic transplants from cadaver pancreas but there are also autologous transplants

for pancreatitis patients who have undergone total pancreatectomy. Islets are digested from deceased-donor pancreases (or the patient pancreas in the case of autologous transplant for pancreatitis) and prepared for infusion through the hepatic portal vein of the patient's liver. Infusing through the hepatic portal vein places the islets in an environment rich in oxygen and nutrients by attaching islets to the walls of small veins**.** One infusion is not always adequate to render the patient independent of exogenous insulin. The patient risks requiring repeat transplantation depending on how the islets respond after infusion. In the case of allogeneic transplants another associated risk is life-long immunosuppression to protect the newly introduced islets from the patient's immune system. Allogenic islet transplantation using cadaveric donor islets is limited by the lack of organ donors and is currently only widely available to patients who cannot sense their hypoglycemia[2]. Overcoming the lack of islet supply, by improving the success of islet transplantation such that fewer islets are necessary per patient, or generating alternative sources of insulin producing cells would open this treatment to additional patients.

Insulin producing cells can potentially be made by differentiating embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) along the pancreatic lineage[3,4]. This could potentially provide an unlimited cell source that could then be used for transplantation. Another potential for transplantation is a cell type that is developmentally related to pancreatic progenitor cells that could be directly reprogrammed *in vivo* or *ex vivo* into an insulin-secreting cell. Reprogramming cells involves providing specific transcription factors to stably change the phenotypic identity into a new cell type. When transcription factors in a vector are delivered to the cells of interest transient expression of these genes can occur. This can induce non stable respecification of the cell type by directly activating target genes. True reprogramming occurs when endogenous genes are activated by the vector's transient gene expression and the new cell

phenotype is stable after the exogenous genes are lost.

- Reprogramming is achieved by delivering specific transcription factors into one somatic cell type to turn it into a different cell type.
- A gene delivery vector is used to get the transcription factors into the beginning cell type.
- Transient re-specification of cells may occur when gene expression from the vector is still present within cells.
- Stable reprogramming occurs only when the new phenotype of the cell is maintained by endogenous gene expression and the vector gene expression is no longer present.

For a number of years researchers have been attempting to reprogram cells from the liver, an endoderm derived organ closely related in embryology to the pancreas, into insulin producing cells[5,6]. Understanding the embryonic development of the liver and pancreas provides perspective on the strategies involved with this direct reprogramming.

The liver and pancreas are closely related during embryonic development because they both arise from the foregut endoderm epithelium[7,8]. Patterning signals that result in the ventral pancreatic bud and adjacent liver bud gaining separate identities include fibroblast growth factors and bone morphogenetic protein signaling from the surrounding mesenchyme and occur at e10 of development in the mouse[9]. Since liver and pancreatic cells arise from common progenitors, it has been proposed that the configuration of chromatin in liver cells may remain more accessible to pancreatic transcription factors than less related organs[10]. Both the liver and pancreas also contain Sox9 expressing progenitor cells during organogenesis and Sox9+ cells persist in ductal regions of both mature organs[11]. Sox9+ embryonic pancreatic ductal cells differentiate into both acinar and endocrine cells of the pancreatic lineage[12,13] and Sox9 stimulates proliferation and survival of pancreatic progenitors during development[14]. Sox9 maintains control of the pancreatic progenitor pool through its regulation of the expression of the transcription factor Ngn3 which in

turn regulates cells fate to be ductal or endocrine[15].

It has been proposed that if at the embryonic stage, the multipotent Sox9+ cells can give rise to insulin+ cells, then Sox9+ cells in pancreatic ducts or small bile ducts of the adult liver could potentially change their identity by being exposed to pancreatic transcription factors and become an insulin-secreting cell type. Extensive research has been done to identify specific transcription factors that can reprogram various cell types into a state that secretes insulin.

This use of exogenous transcription factor expression to change the identity of cells is known as direct reprogramming. Perhaps the best example of this is now considered to be induced pluripotent stem (iPS) cell derivation[16] although examples have now been published from many cell types including neurons, cardiomyocytes, hepatocytes and pancreatic cells[17]. In 2008, Zhou, Q., et al. reported the *in vivo* direct reprogramming of pancreatic exocrine cells into insulin-secreting cells. Using a mouse model, this group reprogrammed exocrine cells into β-like-cells that secrete insulin by using a combination of three separate CMV promoter driven adenovirus vectors encoding Pdx1, Ngn3, and MafA (PNM)[18]. The identification of the PNM combination was made after screening transcription factors with specific cell type expression profiles in the embryonic pancreas[19]. Pdx1 is required during pancreas development and later for proper β-cell function. Ngn3's transient expression during development is needed for the formation of endocrine progenitors and MafA is required for the correct expression of insulin in mature β-cells[20–22].

Figure 1. Ad-PNM vector construct. Adapted from[23].

The design of this current study is based on previously published research from the Dutton laboratory describing the *in vivo* and *in vitro* use of Ad-PNM. The conversion of hepatic cells into insulin expressing cells had been pursued by the overexpression of developmentally relevant transcription factors[23–26] culminating in the generation of glucose-responsive, insulin-secreting cells by directly reprogramming hepatic bile duct cells in diabetic mice[23,25]. Using three transcription factors Pdx1, Ngn3 and MafA in a poly-cistronic adenovirus vector (Ad-PNM) allows high levels of simultaneous protein expression (Figure 1). The intravenous injection of Ad-PNM in immune deficient, diabetic NOD SCID mice[23] and more recently in immune competent, diabetic wild-type mice has resulted in long-term remission of diabetes. The extended restoration of blood glucose control in these animals is achieved by the stable conversion of Sox9+ small bile duct cells into insulin-secreting ducts following transient expression of Ad-PNM. The *de novo* insulin-expressing ducts persist after the exogenous Ad-PNM vector has decayed and is considered a permanent *in vivo* direct reprogramming. Although the ductal cells are clearly not β-cells, their high degree of stable reprogramming and ability to function as replacement insulin producing cells makes them a very attractive proposition for curing type 1 diabetes. The next direction for this research is to test the *in vivo* administration of Ad-PNM in a large animal model and the *in vitro* reprogramming efficiency of Ad-PNM using human cells**.**

Two hypotheses were tested: Specific aim 1 is whether portal vein infusion of Ad-PNM in a non-human primate (NHP) can reprogram Sox9+ bile duct cells into insulin-secreting cells to rescue type 1 diabetes in this large animal model. Also, since human bile duct cells and normal liver tissue are not readily available, another human tissue source containing Sox9+ cells was been utilized for the *in vitro* research. Pancreatic ducts are an unused waste tissue after islet isolation procedures and are readily available through the University of Minnesota Medical Center**.** Specific aim 2 is to isolate, culture, and test human Sox9+ pancreatic ductal cells for their

reprogramming capability with administration of Ad-PNM.

Using a type 1 diabetic, large animal model for the infusion of Ad-PNM is attractive since rhesus macaques are more closely related evolutionarily to humans than mice. This model may provide a better understanding of the potential therapeutic effect Ad-PNM in humans. Using a nonhuman primate model is also necessary for the collection of preclinical data for the safety and efficacy of Ad-PNM before clinical trials in humans can be initiated.

Previously the Dutton laboratory had conducted a single test of Ad-PNM administration into the liver of an immunosuppressed type 2 diabetic cynomolgus macaque, with a 50 day post-infusion follow up. Following administration of Ad-PNM by hepatic portal vein catheter, the animal showed no adverse effects and Ad-PNM viral gene expression was detected for at least 9 days post infusion (Figure 2). Genomic DNA analysis indicated the Ad-PNM vector was present for at least 9 days after infusion indicating successful delivery of the Ad-PNM vector to the liver.

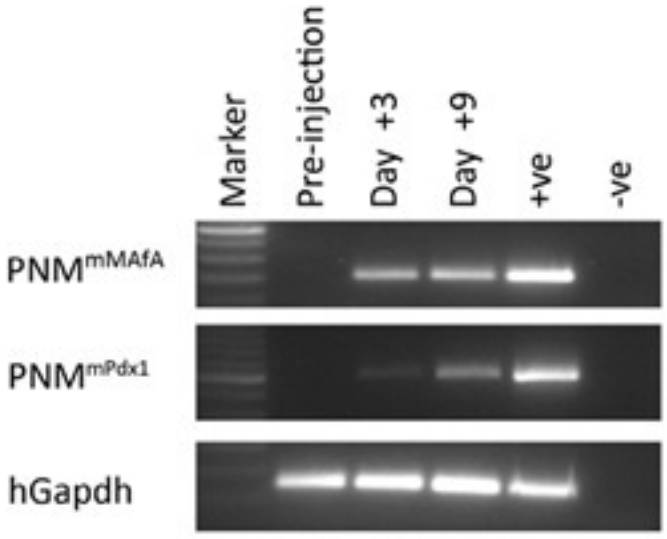

Figure 2. Type 2 diabetic NHP mRNA expression.

The administration of Ad-PNM by infusion through the hepatic portal vein is advantageous to reach the small bile duct cells and is a method in common use for islet transplantation's in humans and NHP's. The hepatic portal vein is unlike other veins in the body, since it does not drain back to the heart. Instead, the hepatic portal vein provides a significant amount of the liver's blood flow along with the hepatic artery. Blood flows from the hepatic portal vein and artery, located at the portal triad of liver lobules, through sinusoids flanking hepatocytes to the central vein, located in the middle of the lobule. It is the central vein that drains deoxygenated blood from the liver and guides it back to the heart. All lobes of the liver are accessed by the branching of the hepatic portal vein[27].

The branching pattern of the hepatic portal vein is mirrored by the bile ducts and the hepatic portal vein supplies oxygenated blood to the bile ducts. The close proximity between the hepatic portal vein and bile duct allows for potentially relatively direct administration of Ad-PNM through a hepatic portal vein catheter to transduce Sox9+ small bile duct cells.

Previous studies have been conducted with NHP hepatic artery or portal vein infusion of adenoviral vectors to test the safety and efficacy of gene delivery. Safety and efficacy were tested with adenoviral infusion into NHP liver during a dose response trial. Liver function was monitored with AST and ALT assays as well as tissue sectioning the liver to observe any detectable morphological damage. The "maximum tolerated dose of an adenovirus vector with significant gene transfer" in rhesus macaques was 5 x 10^12 particles/kg[28]. How the adenoviral vector would spread throughout the NHP body was a concern so biodistribution assays were conducted where DNA was collected from the blood, bone marrow, colon, duodenum, heart, liver, kidney, lung, prostate, spleen lymph node, and testes tissues to test for adenovirus incorporation. Six hours after vector administration the different tissues were harvested and analyzed to measure the number of vector copies per microgram of chromosomal DNA. High

levels of vector copies were detected in the spleen and bone marrow but the highest level was in the liver. Safety was assessed through cytokine analysis and monitoring of AST and ALT levels of the liver[29]. Other groups were especially concerned with the possibility of the adenoviral vector disseminating to the gonads and expressing genes in germ cells[30].

The idea to utilize pancreatic ductal cells as a cell source to reprogram into β-cells is not new. *In vivo* studies in mice have revealed β-cell neogenesis can arise from ductal cells after pancreatic injury. Partial duct ligation (PDL) in adult mice activated Ngn3+ progenitor cells that can differentiate into β-cells *ex vivo* and *in vivo*[31].

Another group of researchers used a different pancreatic injury model to observe which cells contributed to β-cell regeneration. To selectively ablate tissues, mice expressing diphtheria toxin receptor (DTR) at the Rosa26 locus were made to express Cre in the acinar and endocrine tissues of the pancreas. Upon exposure to diphtheria toxin, acinar and endocrine cells were killed leaving the pancreatic ductal tree intact. The surviving cells in the ducts contributed to the regeneration of the endocrine and acinar cells by recapitulating embryonic pancreatic development[32].

After β-cell regeneration was observed after injury to the pancreas, studies utilizing genetic manipulation in mice were performed to understand this phenomena. Lineage tracing assays labeling pancreatic duct cells demonstrated the developmental relatedness between endocrine and ductal tissues. Precursor cells of acinar, duct, and endocrine lineages are Hnf1β+ found in the trunk compartment of the early branching pancreas. When the embryonic duct epithelium is formed the Hnf1β+ cells give rise to ductal and endocrine lineages but not acinar. The Hnf1β+ cells fate is further restricted by the end of gestation since they do not significantly contribute to acinar or endocrine cell populations during neonatal growth nor during the regenerative setting following injury to the pancreas[33]. Solar et al. suggests a restricted plasticity once the ductal

epithelium differentiates.

Controversy has arisen over whether adult pancreatic ducts having facultative β-cell progenitors or not. Many researchers rely on Ngn3 expression as evidence of β-cell neogenesis in adult mice since it is a key determinant of endocrine neogenesis in the pancreas during embryogenesis[34]. However, recent reports show Ngn3 expression also occurs in fully-developed β-cells[35] making lineage tracing research using this marker to show beta cell neogenesis from duct cells less certain.

During human islet transplantation the tissue remaining after islet isolation consists of mainly connective tissue and pancreatic ducts with residual exocrine tissue and islets that were not reclaimed. In immunohistochemical analysis of human donor pancreata it was observed that β-cells with a diameter less than 20μm were located in or along pancreatic ducts. It appeared as though they budded from the ducts[36]. This observation of β-cell neogenesis from the ducts is similar to the studies in mice.

Numerous groups have been utilizing the tissue left at the end of human islet isolation for pancreatic duct research. Isolating the ductal cells from this tissue has been accomplished by using FACS, MACS or both to sort for CK19+ or CD133+ cells[37–39]. Others have utilized culture conditions to isolate, expand, and promote insulin expression in ductal cells[38,40]. Some groups have expanded the ductal cell population using different extracellular matrices and then reprogrammed the cells with adenoviral vectors encoding transcription factors favorable for transition to a pancreactic endocrine lineage[39,41]. Lee et al employed Neurog3, Pdx1, MafA, and Pax6 over-expression in duct cells cultured on matrigel with an extended maturation culture period. Swales et al utilized tissue-culture treated plastic plates for culturing human pancreactic ductal cells. Their aim was to transfect the cells with adenoviral vectors encoding Ngn3 and Myt1 and then profile the mRNA transcripts for endocrine genes. All of these studies reported cell

populations with various levels of insulin secretion and glucose responsiveness.

It is estimated the human pancreas has between one and two million islets. However, it is difficult to isolate adequate quantities consistently to use for clinical transplantation[42]. This current research using human pancreatic ductal cells was undertaken to develop a clinically translational autologous cell product for chronic pancreatitis patients who have undergone total pancreatectomy with islet autotransplant (TP-IAT) but will need β-cell replacement when their autologous islet transplant fails. This work was performed in collaboration with Dr. Melena Bellin, a pediatric endocrinologist at the University of Minnesota's Medical Center. Dr Bellin and her team generously supplied samples and scientific knowledge to this research. Total pancreatectomy (TP) is performed for patients with chronic pancreatitis to relieve pain and is followed by islet autotransplant (IAT) to prevent or minimize post-surgical diabetes by preserving β-cell function[43].

Banking pancreatic ductal cells would be beneficial for patients undergoing TP-IAT because this would save an autologous cell source for potential future use. Once direct reprogramming of pancreatic ductal cells into a β-cell state is achieved with high efficiency, patients who develop exogenous insulin dependence could receive another autologous transplant of insulin-secreting cells. Work on expanding and reprogramming ductal cells from allogeneic donor pancreas to a β-cell-like phenotype would also provide an additional source of insulin expressing cells for transplantation not only for pancreatitis patients but also those living with type 1 diabetes.

Methods

Purification of Ad-PNM

The Ad-PNM vector is amplified in an Ad-293 cells and must to be purified after amplification. Purification began by thawing the -80°C cell/media mixture within 50ml conical tubes at room temperature for 1 hour then in a 37°C water bath until all ice crystals disappeared. The tubes were then subjected to six rounds of thaw, vortex, and freeze in a dry-ice, ethanol bath to lyse the cells and release the Ad-PNM virus particles. The cell/virus suspension was then centrifuged at 3000rpm for 45 minutes to separate the cell debris from the adenovirus that remains in the supernatant. The supernatant containing the virus was poured into a vacuum filter unit. The flow-through was collected into a new 50ml conical tube and kept on wet ice while Cesium Chloride (CsCl) gradients were prepared.

The gradient consisted of 6mL 4M CsCl at the bottom, 6mL 2.2M CsCl in the middle, and about 25mL of the viral suspension on the top in a 38.5mL open-top polyclear centrifuge tube. The CsCl gradients with viral suspensions were centrifuged at 27,000rpm at 4°C overnight. A white band containing the Ad-PNM was observed in the middle of the tube. The top yellow and pink supernatants were aspirated and the white bands were removed with a 5ml pipette and placed into a new 50ml conical tube. An equal amount of saturated CsCl was added to the Ad-PNM suspension and divided equally among new open-top polyclear centrifuge tubes. Next, 4.5mL 4M and 4.5mL 2.2M CsCl were layered on top of 8mL of the saturated CsCl/Ad-PNM mixture to create a gradient in 17mL open-top poly clear centrifuge tube. Before loading the ultra-centrifuge, the tubes were weighed and paired for balancing. Centrifugation at 27,000rpm occurred for 3 hours at 4°C. Two white bands in the middle of the tubes were observed after centrifuging. The lower white band was removed with a 1000 microlitre pipette and placed in a new 15ml conical tube. This stock of Ad-PNM was considered “Hi” titer. The rest of the band

and the second white band was also removed and was considered “Low” titer. The tubes were kept on wet ice until the dialysis components were prepared.

To remove remaining CsCl in suspension in the Ad-PNM stock, the solution was dialysed. Dialysis tubing was prepared by boiling in a 2% sodium bicarbonate solution for 10 minutes, rinsed twice with sterile water, and then boiled in 1mM EDTA solution for 10 minutes, cooled to 4°C and stored in 50% ethanol. The dialysis tubes were rinsed inside and out with sterile water then knots were tied into one end. The Hi and Low titer Ad-PNM were kept separate and pipetted into their respective dialysis tubes and another knot was tied into the other end. Plastic clips were placed on the inner side of the knots. Filled dialysis tubes were kept on wet ice until placed into 1L of PBS 5% glycerol solution with magnetic stir bar in a 4°C cold room. After 1 hour, the tubes were moved into a 2L PBS 5% glycerol solution and kept their overnight. The next day, the tubes were moved into a new 1L PBS 5% glycerol solution and incubated for 1 hour.

The purified Ad-PNM was then removed from the dialysis tubes and stored in aliquots. An insulin syringe was used to poke a hole in the dialysis tubes and remove the Ad-PNM solution. Various volumes were aliquoted into Eppendorf or PCR tubes and kept on wet ice. The tubes were centrifuged, placed on dry-ice to freeze, and stored at -80°C.

Titering of Ad-PNM

After purification of Ad-PNM, the adenovirus was titered using the Adeno-X Rapid Titer Kit[46]. Ad-293 cells were plated at a density of 2.5 x 10^5 cells per well of a 24-well plate in high-glucose DMEM 10% FBS with antibiotics. The next day, Ad-PNM batches were diluted in media from 10^-2 to 10^-6 in duplicate. The media was aspirated from the wells and replaced with 500µl of Ad-PNM dilution. Plates were incubated at 37°C for 48 hours. After two days of Ad-PNM infection, the media was aspirated and the cells were allowed to dry in the BSL2 hood for 5 minutes. The cells were fixed by gently adding 500µl of ice-cold 100% methanol to each well and

incubated at -20°C for 10 minutes. The methanol was aspirated and the wells were rinsed three times with 500µl of PBS + 1% BSA. The final rinse was aspirated then Mouse Anti-Hexon Antibody diluted 1:1000 in PBS + 1% BSA was added to each well and incubated for 1 hour at room temperature. The primary antibody was aspirated and the wells rinsed three times with PBS + 1% BSA. The final rinse was aspirated and Horse Anti-Mouse antibody (HRP conjugate) diluted 1:500 in PBS + 1% BSA was added to each well and incubated for 1 hour at room temperature. During this incubation step, the DAB working solution was made by diluting 10X DAB substrate 1:10 in 1X stable peroxidase buffer and was allowed to come to room temperature. After incubation of the secondary antibody, the wells were aspirated and rinsed three times with PBS + 1% BSA. The final rinse was aspirated then the DAB working solution was added to each well and incubated at room temperature for 10 minutes. The DAB working solution was aspirated and PBS was added to each well. Each virus dilution was visualized at an objective of 20x on an inverted microscope to determine which dilution had 5-50 cells positively stained for the Hexon protein. Pictures were taken of three fields in each well of the dilution that fit the criteria. Counts and calculations were made to determine the titer (infectious units per milliliter) of Ad-PNM.

ifu/ml = (cell/field) x (fields/well) / (volume of Ad-PNM) x (dilution factor)

Functional assay of Ad-PNM

To determine the infection efficiency and function of different batches of Ad-PNM adenovius preparations we used, AR42J-B13 cells (B13s), a rat pancreatic exocrine cell line[44]. Cells were cultured in low-glucose DMEM, 10% FBS at 37°C in 5% CO_2 and the media was replaced every 2 days. For Ad-PNM infection 50,000 B13 cells were plated per 9.6 cm^2 well of a 6-well tissue-culture treated plate Cells were allowed to attach overnight and the media changed before virus infection. A virus dilution was made by adding a 1 µl aliquot of Ad-PNM adenovirus

preparation into 1ml low-glucose DMEM, 10% FBS. 100 µl, 50 µl, and 10 µl of the diluted virus were added to 3 separate wells for the infection of B13 cells overnight. A well of uninfected B13 cells was also plated to use as a negative control. Following the night of infection, the media in the wells was replaced. On the third day after infection, the cells were fixed in preparation for immunohistochemistry. Following antibody detection the cells in at least three fields per well were analyzed at 200x magnification and cells expressing Pdx1 and insulin were counted to calculate the degree of reprogramming.

Preparation of tissue for immunohistochemistry and histology

Whole pieces of tissue were fixed overnight at 4°C in 10% formalin. The formalin was then replaced with a 30% w/v sucrose solution and the tissue was stored in this solution at 4°C until embedding in Optimal Cutting Temperature compound (OCT). OCT blocks were stored at -80°C. 9µm thick tissue sections were cut with a Leica CM3050 S cryostat at -20°C, mounted on Superfrost Plus microscope slides and stored at -80°. Slides were prepared for immunohistochemistry by removing them from -80°C and allowing the slides to return to room temperature. The OCT was washed from the tissue sections by immersing the sides in PBS-T for 5 minutes. Slides were removed from the PBST bath and dabbed on paper towels to remove excess liquid then placed in a dark slide box. If permeablization was required, 100-250 µl of PBS, 1% BSA 1% Tween 20 solution was pipetted onto the slides which were then overlaid with parafilm. for 10 minutes at room temperature. All slides were treated to reduce nonspecific antibody binding with a blocking solution (PBS-T 1% BSA) for 15 minutes, covered with parafilm. Primary antibodies were diluted in blocking solution, pipetted onto the slides, and covered with parafilm to incubate overnight at 4°C. The following day, the slides were washed in a PBS-T bath for 10 minutes, before incubation with secondary antibodies diluted in blocking solution at room temperature for one hour. After incubation with the secondary antibody, the

slides were washed in PBS-T for 10 minutes, before adding DAPI (10mg/ml stock) diluted 1 in 1000 in PBS-T was pipetted on to the slides and incubated at room temperature for 3 minutes. The slides were washed again in PBS-T for 5 minutes, before mounting with Immu-Mount (Thermo Scientific) and a cover-slip. The slides were sealed with clear nail polish painted along the edges of the cover-slip.

Fixing Cells and Immunocytochemistry

Media was removed from cells in culture and the cells fixed for 10 minutes at room temperature by immersion in 10% formalin. The formalin was aspirated and the wells were washed three times over 15 minutes with PBS-T. 2ml of fresh PBS-T was then placed in each well and the plates were stored at 4°C.

Cells were permeablized for 10 minutes with PBS-T 1% BSA 1% Tween 20. Nonspecific antibody binding was reduced by blocking for 30 minutes with a blocking solution (PBS-T 1% BSA). Primary antibodies were diluted in blocking solution and incubated for one hour at room temperature or overnight at 4°C. After incubation, the wells were washed three times with PBST and the secondary antibody diluted in blocking solution was incubated in wells for one hour at room temperature or overnight at 4°C. After incubation the wells were washed three times with PBST and a 1 in 1000 dilution of DAPI (10mg/ml stock) was added to each well.

Table 1. Primary antibodies and dilutions.

Primary Antibody	Dilution
Millipore Anti-Pdx1 rabbit	1:2000
Sigma Anti-Insulin guinea pig	1:250
Millipore Anti-Sox9 rabbit	1:1000
Dako Anti-Cytokeratin 19 mouse	1:500

Table 2. Secondary antibodies and dilutions.

Secondary Antibody	Dilution
Alexa Fluor 488 goat anti-rabbit	1:1000
Alexa Fluor 555 donkey anti-rabbit	1:500
Alexa Fluor 555 donkey anti-mouse	1:500
Alexa Fluor 555 donkey anti-guinea pig	1:500
Rhodamine Concanavalin A	1:100
Alexa Fluor 647 donkey anti-mouse	1:1000

Plaque Forming Units assay

To determine the plaque forming units of the Ad-PNM preparation administered to type 1 diabetic NHP, we performed a PFU assay. Ad-293 cells were thawed and cultured in high-glucose DMEM, 10% FBS with 1x penicillin.streptomycin. Once the cells reached 80% confluency, they were passaged with 0.25% Tyrpsin and replated at a density of 5 x 10^5 cells per well of 6-well tissue culture plates and incubated overnight at 37°C. The next day, Ad-PNM was diluted from 10^{-2} to 10^{-6} in 1 ml volumes of media. Each dilution was carried out in duplicate. The media in the 6-well plates with Ad-293 cells was aspirated and each well was replaced with the different 1 ml Ad-PNM dilutions. Two wells were left with media, no Ad-PNM, as a negative control. To infect the Ad-293 cells with Ad-PNM, the 6-well plates were incubated for 2 hours at 37°C. Meanwhile, an autoclaved solution of 2% agarose in PBS was melted in the microwave, allowed to cool to about 45°C and maintained at this temperature through incubation in a water bath. Media was equilibrated to 37°C. Equal volumes of media and melted agarose were added together and mixed well. The media containing the different dilutions of Ad-PNM was aspirated out of the 6-well plates and 3 ml of agarose/media mix was gently pipetted into each well to overlay the Ad-293 cells. The 6-well plates were tilted to ensure the agarose completely covered the bottom of the wells. The plates were incubated at 37°C[45].

NHP Care

Care for NHP's was provided by the PCRC in accordance with an approved IACUC protocol. Collaboration with Dr. Melanie L. Graham, an expert in diabetes research with non-human primates, and her team allowed for innovative and clinically relevant research[47–49]. Training and surgical procedures to implant the hepatic portal vein catheter occurred at the PCRC under the supervision of Dr. Graham. The induction of diabetes was made through an STZ injection[48]. The PCRC conducted the infusion of Ad-PNM and collection of liver needle biopsies throughout the study. The needle biopsies were used for PCR analysis of the expression of PNM. All laboratory screening panels for the type 1 and 2 diabetic NHP's were collected and analyzed by the PCRC (Appendix).

NHP Liver Biopsy Processing for RNA Extraction

Non-human primate liver biopsies were collected with a 20 gauge needle and transferred into 500µl of Trizol Reagent and immediately frozen at -20°C by staff at the University of Minnesota's Preclinical Research Center (PCRC).The samples were transferred to the Stem Cell Institute, thawed, vortexed, and homogenized with a tissue grinder pestle. The mixture was then centrifuged at 13,000 x g for 1 minute and the supernatant was removed from the remaining particulate pellet and put into a new RNase-free tube for RNA purification.

Cell Processing for RNA Extraction

RNA from cell monolayers was extracted by lysing adherent cells in culture plates with Trizol Reagent through repeated pipetting and mixing within the wells. The cell lysate was then transferred to an RNase-free tube and centrifuged for 1 minute at 13,000 x g. The supernatant was separated from particulates in the bottom of the tube by transfer into a new RNase-free tube.

RNA Extraction

RNA purification involved adding one volume ethanol to one volume of tissue or cell

sample homogenate in Trizol Reagent. The ethanol/Trizol mixture was then loaded into a Zymo-Spin IIC column placed in a collection tube and centrifuged for 1 minute at 13,000 x g to bind the RNA to the column. The flow-through was discarded, and the column was then washed with RNA wash buffer and centrifuged for 30 seconds. Flow-through was discarded and then a DNase I Reaction Mix was added to the column and incubated at room temperature for 15 minutes. Following incubation, the column was centrifuged for 30 seconds and then Direct-zol RNA PreWash was added and centrifuged for 1 minute. Then the RNA wash buffer was added to the column and centrifuged for 1 minute. The RNA in the column was then eluted into a new RNase-free tube by centrifuging 35µl of DNase/RNase-Free water through the column for 1 minute. (Direct-zol RNA MiniPrep). Samples were then analyzed using a NanoDrop Lite Spectrophotometer (Thermo Scientific) to determine concentration of RNA in the sample.

A second DNase treatment was implemented to ensure the samples were DNA-free. In a RNase/DNase-free PCR tube, 2.2ug of purified RNA, DNase, 10x DNase buffer and DEPC water was added and then incubated in a BioRad T100 Thermal cycler at 37°C for 45 minutes. Once the DNase reaction was complete, the tubes were placed in the -80°C freezer and stored until further use.

RNA Quality assay

To test the quality of RNA samples, a bleach ethidium bromide gel was run. A 1.5% agarose 1.15% bleach gel was made with Tris-acetate EDTA (TAE, Sigma Aldrich) buffer. RNA samples were thawed, vortexed, and 2ug of the sample put into a new PCR tube. An equal volume of, denaturing loading dye (Ambion #8546G) was added. The wells of the bleach gel were loaded with the RNA samples.. Gel electrophoresis was run at 100V for 20 minutes.

DNase assay

To ensure previous DNasing steps worked, RNA samples were tested before cDNA

synthesis by using the RNA as a template in a PCR reaction. If no DNA is present the RNA template should not generate a product in this test. All experimental samples as well as positive and negative control reactions were amplified in a Thermocycler for 35 cycles at an annealing temperature of 60°C. Samples were then loaded into wells of a 1.5% agarose gel and run at 110V for 25 minutes. Pictures of the gel were taken with a Gel Logic 212 Pro.

cDNA Synthesis

cDNA was synthesized from RNA samples using SuperScript III First-Strand Synthesis System (Life Technologies). 1µg RNA, random hexamers and dNTPs in a total volume of 13micorlitres were incubated for 5 minutes at 65°C to denature the RNA. Next the cDNA synthesis mix was added to each sample and incubated at room temperature for 10 minutes to anneal the random hexamers to the RNA template. Then, the samples were incubated at 50°C for 50 minutes to enable the Superscript III Reverse Transcriptase to synthesize cDNA. The incubation was terminated at 85°C for 5 minutes and then cooled to 4°C. cDNA samples were stored at -20°C until ready for PCR amplification.

Primer Reconstitution

Primers were purchased from IDT and reconstituted in DEPC water as 50µm stock solutions. The reconstituted primer was heated in a dry bath for 5 minutes at 65°C to ensure the primer was completely resuspended. The primers were then diluted to 10 µm working stock solutions. Stock primers and primer aliquots were stored at -20°C.

Table 3. Primers and sequences.

Gene Name	Sequence, 5'-3'	DNA Bases	Melting Temperature °C	Reference #
mPdx1-Forward	CCG GAC ATC TCC CCA TAC GAA GT	23	60.3	126213555
mPdx1-Reverse	CGC ACA ATC TTG CTC CGG CTC TT	23	62.1	126213548
mMafA-Forward	ATC ATC ACT CTG CCC ACC AT	20	56.2	126213551
mMafA-Reverse	AGT CGG ATG ACC TCC TCC TT	20	57.4	126213552
mNgn3-Forward	CCG GAT GAC GCC AAA CTT ACA	21	57.7	126213553
mNgn3-Reverse	ACA CCA GTG CTC CCG GGA G	19	62.2	126213554
hGapdh-Forward	CCA AAA GGG TCA TCA TCT CTG C	22	55.9	126213546
hGapdh-Reverse	ACT CCT TCC ACG ATA CCA AAG T	22	55.8	126213547
hAlbumin-Forward	TGC TTG AAT GTG CTG ATG ACA GGG	24	59.7	126213541
hAlbumin-Reverse	AAG GCA AGT CAG CAG GCA TCT CAT C	25	61.3	126213542
hCk19-Forward	CGA CTA CAG CCA CTA CTA CAC GA	23	57.7	126213543
hCk19-Reverse 159	CTC ATG CGC AGA GCC TGT	18	58.1	126213545
hCK19-Reverse 307	GCC CCT CAG CGT ACT GAT TT	20	57.4	126213544

PCR

PCR amplification was performed using synthesized cDNA as template and primers for the genes of interest in a BioRad TC-100 Thermal Cycler for 30-38 cycles, and an annealing temperature of 60°C. Once PCR amplification was complete, the sample were centrifuged and loaded into a 1.5% agarose, ethidium bromide, 1x TAE gel for electrophoresis at 110V for 25

minutes. A 1kb ladder was also loaded into the gel.

TP-IAT procedure

A description of the TP-IAT procedure can be found in other publications[50,51].The University of Minnesota Molecular and cellular therapeutics GMP facility performed the islet isolation and purification procedure[52,53].

Tissue Digestion

Human pancreas tissue, following the islet isolation, was provided in UW solution[54]for preservation. Tissue processing was then performed in the SCI in a BSL2 hood under sterile conditions. Most of the UW solution was aspirated from the 50ml conical tube and the tissue transferred to a 10cm dish. The sample was visually assessed for any undesired contents such as sutures, staples, blood clots, vessels, and hard fibrotic pieces. Once these contaminates were removed, the tissue was manually cut up with a scalpel and forceps in preparation for tissue digestion. The sample was placed in a 50ml conical tube and 40ml of DMEM 10%FBS added for five minutes remove lower density debris away from the sample. The media was aspirated and 50ml of EGTA dissociation buffer (500µl 10mM HEPES pH 7.4, 250µl 5mM KCl, 250µl 5mM glucose, 250µl gentamicin, 50µl 0.5mM EGTA, and 48mL PBS) prewarmed to 37°C was added to the sample. The EGTA buffer and sample were incubated for 20 minutes in a 37°C water bath. After centrifuging the sample at 1000rpm for 5 minutes and aspirating the EGTA buffer, 50ml of a digestion buffer containing DMEM 5% FBS, 83.3 mg/ml collagenase type II and 66.7 mg/ml hylauronidase was added to the sample. During a 40 minute incubation in a 37°C water bath the digestion buffer was pipetted to help break apart the tissue. 50 units/ml of dispase was then added to the digestion buffer and incubated with pipetting for another 30-60minutes depending on the consistency of the tissue. The sample was then washed three times with 50ml DMEM 10%FBS. To remove large undigested pieces of human pancreas, the sample was passed through a 100 µm

cell strainer and the cell flow through collected. and cultured overnight in an ultra-low attachment 10cm dish with DMEM 10% FBS, 1 x Pen/Strep, 1x Anti-Anti, and units Gentamicin.

Culture Clean-up

The following day the sample is inspected for aggregation under the microscope. If only a single cell suspension exists, the sample was over digested and will be kept in suspension DMEM 10% FBS for an addition night. When aggregates were present procedures for epithelial ball isolation were initiated. The sample in suspension was collected and passed through a 37 μm reversible cell strainer to remove the single cells. The strainer was then flipped into a new 50ml conical tube and the aggregates were back washed into suspension with Keratinocyte serum-free media (KSFM-Life Technologies) and plated in an ultra-low attachment 10cm dish. The aggregates were left in suspension and cultured in KSFM for 7-10 days with occasional straining with a 37 μm reversible cell strainer to remove the single cells or fibroblasts until distinct clear epithelial balls were present.

Adherent culture of Epithelial cell aggregates

Epithelial cell aggregates were cultured on a plate coated in dry collagen type I, (Corning BioCoat Cellware) at 37°C and in 5% CO2 in KSFM media or DMEM + 10% FBS.

Reprogramming with Ad-PNM

BioCoat plates were used the Ad-PNM infection assay. The epithelial aggregates were divided to make two sub-populations. The infected and uninfected groups were then split 20/80 and plated onto BioCoat plates. The 20% populations for infected and uninfected were used for IHC whereas the 80% populations were for RNA extraction.. BioCoat wells for Ad-PNM infection received 1ml of a virus solution made up of 2ml DMEM 10% FBS and a 1.1 μl aliquot of a specific Ad-PNM batch. Uninfected wells received 1ml of DMEM media only. After overnight infection, the wells were aspirated and 2ml of fresh media were added. On the third day

the 20% wells were fixed in preparation for IHC and the 80% wells were used for RNA extraction.

Expansion of Epithelial Balls

Expansion of the epithelial cells was tested by plating the aggregates on BioCoat plates or in collagen type I. BioCoat plates allowed the cell to adhere and form monolayer patches of epithelial cells. Plating in collagen type I involved preparing tissue-culture plates with a layer of a collagen solution made up of 8 parts type I collagen (4 mg/ml):1 part Williams E 10x media:1 part 0.34M NaOH. The plate was then incubated for 30minutes to set the collagen in a gel-like state. The epithelial cells in suspension were then added on top of the collagen and allowed to attach for two days before aspirating the media and overlaying the well with another 8:1:1 collagen type I solution. This collagen sandwich was incubated for at least 30 minutes before 2ml of KSFM media was added. Media changes were made once a week.

Results

Functional assay of Ad-PNM

The different batches of Ad-PNM were tested for their functionality on AR42J B13 cells. The efficiency of each Ad-PNM batch to reprogram B13 cells to express Pdx1 and insulin was visualized by immunocytochemistry. Uninfected B13 cells were used as a negative control (Figure 3A). Figure 3A shows representative results for Ad-PNM batches A2 through A12 for Pdx1, Insulin, and DAPI. The functional efficiency to reprogram B13 cells was quantified by counting the number of Pdx1+ cells, number of Pdx1+/Ins+ cells and the total number of cells (DAPI+) in at least three different fields at a 200x magnification. The mean percentage of Pdx1+ and Pdx1+/Ins+ cells is shown in (Figure 3B). B13 cells with high Pdx1 expression tend not to express insulin as seen in A11 Ad-PNM (Figure 3A) as well as expression percentage (Figure 3B). Insulin seems to be expressed in B13 cells with levels of Pdx1 protein expression such as A8 Ad-PNM in Figure 3A & B.

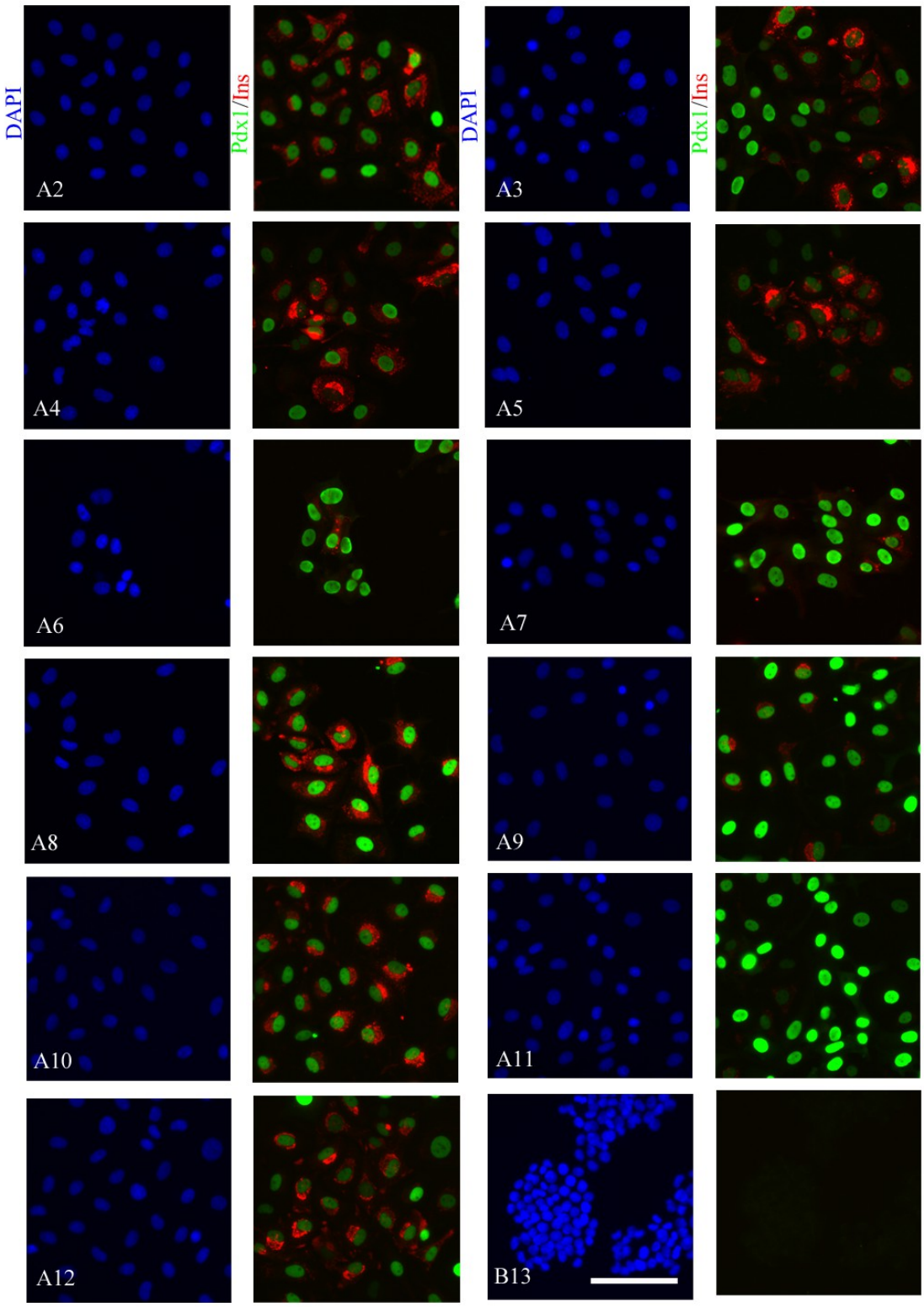
DAPI
Pdx1/Ins
DAPI
Pdx1/Ins
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
B13

Figure 3A. Ad-PNM infection functional assay. Each batch of Ad-PNM (A2-A12) was tested for its functionality by plating 50,000 B13 cells per 9.6 cm^2. Each Ad-PNM was tested in duplicate, incubated for three days and then fixed for immunocytochemistry. Wells of uninfected B13 cells were used as a negative control. All wells were tested for expression of Ins (red) and Pdx1 (green) with DAPI (blue). All pictures were taken with an inverted microscope at 200x, the scale bar is 100 µm.

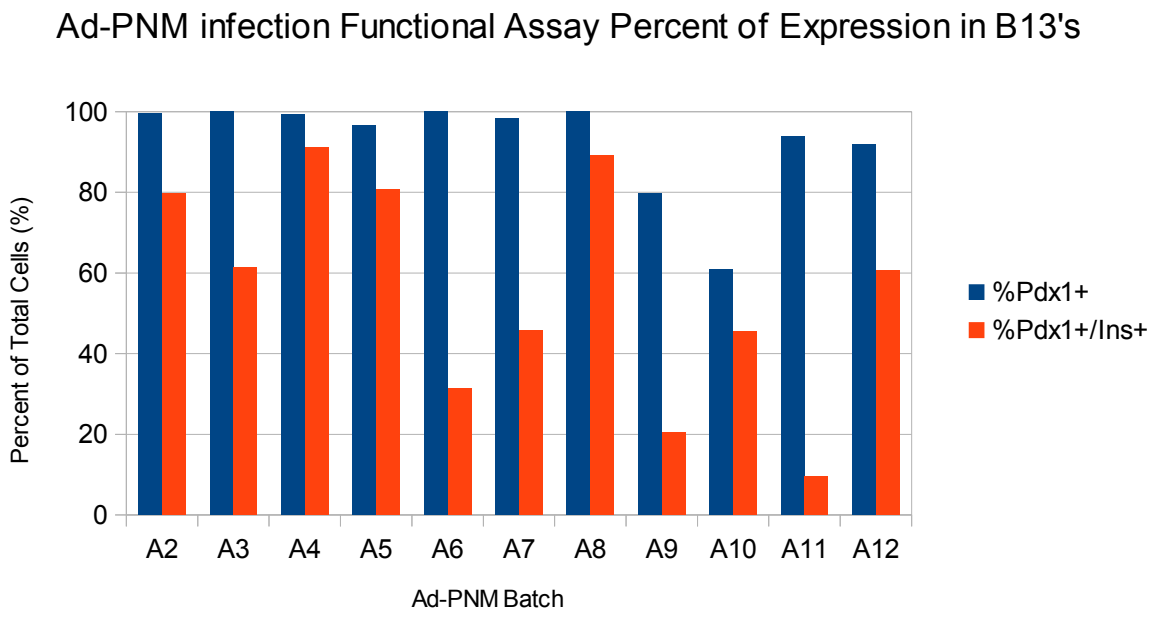

Figure 3B. Percent of B13 cells Pdx1+ and Pdx1+/Ins+ for Ad-PNM batches A2 through A12. At least three fields were counted from 200x magnification similar to those in Figure 3A.

Ad-PNM titer assay

After the purification of Ad-PNM batches, the viruses were titered to determine the number of infectious units per milliliter. The assay titers were calculated for each batch of Ad-PNM (Table 4). Ad-PNM batches with higher titers did not always respecifiy B13 cells into expressing

insulin (A6 Ad-PNM in Table 4, Figures 3A & B).

Table 4. Ad-PNM titers.

Ad-PNM Batch	Titer (ifu/ml)
A2	1.0 x 10^11
A3	1.18 x 10^10
A4	1.2 x 10^11
A5	1.7 x 10^10
A6	1.3 x 10^11
A7	5.9 x 10^10
A8	3.26 x 10^10
A9	8.03 x 10^10
A10	9.59 x 10^9
A11	1.41 x 10^11
A12	1.97 x 10^10
MM	4.14 x 10^10
JI	6.6 x 10^11
JII	3.68 x 10^11
R1	1.0 x 10^12

Functional assay of diluted Ad-PNM

The variation in the amount of Pdx1 and insulin expression in the different batches of Ad-PNM lead us to test dilution assays of the virus batches on B13 cells. A11 Ad-PNM was tested at three different dilutions to see if reprogramming improved with less virus exposure. In Figure 4A immunohistochemistry of cells infected with different titers of A11 Ad-PNM had varied fluorescent intensity of Pdx1 expression. When half the original titer was used to infect B13 cells about the same percentage of cells were Pdx1+ as with the full titer virus (Figure 4B). An increased percentages of Pdx1+ cells also expressed insulin when one-tenth of the original titer of

A11 was used on B13 cells (Figure 4B).

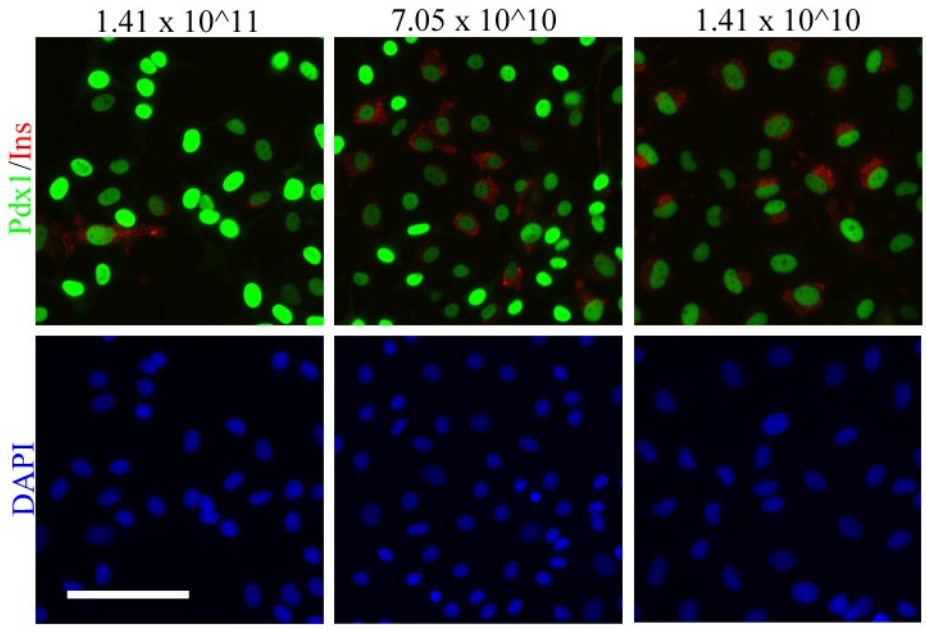

Figure 4A. A11 Ad-PNM infection dilution assay. Dilution assays were performed on the various batches of Ad-PNM to see if there was an optimal titer for reprogramming B13 cells. Scale bar is 100 μm.

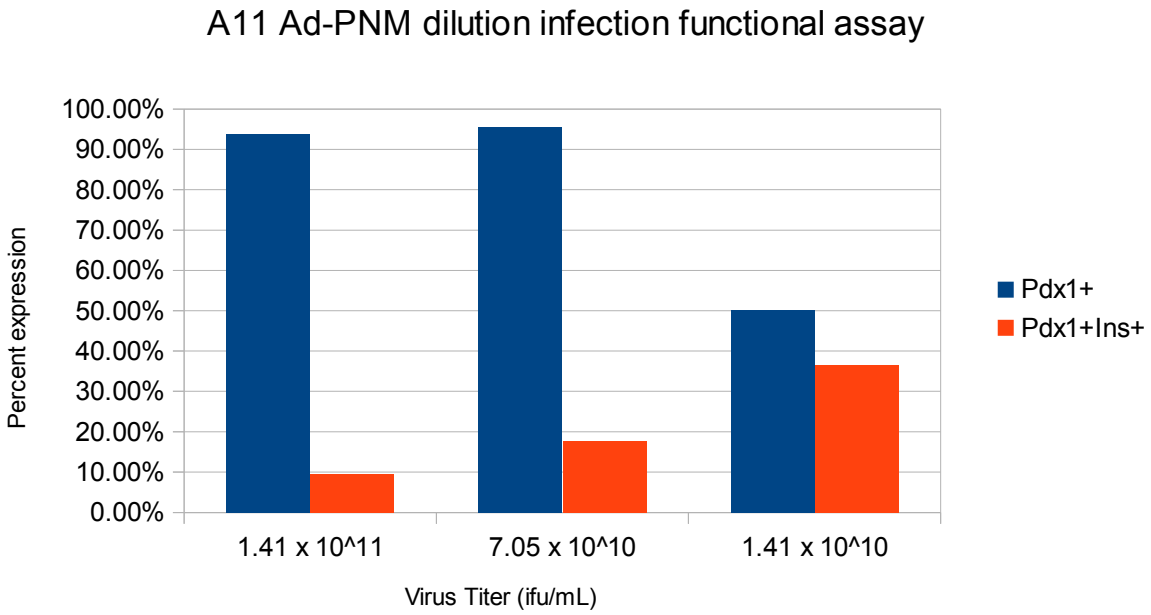

Figure 4B. Percent of B13 cells Pdx1+ and Pdx1+/Ins+ for A11 Ad-PNM dilutions. At least three fields were counted from 200x magnification fields similar to those in Figure 4A.

Functional assay of Ad-PNM for NHP infusion

Based on the Ad-PNM functional assays, combinations of the batches were made to infuse into the portal vein of diabetic NHP's. The Ad-PNM infused in the type 2 diabetic NHP was composed of JI, JII, and A2 and will be referred to as R1. The type 1 diabetic NHP was infused with Ad-PNM made up of A7 and A8 which was named MM (Table 4). Immunohistochemistry of B13 cells infected with R1 revealed relatively low reprogramming efficiency of this batch of Ad-PNM with 24% of cells Pdx1+ (Figure 5 & 6B). A dilution assay was performed with MM on B13 cells. Immunohistochemistry and quantification showed a higher reprogramming efficiency than R1 with 86% of cells Pdx1+ and approximately 70% also positive for insulin expression (Figure 6A & B). The MM Ad-PNM dilution assay revealed Pdx1 and insulin expression

decreased when the infection titer of the virus decreased (Figure 6C).

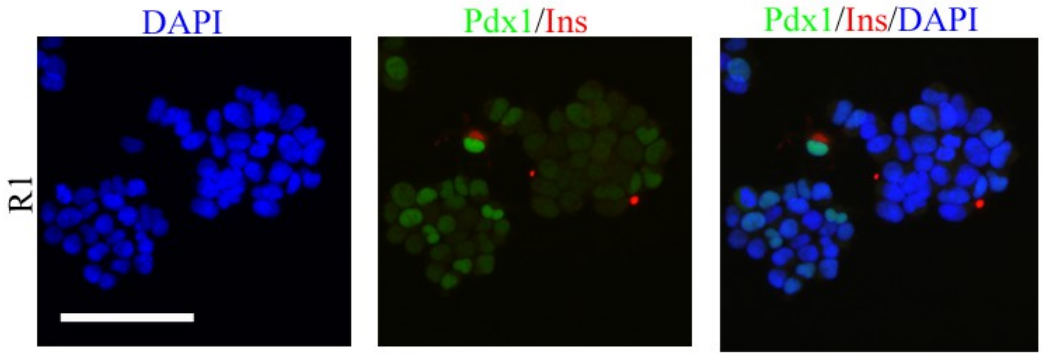

Figure 5. Type 2 diabetic NHP Ad-PNM infection functional assay. To test the infection efficiency of the Ad-PNM batch administered to the type 2 diabetic NHP, B13 cells were plated at a density of 50,000 cells/9.6 cm^2, infected with Ad-PNM and incubated for three days. The cells were fixed and immunocytochemistry was performed to visualize reprogramming events. Scale bar is 100 µm. Insulin (red), Pdx1 (green), DAPI (blue).

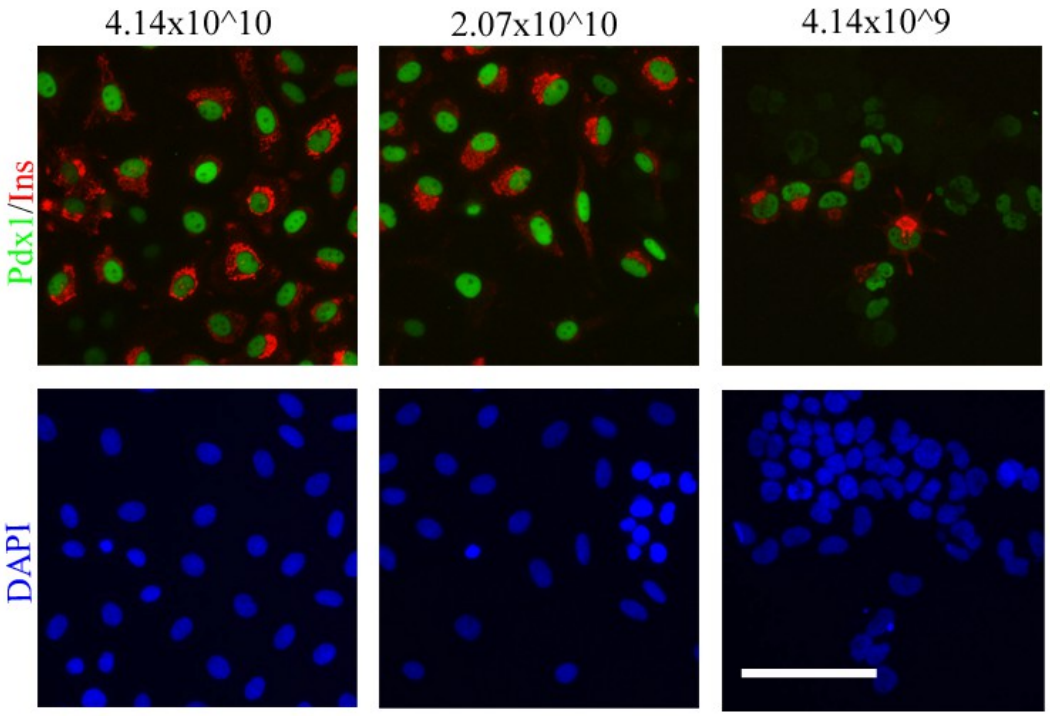

Figure 6A. Type 1 diabetic NHP Ad-PNM infection functional assay. The Ad-PNM batch infused into the type 1 diabetic NHP was tested for functionality at different dilutions. B13 cells were plated in 6-well plates, infected, and incubated for three days before being fixed. Reprogramming events were visualized by immunocytochemistry for Pdx1 (green) and Insulin (red). DAPI (blue) was used for counting cells. Scale bar is 100 µm.

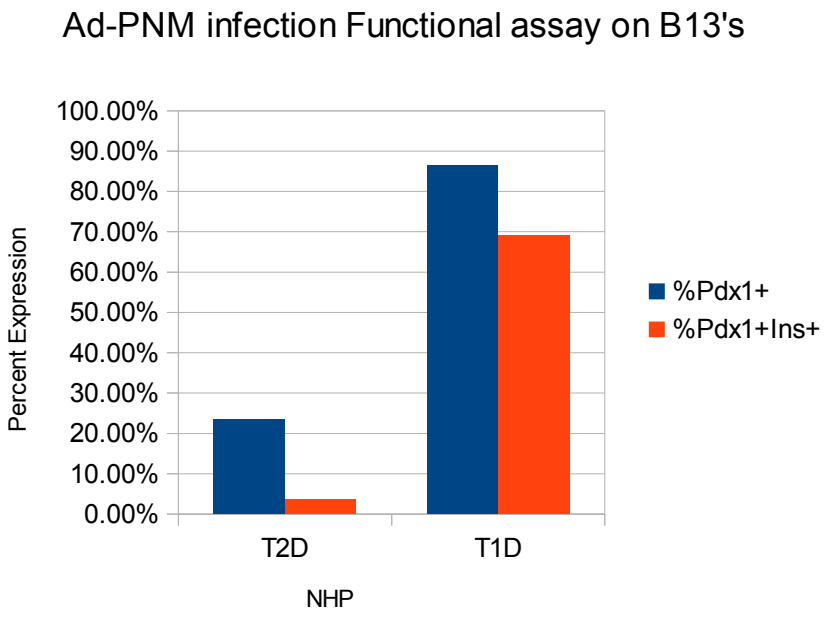

Figure 6B. Percent of B13 cells Pdx1+ and Pdx1+/Ins+ for Ad-PNM batches made for the type 1 and 2 diabetic NHP portal vein infusions. At least three fields were counted from 200x magnification similar to those in Figures 5 & 6A.

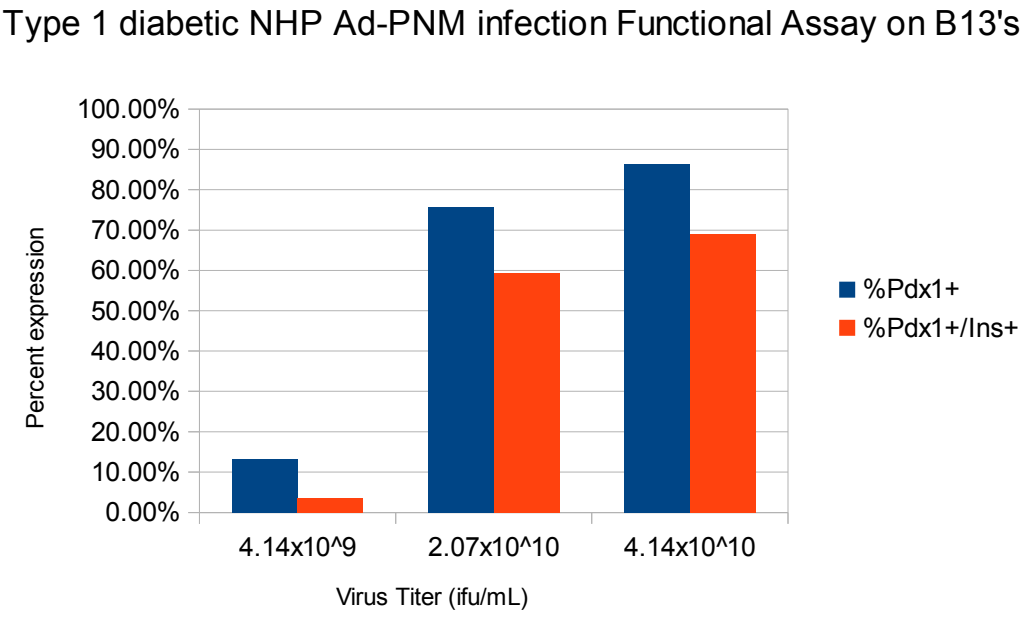

Figure 6C. Percent of B13 cells Pdx1+ and Pdx1+/Ins+ for MM Ad-PNM made for the type 1 diabetic NHP portal vein infusion. At least three fields were counted from 200x magnification similar to those in Figure 6A.

Plaque forming units assay

Multiple attempts were made to conduct a plaque forming units assay to titer the virus in MM Ad-PNM that was infused into the type 1 diabetic NHP. With each attempt, the cultures were contaminated by mold growth before the 12-21 day time point for plaque formation was reached.

Type 2 diabetic NHP Ad-PNM infusion

After analysis of Ad-PNM, it was administered *in vivo* through portal vein catheter to NHP's. The infusion of 5mL of R1 Ad-PNM into the type 2 diabetic NHP, an 18 year old,

cynomolgus macaque weighing 8.5kg was to test the safety and efficacy of Ad-PNM to treat diabetes in a large animal model. Since this NHP was a type 2 diabetic, his islets produced insulin but his muscle and adipose tissue had developed a resistance to insulin leading to a loss of glycemic control. Exogenous insulin was administered to control blood glucose levels that were monitored twice a day (Figure 6D). No adverse effects were observed. Ad-PNM was detected in needle biopsies from the type 2 diabetic NHP liver at three and nine days after infusion (see previous Figure 2). At the time of the type 2 diabetic NHP's necropsy, three one-inch by one-inch liver pieces were obtained. Some of this tissue was prepared for immunocytochemistry and the remaining tissue was digested to isolate bile duct cells.

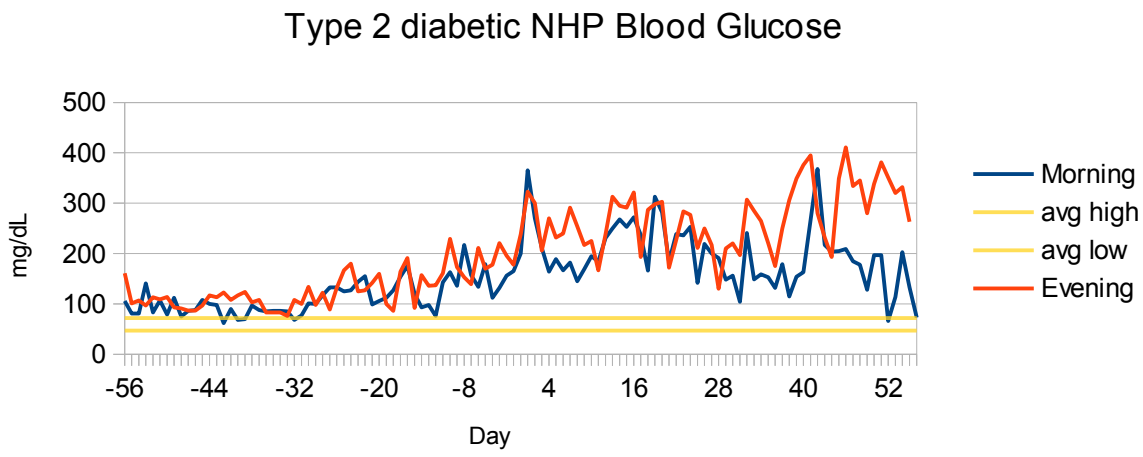

Figure 6D. Type 2 diabetic NHP blood glucose.

<u>*In vitro* NHP bile duct cells</u>

After isolating bile duct cells from the type 2 diabetic NHP liver, three different plating conditions were used. Some of the bile duct cells were plated either in or on type I collagen and kept in culture for 21 days to observe for cell expansion. Both culture conditions were tested for expression of Sox9 protein, a bile duct cell marker. Cells in collagen were passaged (P1) from the

collagen, split 1:2, and plated on tissue-culture treated plastic. One of the P1 in collagen bile duct cell wells was infected with A2 Ad-PNM and kept in culture an additional three days. The other well of P1 in collagen bile duct cells was fixed fro imunnohistochemistry the day after passaging. Bile duct cells on collagen were fixed without passaging. Cells plated in and on collagen had Sox9 expressing cells (Figure 7A). The P1 in collagen bile duct cells infected with A2 Ad-PNM were fixed and tested for Pdx1 expression after three days in culture (Bottom row of Figure 7A). Ad-PNM infected and uninfected NHP bile duct cells cultured in collagen were counted to quantify the percent expression of either Sox9+ or Pdx1+ cells (Figure 7B).

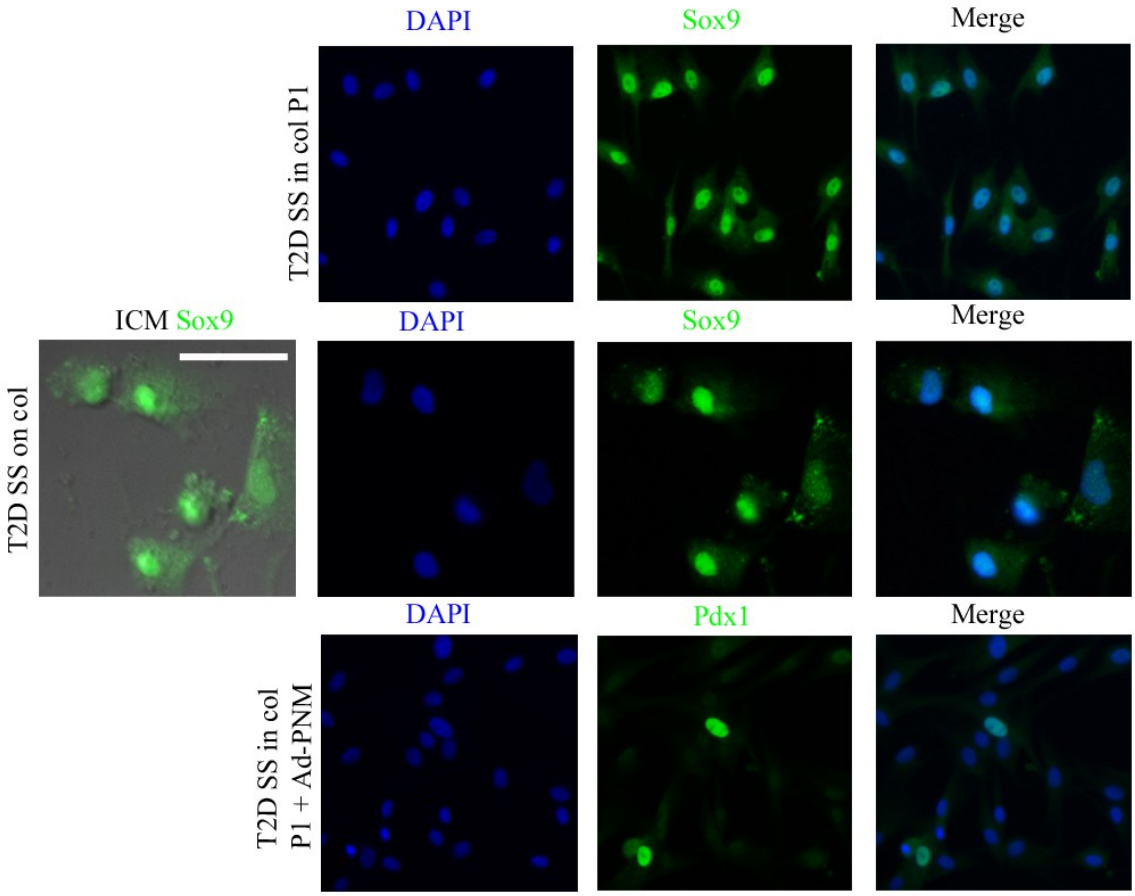

Figure 7A. In vitro type 2 diabetic NHP bile duct cells cultured with type I collagen. Cells

isolated from NHP liver expressed Sox9. These cells also have the capacity to be infected with Ad-PNM in vitro since Pdx1 was expressed in some of the bile duct cells after administration of Ad-PNM. Scale bar 100 µm.

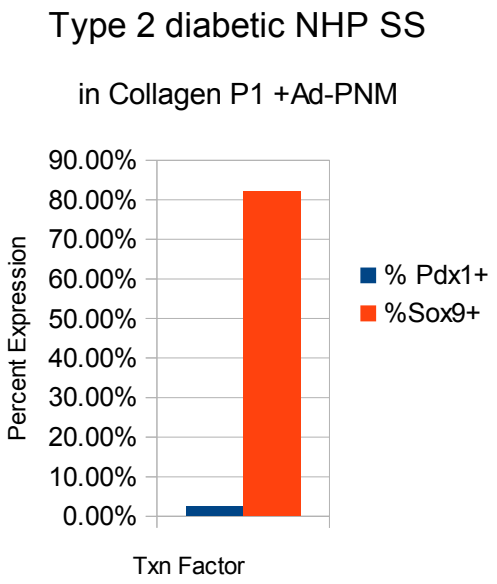

Figure 7B. Percent expression of Pdx1 or Sox9 in NHP bile duct cells after in vitro culture in collagen. At least three 200x magnification fields were counted similar to those in Figure 7A.

Another fraction of the isolated NHP bile duct cells were plated directly on tissue-culture treated plastic plates. This fraction was divided into three sub-populations of cells that were i) uninfected, cultured for four days, ii) infected with A2 Ad-PNM, cultured for four days, and iii) infected with A2 Ad-PNM, cultured for ten days. Each sub-population was fixed and tested for Pdx1 and insulin expression. The uninfected population had no expression of Pdx1 or insulin (data not shown). The infected population cultured for four days had robust Pdx1 and insulin expression (Left column of Figure 8). However, the infected population cultured for ten days had

no insulin expression but retained Pdx1 expression (Right column of Figure 8).

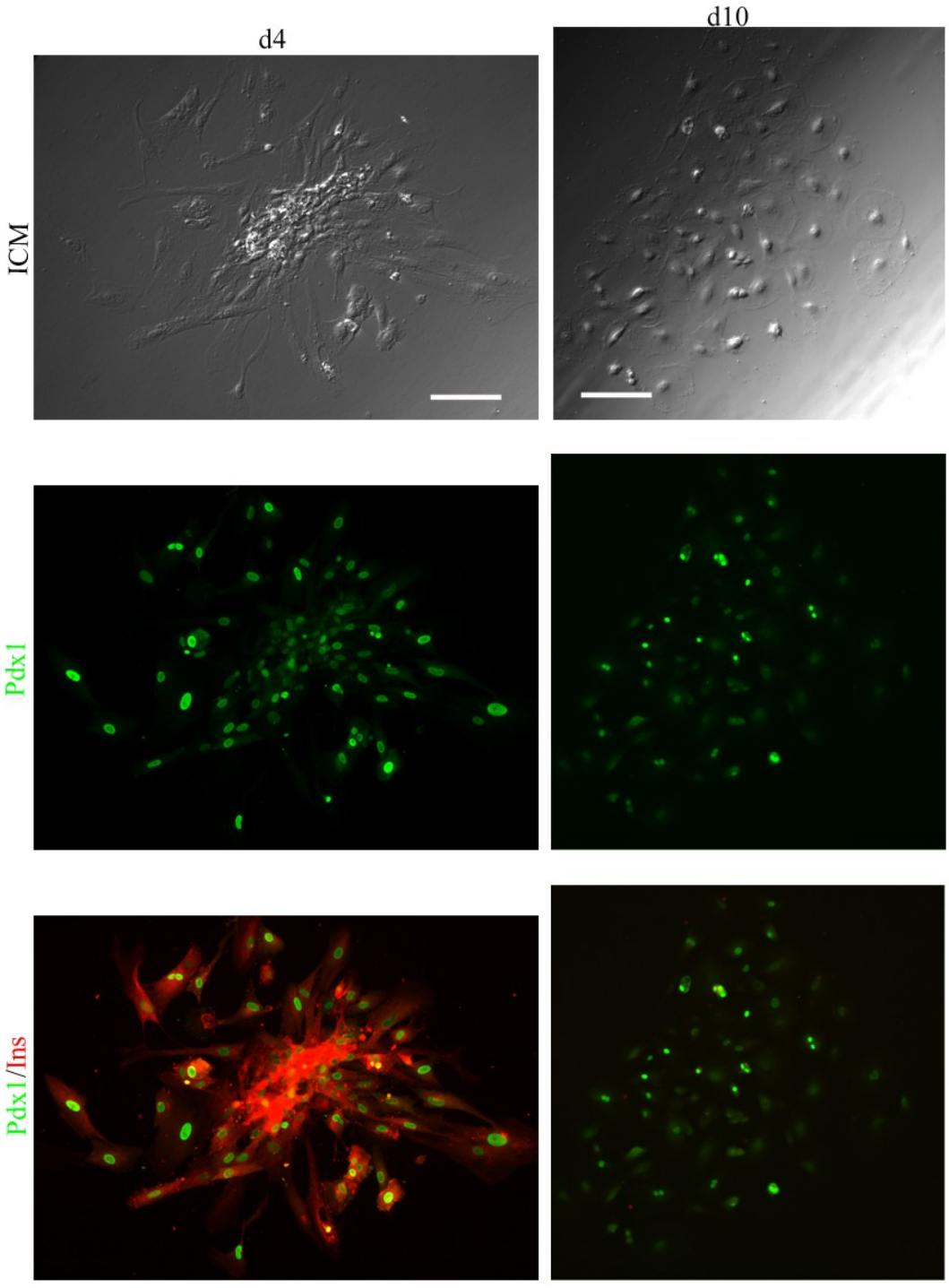

Figure 8. Ad-PNM infection of NHP bile duct cells. Type 2 diabetic NHP bile duct cells infected in vitro with Ad-PNM express insulin and Pdx1 after four days in culture. After 10 days in culture, Pdx1 expression is still evident but no insulin expression is detected. ICM images are provided to give context to cell morphology. Scale bars 100μm.

Type 1 diabetic NHP Ad-PNM infusion

The type 1 diabetic NHP was a 5 year and 8 month old cynomolgus macaque weighing about 5.5kg. To simulate type 1 diabetes in this NHP, STZ had been administered to ablate pancreatic islets and thus cease all endogenous insulin production[48]. When the type 1 diabetic NHP was made exogenous insulin dependent, blood glucose readings were taken in the morning and evening throughout the study (Figure 9A). Seven days prior to MM Ad-PNM infusion, two needle biopsies from Thresh's liver were taken for mRNA expression analysis (Figure 9B). The WT NHP lane was an unrelated, non-diabetic control liver sample and was not expected to have products for Ad-PNM^Pdx1, Ad-PNM^MafA, or human insulin. However, we did expect to see a band for albumin since the mRNA was from liver tissue. No Ad-PNM transcripts or insulin mRNA expression were seen in the day -7 sample from the type 1 diabetic NHP. Gapdh expression was used as a control for the PCR reaction for all samples. Positive (+ve) and negative (-ve) control samples are in the right two lanes. Human islet cDNA was used as a positive control sample for the insulin primer and cDNA from B13 cells infected with Ad-PNM was used as positive controls for the mouse Pdx1 and Mafa primers. No cDNA template was added to the PCR reactions for negative controls (Figure 9B).

On day zero, a total of 6mL of MM Ad-PNM was administered to the type 1 diabetic NHP through his portal vein catheter. In anticipation of insulin production from transiently respecified hepatocytes, the administration of exogenous insulin was reduced. At day 3, two needle biopsies

from Thresh's liver were collected. mRNA was extracted from the biopsies and cDNA prepared to test for Ad-PNM transcripts as well as insulin mRNA expression. The day +3 samples indicated expression of Ad-PNM^Pdx1, Ad-PNM^MafA and insulin. Detection of Ad-PNM transcripts meant the infusion into the liver was successful and the exogenous gene expression had remained in the liver for three days. Detection of insulin mRNA at +3days suggests some cells in the liver were respecified by Ad-PNM and had began to express insulin (Figure 9B). At day 3, an adverse event occurred in which the type 1 diabetic NHP developed ketones (see BETA-HYDROXYBUTYRATE{BHY} pathology: 1.40 mmol/L in Appendix) because endogenous insulin expression from cells reprogrammed by Ad-PNM as well as exogenous insulin administration was not sufficient to stimulate glucose transport into cells. The PCRC addressed the event by increasing exogenous insulin levels and administering intravenous saline. Two needle biopsies of the type 1 diabetic NHP liver were collected on day +10 for mRNA extraction and cDNA preparation to test for Ad-PNM and insulin expression. By this time point no Ad-PNM^Pdx1, Ad-PNM^MafA, or insulin mRNA expression was detected (Figure 9B). Two different reasons could account for the absence of gene expression. First, the needle biopsies may not have collected liver tissue that was exposed to Ad-PNM. Second, expression of Ad-PNM had been transient and cleared from the liver. In conclusion, Ad-PNM was successfully infused into the liver of a NHP and insulin expression was detected. The type 1 diabetic NHP was euthanized 35 days after infusion. Final mRNA expression has not been prepared.

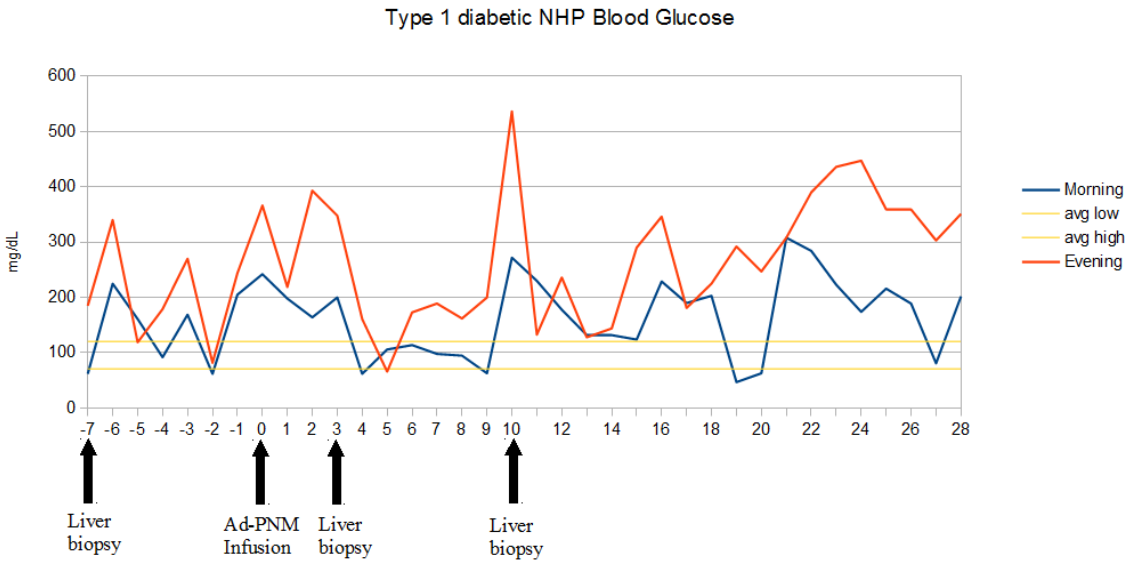

Figure 9A. Type 1 diabetic NHP blood glucose levels. The blue line indicates morning blood glucose levels and the red line evening blood glucose levels. The upper and lower yellow horizontal lines indicate the average range (47-72 mg/dL) of blood glucose numbers for wild type cynomolgus macaques[55].

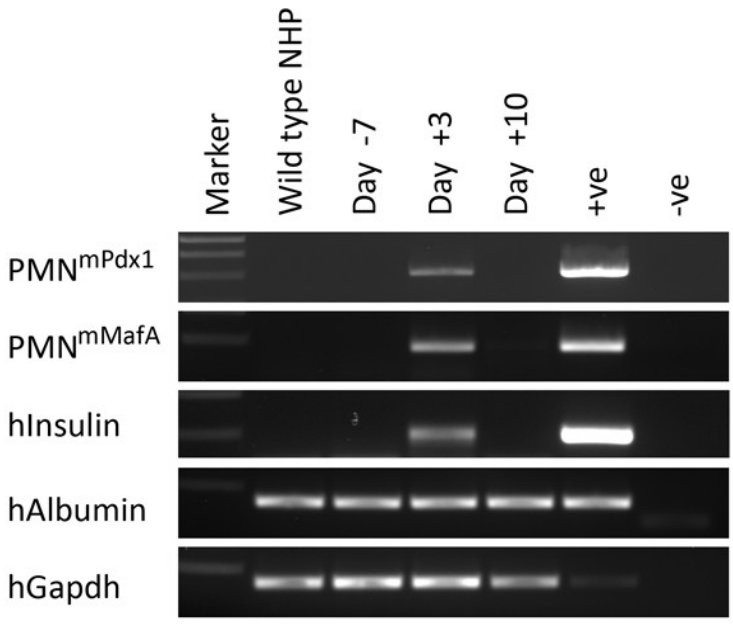

Figure 9B. Type 1 diabetic NHP qualitative cDNA expression.

Identifying ductal cells in the liver and pancreas

Since NHP or human liver samples were not available on a regular basis, human pancreatic ducts were characterized. Frozen sections of NHP liver, human liver, and human pancreas were prepared and tested for Sox9 protein expression. All three tissues have Sox9+ cells lining the ducts (Figure 10). Cytokeratin 19 is an intermediate filament specific to epithelial ductal cells and is co-localized with Sox9+ cells in NHP liver and human liver sections. Rhodamine labeled Concanavalin A (Con A), gave context to the acinar tissue around the human pancreatic ducts stained with Sox9 (Figure 10). A common cell population that is Sox9+ was found in liver bile ducts and pancreas ducts. Human pancreatic tissue was readily available after islet isolation for IAT.

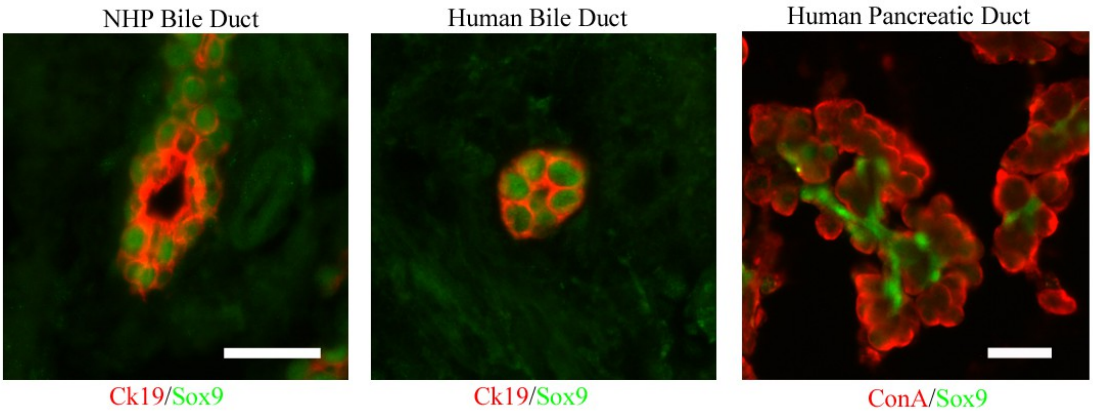

Figure 10. Sections of NHP liver, human liver, and human pancreas showing Sox9+ ductal cells. 50μm scale bars

Following human islet autotransplantation, isolation of pancreatic duct cells from the unused tissue was performed to assess for cell expansion and ability for Ad-PNM reprogramming into an insulin-secreting cell type.

Isolation of human pancreatic ductal cells

Fifteen patients underwent TP-IAT procedures through the University of Minnesota's Medical Center. Following each islet-isolation, pancreas tissue not used for transplant was stored in UW solution at 4°C. When samples arrived in the laboratory, they were visually assessed to remove undesired sutures or fibrotic tissue. Each sample varied in total volume and digestion following islet-isolation (Figure 11). The variation among samples may contribute to the success of epithelial cell isolation (Table 5).

Table 5. Post-islet isolation samples.

Patient ID	Successful Epithelial Ball Isolation
1-6/20/2014	Yes
2-6/30/2014	No
3-7/7/2014	Yes
4-7/11/2014	Yes
5-7/14/2014	No
6-7/18/2014	Yes
7-7/21/2014	Yes
8-8/11/2014	No
9-8/18/2014	Yes
10-8/22/2014	No
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13-9/29/2014	No
14-10/13/2014	Yes
15-10/27/2014	Yes

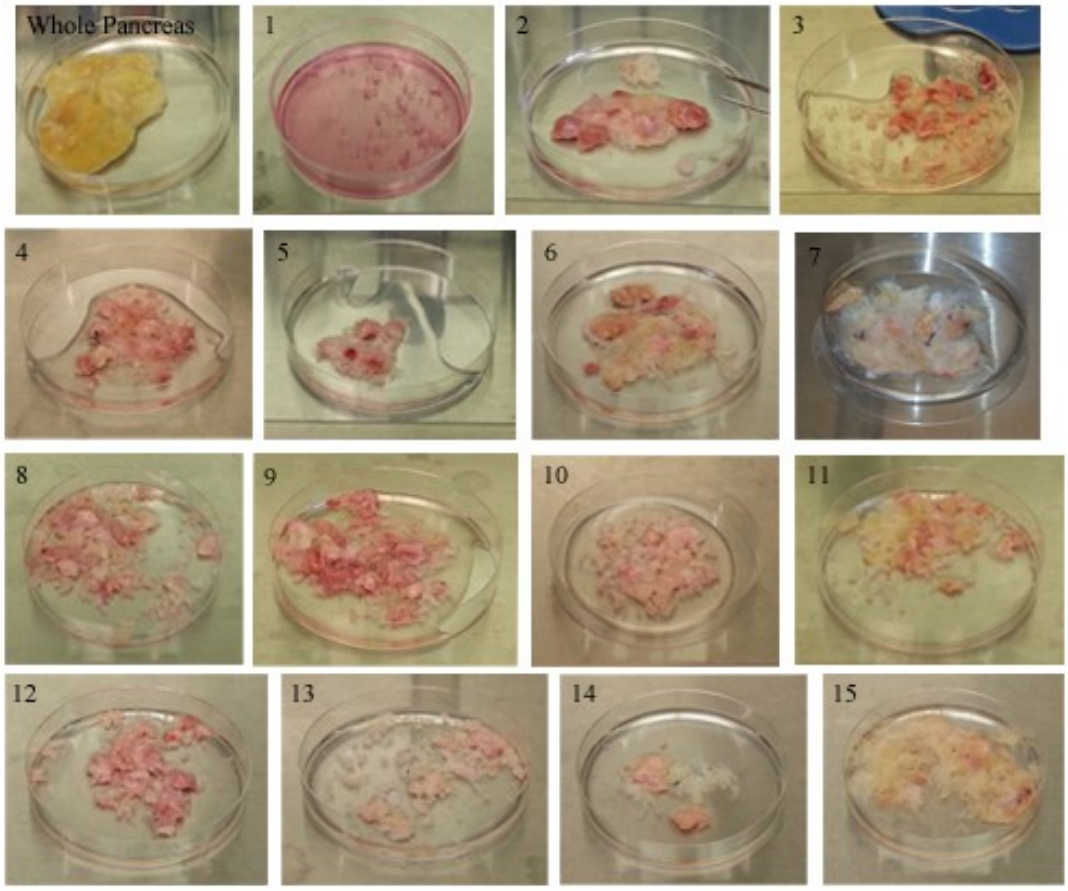

Figure 11. Human pancreatic tissue after islet-isolation. Images of samples 1-15 after TP-IAT in 10cm dishes. Part of a human pancreas not put through an islet-isolation protocol is pictured for comparison.

The digestion and isolation protocol was gradually refined with each sample to the point where clean epithelial cell aggregates were formed (Figure 12). Immunohistochemistry detecting Sox9 and Ck19 in cells in adherent culture indicated that the epithelial cell aggregates contained ductal cells. The co-localization of Sox9 and Ck19 in cells isolated from the first patient is shown in Figure 13.

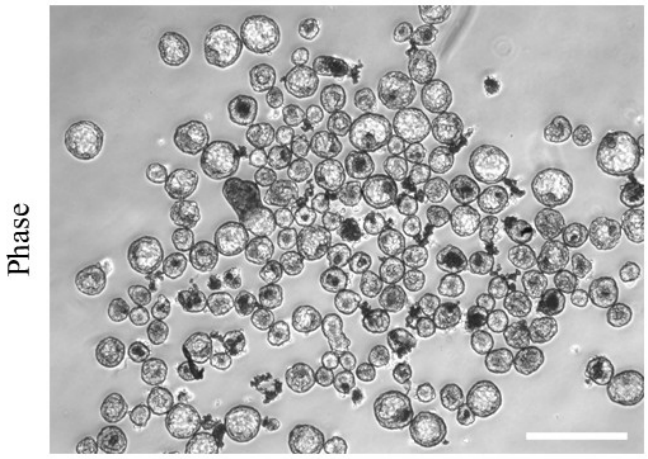

Figure 12. Isolated human pancreatic ductal epithelial cell aggregates. Phase contrast picture of human pancreatic ductal epithelial cells in suspension from the 6th patient. Scale bar is 500μm.

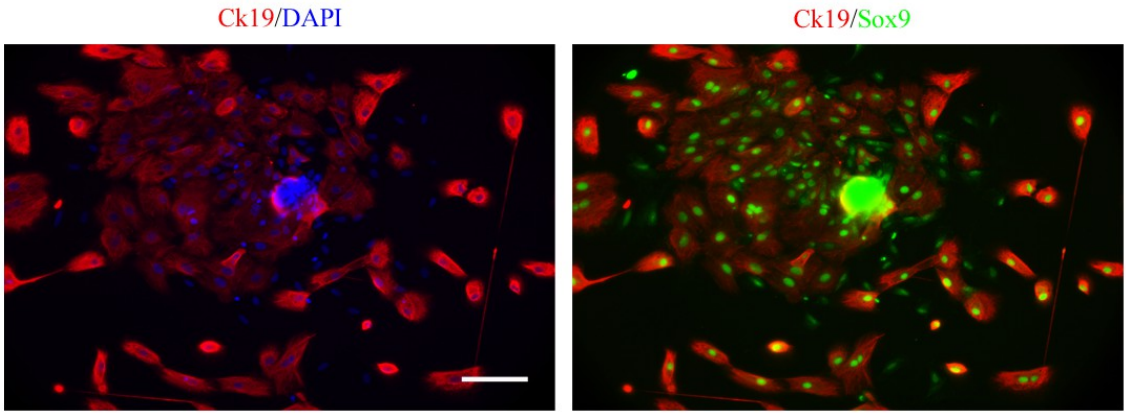

Figure 13. Pancreatic ductal cells from the 1st human patient sample. Epithelial ductal cells were successfully isolated from human post-islet isolation pancreas tissue. Sox9/Ck19 double positive cells were detected. 100μm scale bar.

Reprogramming human pancreatic ductal cells

After confirming the protocol isolated pancreatic ductal cells, the reprogramming of this cell type was attempted. The epithelial cell aggregates were lightly trypsinized, divided into two

sub-populations, and plated on Biocoat plates with collagen coating. One population was infected with A8 Ad-PNM while the other remained uninfected. Some epithelial aggregates and single cells adhered in each sub-population, but the majority failed to adhere to the plate. After four days in culture, attached cells were fixed. The uninfected population had no Pdx1 or insulin expression. The infected population had many Pdx1+ cells (Large picture in Figure 14) and scattered had insulin+ cells (Right inset in Figure 14). The cell type that expressed Pdx1 also expressed Ck19, which suggests Ad-PNM infected ductal cells (Left inset in Figure 14).

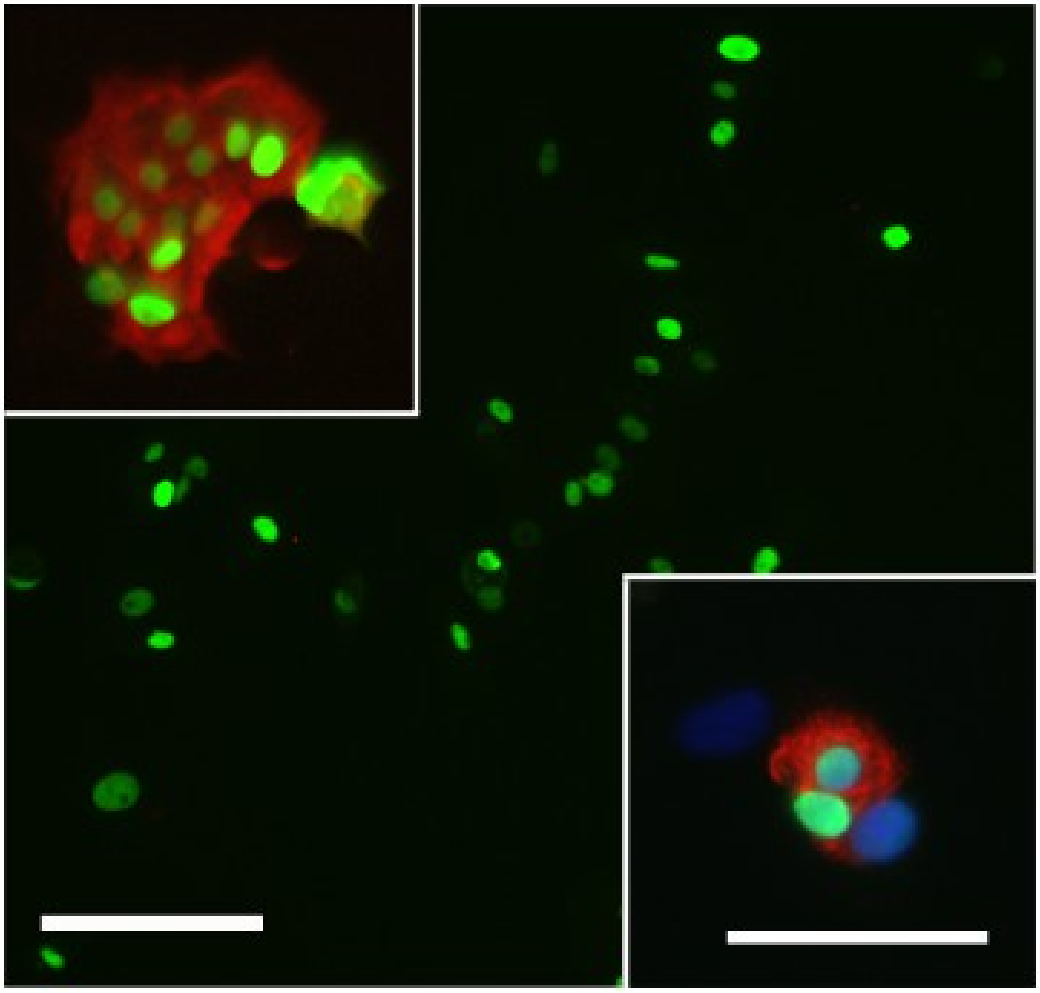

Figure 14. Ad-PNM infection of human pancreatic ductal cells. In the large picture Pdx1+ cells from the fifteenth patient are shown after Ad-PNM infection and four days of culture. Insulin/Pdx1 double positive cells can be seen in the right inset picture. 100μm scale bar in large figure. 50μm scale bar in insets.

Discussion

This research showed Ad-PNM can be delivered safely and effectively in a large NHP animal model. In the experiment with Thresh Ad-PNM transcripts and insulin expression were detected in the liver which was the targeted tissue. It was also shown that Sox9+ cells were found in bile ducts of the liver and ducts in the pancreas. Ad-PNM can infect human pancreatic ductal cells isolated from pancreatic tissue discarded after islet isolation.

The next phase of the work with the NHP large animal model would include portal vein infusion of Ad-PNM and an additional treatment to attempt to enhance reprogramming within the liver. Past research in the Dutton Laboratory has shown a peroxisome-proliferator-activated receptor (PPAR) agonist, WY14643, also known as pirinixic acid, will cause cell proliferation and enhance direct reprogramming in mouse livers when administered through the GI tract two days prior and two days post Ad-PNM infusion through the tail vein[25]. It would be expected that administering WY14643 in a similar manner as the mice, to an STZ treated NHP and infusing a greater volume of Ad-PNM may reprogram more hepatocytes initially but also reprogram bile ducts into stable insulin-secreting structures. The direct reprogramming to insulin-secreting ducts may provide enough insulin expression to be clinically relevant if sufficient C-peptide levels are attained. Another avenue to improve reprogramming would involve searching for more and/or other transcription factors to promote the Sox9+ cells to become more β-cell-like.

Further work on reprogramming human pancreatic ductal cells is necessary for their clinical translation. Improvement in reprogramming with other transcription factors could help as well as optimizing culture conditions. Once reprogramming efficiency was improved, STZ nude mice would be used for transplantation of the human pancreatic ductal cells under the kidney capsule to see if diabetes in a small animal model could be rescued. An expansion phase would also be needed to generate enough cells for reprogramming to produce enough insulin to be

clinically relevant and transplanted in a similar manner as islets or encapsulated and placed in the body. Human pancreatic ductal cells may also be a good source to make iPSCs with the intention of differentiating the pluripotent cells into β-cells with a protocol like the one found in[3]. The epigenetics of the pancreatic ductal cells may retain epigenetic memory from the pancreatic lineage when iPSCs are generated[56] and these iPSCs may differentiate more readily into pancreatic progenitors and ultimately β-cells than iPS cell lines derived from other tissues.

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Appendix

Primate No.: Riley 02AP2
Species: Male Cynomolgus Macaque
Birthdate: 12/01/1995
Protocol No.: 1303A30459
Permanent Identification (Tattoo): 02AP2
Barcode: B000 0000 8266

			Blood Glucose		Insulin / Weight	INSULIN	Weight
			Morning	Evening			
POD	Date	EVENTS	mg/dL	mg/dL	units/kg	units	kg
T2D	10/4/2013	Weight					8.54
T2D	10/8/2013	Weight					8.54
T2D	10/10/2013	Weight					8.48
-56	10/14/2013	Start BG BID	106	161	0.00	0.0	
-55	10/15/2013		81	101	0.00	0.0	
-54	10/16/2013	R VAP(IVC) Removal, L and splenic VAP placement	81	107	0.00	0.0	8.26
-53	10/17/2013		141	97	0.00	0.0	
-52	10/18/2013		83	113	0.00	0.0	
-51	10/19/2013		107	109	0.00	0.0	
-50	10/20/2013		79	114	0.00	0.0	
-49	10/21/2013		112	93	0.00	0.0	
-48	10/22/2013		75	91	0.00	0.0	8.20
-47	10/23/2013		86	87	0.00	0.0	
-46	10/24/2013	MMTT	88	87	0.00	0.0	8.42
-45	10/25/2013		108	97	0.00	0.0	
-44	10/26/2013		100	117	0.00	0.0	
-43	10/27/2013		98	113	0.00	0.0	
-42	10/28/2013		62	123	0.00	0.0	
-41	10/29/2013		90	108	0.00	0.0	8.44
-40	10/30/2013		68	117	0.00	0.0	
-39	10/31/2013		70	124	0.00	0.0	8.42
-38	11/1/2013		97	103	0.00	0.0	
-37	11/2/2013		88	108	0.00	0.0	
-36	11/3/2013		85	83	0.00	0.0	
-35	11/4/2013		86	83	0.00	0.0	
-34	11/5/2013		86	83	0.00	0.0	8.48
-33	11/6/2013		85	76	0.00	0.0	
-32	11/7/2013		68	108	0.00	0.0	8.30
-31	11/8/2013		77	100	0.00	0.0	
-30	11/9/2013		101	134	0.00	0.0	
-29	11/10/2013		100	98	0.00	0.0	
-28	11/11/2013		117	122	0.00	0.0	8.50
-27	11/12/2013		133	89	0.00	0.0	8.57
-26	11/13/2013		133	132	0.00	0.0	
-25	11/14/2013		125	167	0.00	0.0	
-24	11/15/2013		127	180	0.00	0.0	8.54
-23	11/16/2013		144	125	0.00	0.0	
-22	11/17/2013		155	127	0.00	0.0	
-21	11/18/2013		99	142	0.00	0.0	
-20	11/19/2013		106	160	0.00	0.0	8.63
-19	11/20/2013		112	100	0.00	0.0	
-18	11/21/2013	IVGTT K=1.16	127	86	0.00	0.0	8.31
-17	11/22/2013		154	164	0.00	0.0	
-16	11/23/2013		176	191	0.00	0.0	
-15	11/24/2013		120	92	0.00	0.0	
-14	11/25/2013		93	157	0.00	0.0	
-13	11/26/2013		98	136	0.00	0.0	8.64
-12	11/27/2013		76	137	0.00	0.0	
-11	11/28/2013		143	161	0.00	0.0	8.63
-10	11/29/2013		163	229	0.00	0.0	
-9	11/30/2013		136	173	0.00	0.0	
-8	12/1/2013		217	153	0.00	0.0	
-7	12/2/2013		157	139	0.00	0.0	
-6	12/3/2013		134	211	0.00	0.0	8.88
-5	12/4/2013		179	170	0.00	0.0	
-4	12/5/2013		112	178	0.00	0.0	8.71
-3	12/6/2013		132	221	0.00	0.0	
-2	12/7/2013		156	196	0.00	0.0	
-1	12/8/2013		165	178	0.00	0.0	

0	12/9/2013	Infusion	200	238	0.00	0.0	8.42
1	12/10/2013		365	323	0.00	0.0	8.59
2	12/11/2013		270	300	0.00	0.0	8.80
3	12/12/2013	Liver Biopsy	211	206	0.00	0.0	8.61
4	12/13/2013		164	270	0.00	0.0	8.51
5	12/14/2013		189	232	0.00	0.0	
6	12/15/2013		167	240	0.00	0.0	
7	12/16/2013		182	291	0.00	0.0	8.32
8	12/17/2013		145	254	0.00	0.0	
9	12/18/2013	Liver Biopsy	169	217	0.00	0.0	8.39
10	12/19/2013		195	225	0.00	0.0	8.40
11	12/20/2013		184	167	0.00	0.0	8.31
12	12/21/2013		231	238	0.00	0.0	
13	12/22/2013		250	313	0.00	0.0	
14	12/23/2013		268	295	0.00	0.0	
15	12/24/2013		253	291	0.00	0.0	
16	12/25/2013		272	321	0.00	0.0	8.41
17	12/26/2013		237	193	0.00	0.0	
18	12/27/2013		166	287	0.00	0.0	8.11
19	12/28/2013		313	298	0.00	0.0	
20	12/29/2013		282	303	0.00	0.0	
21	12/30/2013	Liver Biopsy	184	172	0.00	0.0	8.31
22	12/31/2013		239	227	0.00	0.0	
23	1/1/2014		236	284	0.00	0.0	
24	1/2/2014		253	277	0.00	0.0	8.39
25	1/3/2014		142	211	0.00	0.0	8.35
26	1/4/2014		219	250	0.00	0.0	
27	1/5/2014		200	217	0.00	0.0	
28	1/6/2014	MMTT	191	130	0.00	0.0	8.47
29	1/7/2014		148	210	0.00	0.0	
30	1/8/2014		156	220	0.00	0.0	8.32
31	1/9/2014		104	197	0.00	0.0	
32	1/10/2014		241	307	0.00	0.0	8.47
33	1/11/2014		149	285	0.00	0.0	
34	1/12/2014		159	265	0.00	0.0	
35	1/13/2014		153	221	0.00	0.0	8.56
36	1/14/2014		132	175	0.00	0.0	
37	1/15/2014		179	250	0.00	0.0	8.55
38	1/16/2014		115	306	0.00	0.0	
39	1/17/2014		154	349	0.00	0.0	8.60
40	1/18/2014		163	376	0.00	0.0	
41	1/19/2014		267	395	0.00	0.0	
42	1/20/2014		368	280	0.00	0.0	
43	1/21/2014		217	232	0.00	0.0	8.54
44	1/22/2014		204	193	0.00	0.0	8.58
45	1/23/2014		205	349	0.00	0.0	
46	1/24/2014		209	411	0.23	2.0	8.53
47	1/25/2014		185	334	0.70	6.0	
48	1/26/2014		178	345	0.82	7.0	
49	1/27/2014		128	280	0.80	7.0	8.75
50	1/28/2014		197	338	0.91	8.0	
51	1/29/2014		197	381	1.03	9.0	
52	1/30/2014		66	351	1.03	9.0	
53	1/31/2014		114	320	1.13	10.0	8.88
54	2/1/2014		203	332	1.13	10.0	
55	2/2/2014		133	263	0.56	5.0	
56	2/3/2014	Euthanasia	73	ND	0.00	0.0	8.52

Primate No.: Riley 02AP2
Species: Male Cynomolgus Macaque
Birthdate: 12/01/1995
Protocol No.: 1303A30459
Permanent Identification (Tattoo): 02AP2
Barcode: B000 0000 8266

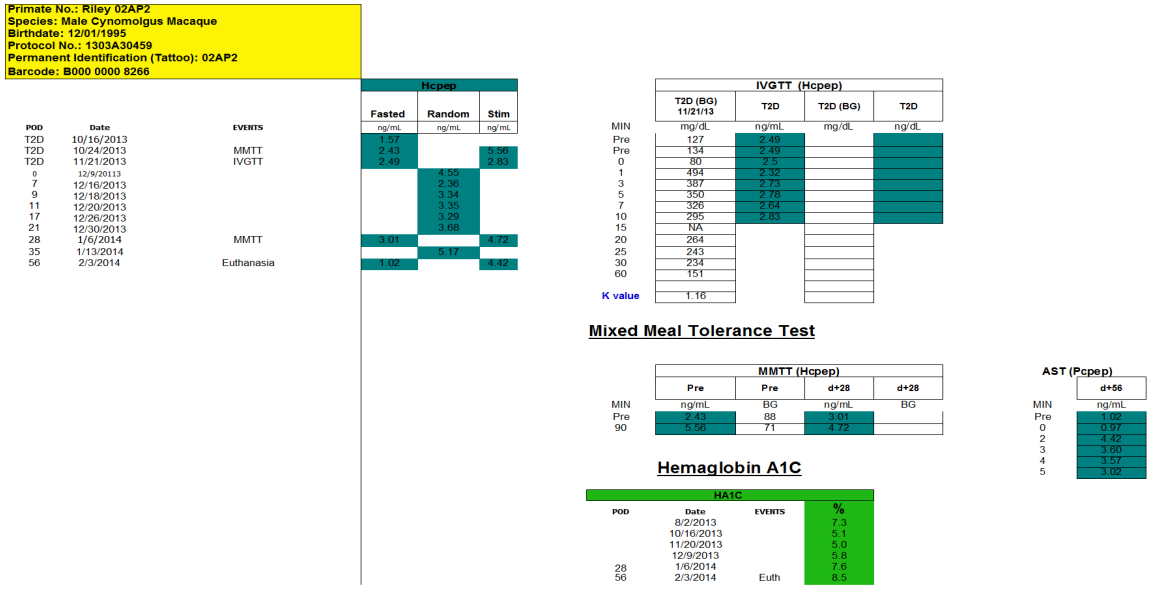
			FK506			FK506/Weight	MMF			MMF/Weight	
			AM	PM	Trough	Dose	AM	PM	Trough	Dose	
POD	Date	EVENTS	mg	mg	ug/L	mg/kg	mg	mg	ug/L	mg/kg	
-7	12/2/2013		5.0	5.0		0.86	0	500		0.02	8.63
-6	12/3/2013		5.0	5.0		0.89	0	500		0.02	8.88
-5	12/4/2013		5.0	5.0		0.89	0	500		0.02	
-4	12/5/2013		5.0	5.0		0.89	0	500		0.00	
-3	12/6/2013		5.0	5.0		0.89	0	500		0.00	
-2	12/7/2013		5.0	5.0		0.89	0	500		0.00	
-1	12/8/2013		5.0	5.0		0.89	0	500		0.00	
0	12/9/2013		5.0	5.0	12.5	0.84	0	500	2.49	0.00	8.42
1	12/10/2013		2.5	2.5		1.68	0	500		0.02	8.59
2	12/11/2013		2.5	2.5		1.72	0	500		0.02	8.80
3	12/12/2013		2.5	2.5		1.76	0	500		0.02	8.61
4	12/13/2013		2.5	2.5		1.72	0	500		0.02	
5	12/14/2013		2.5	2.5		1.72	0	500		0.02	
6	12/15/2013		2.5	2.5		1.72	0	500		0.02	
7	12/16/2013		2.5	2.5	7.2	1.72	0	500	2.83	0.02	
8	12/17/2013		2.5	2.5		0.00	0	500		0.00	
9	12/18/2013		2.5	2.5		0.00	0	500		0.00	
10	12/19/2013		2.5	2.5		0.00	0	500		0.00	
11	12/20/2013						0	500		0.00	
12	12/21/2013						0	500		0.00	
13	12/22/2013						0	500		0.00	
14	12/23/2013						0	500		0.00	
15	12/24/2013						0	500		0.00	
16	12/25/2013						0	500		0.00	
17	12/26/2013						0	500		0.00	
18	12/27/2013						0	500		0.00	
19	12/28/2013						0	500		0.00	
20	12/29/2013						0	500		0.00	
21	12/30/2013				4.8		0	500	2.35	0.00	
22	12/31/2013						0	500		0.00	
23	1/1/2014						0	500		0.00	
24	1/2/2014						0	500		0.00	
25	1/3/2014						0	500		0.00	
26	1/4/2014						0	500		0.00	
27	1/5/2014						0	500		0.00	
28	1/6/2014				<3.0		0	500	8.81	0.00	
29	1/7/2014						0	500		0.00	

Primate No.: Riley 02AP2
Species: Male Cynomolgus Macaque
Birthdate: 12/01/1995
Protocol No.: 1303A30459
Permanent Identification (Tattoo): 02AP2
Barcode: B000 0000 8266

ND=Quality non sufficient

Clinical Pathology Laboratory-UMN

POD	T2D	T2D	0	1	2	7	21	35	56
date	10/16/2013	11/21/2013	12/9/2013	12/10/2013	12/11/2013	12/162013	12/30/2013	1/13/2014	2/3/2014
EVENTS	VAP Placements								Euthanasia
WBC (x10³/µL)	8.5	12.56	7.06	7.57	13.11	15.6	9.02	14.25	12.41
RBC (x10⁶/µL)	7.69	7.17	7.29	7.4	7.63	7.07	6.81	6.67	6.92
HGB (g/dL)	17.1	15.7	15.6	16.2	16.6	15.3	14.7	14.7	14.9
HCT (%)	54.1	51.6	50.4	53.2	53.9	48.7	46.7	48.1	48.2
MCV (fL)	70.3	72	69.1	71.9	70.6	68.9	68.6	72.1	69.7
MCH (pg)	22.2	21.9	21.3	21.9	21.8	21.7	21.5	22	21.5
MCHC (g/dL)	31.5	30.4	30.9	30.5	30.9	31.5	31.4	30.5	30.8
RDW (%)	13.2	13.1	13.3	13.5	13.3	13.6	14.9	15.1	14
Plt (x10³/µL)	179	304	175	210	230	283	227	274	199
MPV (fL)	11.8	11.8	11.5	11.6	11.7	12.2	11.7	11.8	11.2
PCT%	64.1	68	67.9	70.3	73.1	64	68.6	68.2	66.8
PDW%	0.21	0.36	0.2	0.24	0.27	0.34	0.27	0.32	0.22
%BAND	60	42.99	65.01	54.03	52.02	58.97	54.99	36.98	70.99
%NEUT	0	0	0	0	0	0	0	0	0
%LYMPH	32	48.96	32.01	33.95	36	28.97	36.03	48.98	22.97
%MONO	5.06	5.02	0	10.96	9	11.03	5.99	7.02	5
%EOS	2	1.99	1.98	1.06	0.99	0	1	4.98	0.97
%BASO	1.06	1.04	0.99	0	1.98	1.03	2	2.04	0
#NEUT (10³cells/µL)	5.1	5.399544	4.589706	4.090071	6.819822	9.19932	4.960098	5.26965	8.809859
#BAND (10³cells/µL)	0	0	0	0	0	0	0	0	0
#LYMPH (10³cells/µL)	2.72	6.149376	2.259906	2.570015	4.7196	4.51932	3.249906	6.97965	2.850577
#MONO (10³cells/µL)	0.4301	0.630512	0	0.829672	1.1799	1.72068	0.540298	1.00035	0.6205
#EOS (10³cells/µL)	0.17	0.249944	0.139788	0.080242	0.129789	0	0.0902	0.70965	0.120377
#BASO (10³cells/µL)	0.0901	0.130624	0.069894	0	0.259578	0.16068	0.1804	0.2907	0
RBC morphology	Rare polychromasia	Normal RBC morphology	Normal RBC morphology	1+ anisocytosis, rare howell jolly bodies, rare polychromasia, rare disrupted RBCs	Rare polychromasia	Normal RBC morphology	Normal RBC morphology	Normal RBC morphology	Normal RBC morphology
WBC Comment	2+ lobulated lymphocytes	2+ lobulated lymphocytes	1+ lobulated lymphocytes	No Comments	Rare lobulated lymphocytes	Rare lobulated lymphocytes	3+ lobulated lymphocytes	3+ lobulated lymphocytes	1+ lobulated lymphocytes
BUN (mg/dL)	10	14	15	12	12	18	11	17	14
Creat (mg/dL)	1.1	0.8	0.8	0.7	0.7	0.8	0.7	0.8	0.7
Ca (mg/dL)	7	9.4	8.7	9	8.9	9.2	9.4	10.1	9.1
Phos (mg/dL)	4.7	4.4	3.4	15.4	3.7	5	3.7	5	5.9
Mg (mg/dL)	1.5	2	1.2	1.1	1.2	1.3	1.5	1.8	2
TP (g/dL)	4.3	7.5	6.7	6.7	6.3	7.3	6.8	7.2	7.1
Alb (g/dL)	2.1	3.2	3.2	2.9	2.7	3.2	3.2	3.5	3.3
Glob (g/dL, calc)	2.2	4.3	3.5	3.8	3.6	4.1	3.6	3.7	3.8
Na (mmol/L)	148	147	143	135	141	141	143	145	145
Cl (mmol/L)	119	107	106	100	103	102	106	103	107
K (mmol/L)	4.4	3.9	3.6	3.7	3.5	3.4	3.6	3.7	3.1
HCO3 (mmol/L)	23.2	17.5	26.3	20.2	23.9	21.9	27.2	18.3	24.5
Osmol (calc.)	293	294	292	283	293	288	290	294	288
An Gap	10	26	14	19	18	21	13	27	17
T. Bili (mg/dL)	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
ALP (U/L)	113	129	120	148	155	131	133	138	126
GGT (U/L)	31	60	52	46	44	48	54	58	60
ALT (U/L)	83	182	69	61	97	70	95	75	130
AST (U/L)	55	42	14	17	22	20	19	26	33
CK (U/L)	2133	233	135	388	293	179	503	221	326
Gluc (mg/dL)	85	126	201	342	310	183	207	169	71
Chol (mg/dL)	57	127	112	126	178	155	145	173	164
Amy (U/L)	158	179	219	143	118	141	153	150	161
Lip (U/L)	16	15	15	20	23	15	16	12	15
Trig (mg/dL)	88	107	374	695	1072	361	462	460	182
LIH	0 0 0	0 0 0	0 0 0	1 0 1	0 0 1	0 0 0	0 0 0	0 0 0	0 0 1



Primate No.: Riley 02AP2
Species: Male Cynomolgus Macaque
Birthdate: 12/01/1995
Protocol No.: 1303A30459
Permanent Identification (Tattoo): 02AP2
Barcode: B000 0000 8266

POD	Date	EVENTS	Hcpep Fasted ng/mL	Hcpep Random ng/mL	Hcpep Stim ng/mL
T2D	10/16/2013		1.67		
T2D	10/24/2013	MMTT	2.43		5.56
T2D	11/21/2013	IVGTT	2.49		2.83
0	12/9/20113			4.55	
7	12/16/2013			2.36	
9	12/18/2013			3.34	
11	12/20/2013			3.35	
17	12/26/2013			3.29	
21	12/30/2013			3.68	
28	1/6/2014	MMTT	3.01		4.72
35	1/13/2014			5.17	
56	2/3/2014	Euthanasia	1.02		4.42

IVGTT (Hcpep)

MIN	T2D (BG) 11/21/13 mg/dL	T2D ng/mL	T2D (BG) mg/dL	T2D ng/dL
Pre	127	2.49		
Pre	134	2.49		
0	80	2.5		
1	494	2.32		
3	387	2.73		
5	350	2.78		
7	326	2.64		
10	295	2.83		
15	NA			
20	264			
25	243			
30	234			
60	151			
K value	1.16			

Mixed Meal Tolerance Test

MMTT (Hcpep)

MIN	Pre ng/mL	Pre BG	d+28 ng/mL	d+28 BG
Pre	2.43	88	3.01	
90	5.56	71	4.72	

AST (Pcpep)

MIN	d+56 ng/mL
Pre	1.02
0	0.97
2	4.42
3	3.60
4	3.57
5	3.02

Hemaglobin A1C

HA1C

POD	Date	EVENTS	%
	8/2/2013		7.3
	10/16/2013		5.1
	11/20/2013		5.0
	12/9/2013		5.8
28	1/6/2014		7.6
56	2/3/2014	Euth	8.5

Primate No.: Riley 02AP2
Species: Male Cynomolgus Macaque
Birthdate: 12/01/1995
Protocol No.: 1303A30459
Permanent Identification (Tattoo): 02AP2
Barcode: B000 0000 8266

Cytokine Results

Animal ID	Riley 02AP2	Riley 02AP2	Riley 02AP2	Riley 02AP2	Riley 02AP2	Riley 02AP2	Riley 02AP2	Riley 02AP2	Riley 02AP2
Collection date	10/16/13	12/9/13	12/12/2013	12/16/2013	12/20/2013	12/30/2013	1/13/2014	1/6/2014	2/3/2014
Test condition	Fasted	Random	Random	Random	Random	Random	Random	fasted	fasted
Study ID	MGTC	MGTC	MGTC	MGTC	MGTC	MGTC	MGTC	MGTC	MGTC
Timepoint	pre	pre d + 0	post d + 3	post d + 7	post d + 11	post d + 21	post d + 35	post d + 78	post d + 56
D-Lab #	D13-046924	D13-055996	D13-057831	D13-057832	D13-057845	D14-001077	D14-002397	D14-001084	D14-005571
GM-CSF pg/mL	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
TGFa pg/mL	5.34	<2.4	<2.4	3.44	<2.4	<2.4	2.48	4.28	2.68
G-CSF pg/mL	170.79	4.75	11.85	LBC	7.98	11.41	6.74	20.49	12.62
IFNg pg/mL	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
IL-2 pg/mL	19.09	3.56	4.91	3.04	3.56	4.37	4.1	7.11	3.13
IL-10 pg/mL	70.72	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2
IL-15 pg/mL	4.63	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	3.33	<2.4
sCD40L pg/mL	286.4	43.3	377.18	195.25	103.87	490.56	940.19	811.49	15.41
IL-17A pg/mL	<2.4	4.03	6.03	8.35	6.39	5.54	6	8.3	6.08
IL-1ra pg/mL	28.85	2.7	6.11	8.98	2.7	5.57	4.55	13.59	2.88
IL-13 pg/mL	12.83	4.32	17.41	4.32	<2.4	12.15	13.53	24.84	<2.4
IL-1b pg/mL	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
IL-4 pg/mL	<4.9	<4.9	<4.9	<4.9	<4.9	<4.9	<4.9	<4.9	<4.9
IL-5 pg/mL	<2.4	4.39	5.68	8.89	7.01	7.01	9.58	11.16	5.12
IL-6 pg/mL	471.96	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
IL-8 pg/mL	726.7	267.64	566.94	410.01	275.96	615.06	748.02	977.11	79.31
MIP-1a pg/mL	10.88	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
MCP-1 pg/mL	3758.68	282.82	655.02	397.6	333.92	562.49	569.64	734.38	212.3
TNF-a pg/mL	12.73	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
MIP-1b pg/mL	2.84	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
IL-12/23 (p40) pg/mL	3.72	<2.4	3.65	11.4	5.6	3.65	6.01	9.38	<2.4
VEGF pg/mL	220.93	70.09	93.69	93.69	60.07	79.76	74.97	*N/A	*N/A
IL-18 pg/mL	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2	<12.2

*N/A= excluded due to poor s

Primate No.: Thresh 13GP10
Species: Cynomolgus Macaque
Birthdate: 2/6/2009
Protocol No.: MGTC
Permanent Identification (Tattoo): AR688C
Barcode: 001177261010

POD	Date	EVENTS	Blood Glucose Morning mg/dL	Blood Glucose Evening mg/dL	Insulin / Weight units/kg	INSULIN units	Weight kg
Transfer onto protocol. Hx - VAP (IVC), VAP (portal), diabetes induction (STZ), intraportal islet xenograft (suspect rejection/egraftment failure). All clinical path normal, normal physical exam, ok'd for transfer							
-7	9/29/2014	Liver Biopsy	62	185	1.15	3.0	
-6	9/30/2014		225	340	1.15	8.5	
-5	10/1/2014		161	119	1.15	7.0	
-4	10/2/2014		92	179	1.15	6.5	5.63
-3	10/3/2014		169	270	1.33	7.5	
-2	10/4/2014		62	82	1.07	6.0	
-1	10/5/2014		205	244	1.15	6.5	
0	10/6/2014	Ad-PNM Infusion	242	366	0.00	0.0	
1	10/7/2014		198	219	0.53	3.0	
2	10/8/2014		164	393	0.55	3.0	5.42
3	10/9/2014	Liver Biopsy	200	348	0.74	4.0	
4	10/10/2014		62	161	1.12	6.0	5.38
5	10/11/2014		106	66	1.02	5.5	
6	10/12/2014		114	173	0.84	4.5	
7	10/13/2014	MMTT	98	189	1.30	7.0	
8	10/14/2014		95	162	1.02	5.5	
9	10/15/2014		63	200	1.04	5.5	5.28
10	10/16/2014	Liver Biopsy	272	536	0.95	5.0	
11	10/17/2014		230	133	1.12	6.0	5.38
12	10/18/2014		178	236	1.12	6.0	
13	10/19/2014		133	128	0.84	4.5	
14	10/20/2014		131	144	1.30	7.0	
15	10/21/2014		124	290	1.21	6.5	
16	10/22/2014		229	346	1.39	7.5	
17	10/23/2014		190	181	1.12	6.0	5.36
18	10/24/2014		203	225	1.12	6.0	
19	10/25/2014		47	292	1.21	6.5	
20	10/26/2014		63	247	1.03	5.5	
21	10/27/2014		308	308	1.49	8.0	
22	10/28/2014		284	390	1.59	8.5	
23	10/29/2014		223	436	1.49	8.0	
24	10/30/2014		174	447	1.39	8.0	5.77
25	10/31/2014		216	359	1.39	8.0	
26	11/1/2014		189	359	1.39	8.0	
27	11/2/2014		81	303	0.52	3.0	
28	11/3/2014	IVGTT	202	351	1.60	9.0	5.61

Primate No.: Thresh 13GP10
Species: Cynomolgus Macaque
Birthdate: : 2/6/2009
Protocol No.: MGTC
Permanent Identification (Tattoo): AR688C
Barcode: 001177261010

N/A=Not Applicable
WNL=Within Normal Limits
NWNL=Not Within Normal Limits

				Feces										
				Type (%)										
POD	Date	AM/PM	EVENTS	1	2	3	4	Color	Urine	Gait, Posture and Appearance	Attitude and Activity Level	Pain and/or Distress	Treatments	Comments
Pstz	9/15/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/16/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/17/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/18/2014	AM		50	50	0	0	Typical	Typical	WNL	WNL	None		
		PM		0	0	0	0	N/A	N/A	WNL	WNL	None		No Feces Present
Pstz	9/19/2014	AM		50	25	25	0	Typical	Typical	WNL	WNL	None	Given BSSC + 1FT	
		PM		50	50	0	0	Typical	Typical	WNL	WNL	None	Given BSSC + 1FT	
Pstz	9/20/2014	AM		90	10	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/21/2014	AM		80	0	20	0	Typical	Typical	WNL	WNL	None	Given BSSC + 1FT	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/22/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/23/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/24/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/25/2014	AM		50	50	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/26/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		90	10	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/27/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/28/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/29/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	9/30/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None	Start MMF (1 tab)	
		PM		0	0	0	0	N/A	Typical	WNL	WNL	None		No feces present
Pstz	10/1/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
Pstz	10/2/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	10/3/2014	AM		50	50	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	10/4/2014	AM		90	10	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
Pstz	10/5/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
0	10/6/2014	AM		80	10	10	0	Typical	Typical	WNL	WNL	None	APAP(1tab) DPH prior 1tab to infusion	OK for MMF
		PM		0	0	0	0	N/A	Typical	WNL	WNL	None		No feces present/ recheck BG @430pm
1	10/7/2014	AM		0	20	80	0	Typical	Typical	WNL	WNL	None	2mg (1tab) Lop-12	HOLD AM MMF
		PM		0	50	50	0	Atypical	Typical	WNL	WNL	None	2mg (1tab) Lop-12 & 1 tab BSSC	Red Brick Feces Color/ Hold MMF
2	10/8/2014	AM		0	0	100	0	Atypical	Typical	WNL	WNL	None	2mg (1tab) Lop-12 & 1 FT PO, Sidekick	Red Brick Feces Color/ Hold MMF/slight drop
		PM		0	0	100	0	Atypical	Typical	WNL	WNL	None		HOLD PM MMF
3	10/9/2014	AM		10	20	70	0	Typical	Typical	NWNL	WNL	None	150ml saline IV	Dehydrated - MILD
		PM		0	0	0	0	N/A	Typical	WNL	WNL	None	Ordered followup labs, BUP 0.3m/kg, increase insulin	@ 3:06pm NO feces present. @5pm indicati exam by PI + veterinarian - suspect ketosis. confirm
4	10/10/2014	AM		60	20	20	0	Typical	Typical	WNL	WNL	None	Give ORS via syringe	HOLD AM MMF
		PM		0	25	75	0	Typical	Typical	WNL	WNL	None	Give ORS via syringe/260mg char PO	Hold MMF
5	10/11/2014	AM		0	50	50	0	Typical	Typical	WNL	WNL	None	Give Charcocap (PO)	HOLD AM MMF
		PM		0	25	25	50	Typical	Typical	WNL	WNL	None	Give Charcocap (PO) LOP-12 2mg PO	Hold MMF
6	10/12/2014	AM		25	25	25	25	Typical	Typical	WNL	WNL	None	2mg LOP-12	Hold MMF
		PM		0	0	100	0	Typical	Typical	WNL	WNL	None	1 Charcocap	Hold MMF
7	10/13/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None	Charcocap post MMTT	HOLD AM MMF
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None	Charcocap	OK for MMF
8	10/14/2014	AM		0	40	60	0	Typical	Typical	WNL	WNL	None	2mg LOP-12 @ 10:15, 1:00p, 4:00p	OK for MMF
		PM		80	20	0	0	Typical	Typical	WNL	WNL	None		
9	10/15/2014	AM		65	10	25	0	Typical	Typical	WNL	WNL	None	LOP-14	OK for MMF
		PM		0	0	0	0	N/A	Typical	WNL	WNL	None		
10	10/16/2014	AM		50	50	0	0	Typical	Typical	WNL	WNL	None		NO MMF/Liver Biopsy
		PM		50	50	0	0	Typical	Typical	WNL	WNL	None	ZOF-17 (2mg)	OK for MMF
11	10/17/2014	AM		70	0	30	0	Typical	Typical	WNL	WNL	None	Charcocap AM/Noon/PM	Hold MMF
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		OK for MMF
12	10/18/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		OK for MMF
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
13	10/19/2014	AM		70	30	0	0	Typical	Typical	WNL	WNL	None		OK for MMF
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
14	10/20/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		OK for MMF
		PM		0	0	0	0	N/A	Typical	WNL	WNL	None		No Feces present
15	10/21/2014	AM		80	20	0	0	Typical	Typical	WNL	WNL	None	1 tab BSSC	OK for MMF
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
16	10/22/2014	AM		50	50	0	0	Typical	Typical	WNL	WNL	None	1 BSSC (262 mg)	OK for MMF
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		Hold MMF
17	10/23/2014	AM		75	25	0	0	Typical	Typical	WNL	WNL	None		HOLD AM MMF
		PM		0	100	0	0	Typical	Typical	WNL	WNL	None		Hold MMF
18	10/24/2014	AM		70	0	20	10	Typical	Typical	WNL	WNL	None	CHAR-15 1cap BID	MMF PM ONLY
		PM		50	0	0	50	Typical	Typical	WNL	WNL	None		Hold MMF
19	10/25/2014	AM		25	0	75	0	Typical	Typical	WNL	WNL	None	LOP-14 2mg BID	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		HOLD MMF
20	10/26/2014	AM		50	0	50	0	Typical	Typical	WNL	WNL	None	BSSC 262 mg	
		PM		50	50	0	0	Typical	Typical	WNL	WNL	None		Hold MMF
21	10/27/2014	AM		50	25	25	0	Typical	Typical	WNL	WNL	None	BSSC 262 mg	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		
22	10/28/2014	AM		90	10	0	0	Typical	Typical	WNL	WNL	None	BBAC-20 1.0g	
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		OK for MMF
23	10/29/2014	AM		100	0	0	0	Typical	Typical	WNL	WNL	None		75%-1 25% - 3 @ 8:26am
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None		OK for MMF
24	10/30/2014	AM		60	30	10	0	Typical	Typical	WNL	WNL	None	BSSC 262 mg LOP-14; ok for sidekick	
		PM		0	50	50	0	Typical	Typical	WNL	WNL	None	sidekick only	HOLD MMF; NO SF juice
25	10/31/2014	AM		0	100	0	0	Typical	Typical	WNL	WNL	None		
		PM		0	0	0	0	N/A	Typical	WNL	WNL	None	OK for MMF	100% - 1 @ 3:25pm
26	11/1/2014	AM		90	10	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
27	11/2/2014	AM		95	5	0	0	Typical	Typical	WNL	WNL	None		
		PM		100	0	0	0	Typical	Typical	WNL	WNL	None	OK for MMF	
28	11/3/2014	AM		90	10	0	0	Typical	Typical	WNL	WNL	None		
		PM		0	100	0	0	Typical	Typical	WNL	WNL	None		HOLD MMF

Primate No.: Thresh 13GP10
Species: Cynomolgus Macaque
Birthdate: 2/6/2009
Protocol No.: MGTC
Permanent Identification (Tattoo): AR688C
Barcode: 001177261010

			MMF			MMF/Weight
			AM	PM	Trough	Dose
POD	Date	EVENTS	mg	mg	mg/L	mg/kg
-6	9/30/2014		500	500		0.00
-5	10/1/2014		500	500		0.00
-4	10/2/2014		500	500	5.2	177.62
-3	10/3/2014		500	500		177.62
-2	10/4/2014		500	500		177.62
-1	10/5/2014		500	500		177.62
0	10/6/2014		500	500		177.62
1	10/7/2014		0	0	7.99	0.00
2	10/8/2014		0	0		0.00
3	10/9/2014		0	0		0.00
4	10/10/2014		0	0		0.00
5	10/11/2014		0	0		0.00
6	10/12/2014		0	0		0.00
7	10/13/2014		0	0	<0.25	0.00
8	10/14/2014		250	250		92.94
9	10/15/2014		250	250		94.70
10	10/16/2014		250	250	2.10	94.70
11	10/17/2014		250	250		92.94
12	10/18/2014		250	250		92.94
13	10/19/2014		250	250		92.94
14	10/20/2014		250	250	8.62	92.94
15	10/21/2014		250	250		92.94
16	10/22/2014		250	250		92.94
17	10/23/2014		0	0		0.00
18	10/24/2014		0	0		0.00
19	10/25/2014		0	0		0.00
20	10/26/2014		0	0		0.00
21	10/27/2014		0	0		0.00
22	10/28/2014		0	250	<0.25	46.64
23	10/29/2014		0	250		46.64
24	10/30/2014		0	0		0.00
25	10/31/2014		0	250		43.33
26	11/1/2014		0	250		43.33
27	11/2/2014		0	250		43.33
28	11/3/2014		0	0	2.00	0.00

Primate No.: Thresh 13GP10
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NE=Not Evaluated
ND=Quality non sufficient

Clinical Pathology Laboratory-UMN

POD	Q	Pre	Pstz	0	1	2	3	7	10	22
date	8/6/2013	1/13/2014	7/3/2014	10/6/2014	10/7/2014	10/8/2014	10/9/2014	10/13/2014	10/16/2014	10/28/2014
EVENTS		IVC		Infusion					Liver Biopsy	
WBC (x10³/µL)	8.21	6.71	6.41	13.24	5.63	7.01	11.62	10.49	9.17	6.38
RBC (x10⁶/µL)	6.47	5.77	5.69	6.82	6	6.48	5.98	5.83	5.07	5.61
HGB (g/dL)	13.1	11.5	11.8	14.7	12.8	13.7	12.7	12.3	10.7	11.9
HCT (%)	44.8	40.1	41.1	49.6	44.4	47	43.3	41.1	37.3	42.5
MCV (fL)	69.3	69.6	72.2	72.8	74	72.5	72.3	70.4	73.5	75.7
MCH (pg)	20.3	20	20.8	21.6	21.4	21.2	21.3	21.1	21.2	21.2
MCHC (g/dL)	29.2	28.7	28.8	29.7	28.9	29.3	29.4	29.9	28.8	28
RDW (%)	14.4	14.6	14.4	13.3	13.5	13.5	13.4	13.1	14.1	15.4
Plt (x10³/µL)	468	440	406	563	445	497	495	537*	398	591
MPV (fL)	7.6	7.6	7.8	7.5	7.7	7.5	7.4	8	7.3	7.9
PCT%	49.7	46.7	54.4	48.5	49.8	56.1	47.3	50.3	50.5	47.2
PDW%	0.36	0.33	0.32	0.42	0.34	0.37	0.36	0.43	0.29	0.47
%NEUT	24	8.05	19.97	65.03	60.04	30.96	77.02	36.03	37.95	21
%BAND	0	0	0	0	0	0	0	0	0	0
%LYMPH	67.97	87.03	70.98	32.02	31.08	60.06	19.02	55.96	54.96	71
%MONO	3.05	1.04	2.03	3.02	9.06	6.99	3.96	6.01	4.03	4.08
%EOS	4.99	4.02	5.93	0	0	2	0	0.95	3.05	4.08
%BASO	0	0	0.94	0	0	0	0	0.95	0	0
#NEUT (10³cells/µL)	1.9704	0.540155	1.280077	8.609972	3.380252	2.170296	8.949724	3.779547	3.480015	1.3398
#BAND (10³cells/µL)	0	0	0	0	0	0	0	0	0	0
#LYMPH (10³cells/µL)	5.580337	5.839713	4.549818	4.239448	1.749804	4.210206	2.210124	5.870204	5.039832	4.5298
#MONO (10³cells/µL)	0.250405	0.069784	0.130123	0.399848	0.510078	0.489999	0.460152	0.630449	0.369551	0.260304
#EOS (10³cells/µL)	0.409679	0.269742	0.380113	0	0	0.1402	0	0.099655	0.279685	0.260304
#BASO (10³cells/µL)	0	0	0.060254	0	0	0	0	0.099655	0	0
RBC morphology	Normal RBC morphology	Normal RBC morphology	Rare polychromasia	Normal RBC morphology	Normal RBC morphology	2+ disrupted RBCs	1+ echinocytes	anisocytosis, rare echinocytes, rare	Normal RBC morphology	1+ anisocytosis, rare codocytes, rare polychromasia
WBC Comment	1+ lobulated lymphocytes	1+ lobulated lymphocytes	No Comments	1+ reactive lymphocytes, rare lobulated lymphocytes	1+ lobulated lymphocytes, rare reactive lymphocytes	Rare lobulated lymphocytes, 1+ reactive lymphocytes	Rare lobulated lymphocytes	Rare reactive lymphocytes, 1+ lobulated lymphocytes	2+ reactive lymphocytes, 1+ lobulated lymphocytes	Rare reactive lymphocytes, 1+ smudged WBCs, 1+ lobulated lymphocytes
BUN (mg/dL)	17	14	23	11	20	19	19	13	20	20
Creat (mg/dL)	0.7	1.1	0.9	0.8	1.1	0.8	1	0.6	0.7	0.7
Ca (mg/dL)	10.5	9.5	10.3	10	9.5	9.8	9.3	9.6	9.9	10.6
Phos (mg/dL)	5.2	5.5	4	5.1	6.9	4.6	7.6	2.6	2.7	2
Mg (mg/dL)	1.8	1.7	1.8	1.7	1.6	1.6	1.6	1.7	1.8	1.7
TP (g/dL)	7.4	5.8	6.5	7.1	6.7	7.4	7.2	6.8	7	7.2
Alb (g/dL)	4.6	3.8	3.9	4.1	3.8	3.9	4	3.8	3.8	3.9
Glob (g/dL, calc)	2.8	2	2.6	3	2.9	3.5	3.2	3	3.2	3.3
Na (mmol/L)	146	144	142	138	134	135	131	142	144	139
Cl (mmol/L)	107	111	107	103	100	103	101	107	109	107
K (mmol/L)	4.4	4.3	3.9	4.7	4.6	4.5	3.9	3.8	3.7	4.2
HCO3 (mmol/L)	24.3	23.5	24.3	23.5	15.8	16.8	17.7	25.2	25	22.8
Osmol (calc.)	289	285	293	285	281	277	278	284	300	295
An Gap	19	14	15	16	23	20	16	14	14	13
T. Bili (mg/dL)	0.2	0.1	0.1	0.2	0.3	0.2	0.4	0.2	0.1	0.1
ALP (U/L)	444	330	282	273	286	245	294	369	344	289
GGT (U/L)	143	112	153	96	83	83	94	127	123	110
ALT (U/L)	81	24	170	46	42	40	48	90	79	78
AST (U/L)	38	25	56	19	25	24	73	46	35	27
CK (U/L)	265	501	358	119	329	116	5421	149	294	121
Gluc (mg/dL)	50	52	213	269	279	189	328	104	285	359
Chol (mg/dL)	84	62	104	77	69	84	90	69	72	74
Amy (U/L)	269	280	180	164	137	205	184	184	188	196
Lip (U/L)	37	34	29	18	16	23	31	21	21	22
Trig (mg/dL)	36	71	72	144	387	477	111	90	105	105
LIH	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	2 0 1	0 0 2	0 0 0	0 0 0	0 0 0
BHY (mmol/L)							1.40			

Primate No.: Thresh 13GP10
Species: Cynomolgus Macaque
Birthdate: 2/6/2009
Protocol No.: MGTC
Permanent Identification (Tattoo): AR688C
Barcode: 001177261010

Indicates Siemens RIA (historical kit)

POD	Date	EVENTS	Hcpep (Millipore) Fasted ng/mL	Random ng/mL	Stim ng/mL
Pre	2/3/2014	MMTT	0.46		16.72
Pre	2/5/2014	IVGTT	1.23		10.17
Pre	4/7/2014	STZ			
Pstz	5/6/2014	IVGTT	0.39		0.28
Pstz	9/23/2014	MMTT	<0.16		0.25
0	10/6/2014	Infusion		0.21	
1	10/7/2014			0.19	
2	10/8/2014			<0.16	
3	10/9/2014			<0.16	
4	10/10/2014			<0.16	
7	10/13/2014	MMTT	<0.16		0.26
10	10/16/2014			<0.16	
14	10/20/2014	MMTT	<0.16		0.23
18	10/24/2014			<0.16	
22	10/28/2014			<0.16	
25	10/31/2014			<0.16	
28	11/3/2014	IVGTT	<0.16		0.17

IVGTT (Hcpep)

	Pre STZ	2/5/2014	Post STZ	5/6/2014	d+28	11/3/2014
MIN	mg/dL	ng/mL	mg/dL	ng/dL	mg/dL	ng/dL
Pre	55	1.23	184	0.39	202	<0.16
Pre	59	1.18	190	0.36	198	<0.16
0	55	1.55	188	0.32	188	<0.16
1	539	7.77	509	0.28	569	0.17
3	295	7.83	424	0.25	411	<0.16
5	249	8.17	370	0.25	369	<0.16
7	230	8.12	384	0.26	346	<0.16
10	204	10.17	348	0.17	337	<0.16
15	139		283		316	
20	120		294		314	
25	99		292		304	
30	76		288		318	
60	39		283		288	
K value	4.94		0.95		0.79	

MMTT (Hcpep)

	Pre STZ	2/3/2014	Post STZ	9/23/2014	d+7	10/13/2014
MIN	mg/dL	ng/mL	mg/dL	ng/dL	mg/dL	ng/dL
Pre	52	0.46	191	<0.16	98	<0.16
90	55	16.72	456	0.25	291	0.26

HA1C

POD	Date	EVENTS	%	BG
Pre	2/11/2014		3.8	44
Pre	4/4/2014		3.7	44
Pstz	9/22/2014		6.8	222
0	10/6/2014	Infusion	7.1	242
28	11/3/2014		7.7	351