CHIEF SCIENTIST OFFICE

CODE: TCS/17/31

RESEARCH PROJECT BRIEFING


# Preclinical testing of GMP grade mesenchymal stromal cells to promote the long-term engraftment and function of human islets.

## AIMS

The transplantation of insulin producing cells – termed islets – into the liver of someone with Type 1 diabetes is a treatment that can stabilise blood glucose control and improve quality of life. There are a number of issues: firstly it is not possible to grow islets in a lab. Secondly, during the process of islet isolation from donated pancreases there is considerable loss of islets. Thirdly, the majority of islets are subsequently lost after the transplant through inflammation and poor blood vessel formation of islets into the liver. Typically islets from 2 to 3 donor pancreases are required to impact blood glucose control. Since there are not enough pancreases to meet requirements there is an urgent need to improve islet transplantation outcomes.

“Mesenchymal stromal cells” (MSCs) are a form of cell therapy. A cell therapy is a therapy in which viable cells are injected, grafted or implanted into a patient in order to achieve a beneficial therapeutic effect. Here it was to improve islet transplantation by decreasing loss of islets and improving their blood vessel formation and supply to the surrounding tissue that they are implanted in.

The aim of this project was to determine if transplantation of islets in combination with these MSCs improved islet transplantation outcomes in a mouse with diabetes.

Improved islet transplantation outcomes would mean that less islets would be required to control blood glucose levels for each patient with Type 1 diabetes.

## KEY FINDINGS

- Scottish National Blood Transfusion Service (SNBTS) successfully manufactured MSCs from umbilical cord (donated from Tommy's biobank). Such cells which are not manufactured from the patient receiving the transplants are termed allogeneic cells. Investigators in the past have manufactured MSCs from other tissues such as bone marrow and fat and the beneficial effects on insulin secretion have been less marked.
- It is important to note that MSCs are not islet cells and do not secrete insulin, nor do they turn into cells that secrete insulin, but rather they improve the transplantation of any islets that have been delivered to the site of transplantation: which in humans and in our mouse was in the liver.
- We showed that these MSCs were anti-inflammatory and had properties which would promote the formation of blood vessels between islets and liver (termed vascularisation). We reasoned that MSCs would reduce the loss of islets from inflammation and death and lead to better vascularisation of islets thereby again reducing the numbers of islets required for good blood glucose control thereby improving islet transplant outcomes.

- We transplanted islets into a mouse that was induced to have high blood glucose concentrations using a chemical called streptozotocin thereby emulating the diabetic state (termed a diabetic mouse model). Islets were then transplanted at a dose that we knew would not typically cure the mouse as we wanted to test that with the addition of MSCs and the same dose of islets a cure could be achieved. We compared the transplant results in these mice with a group of diabetic mice that received islets and MSCs. In the islet alone group no mice were cured as predicted but in the islet plus MSC group 6 out of the 8 mice were cured.
- All the mice that received MSCs had better evidence of insulin secretion from the islets that were transplanted.

## WHAT DID THE STUDY INVOLVE?

The study involved manufacturing MSCs at SNBTS. Although the starting material was derived from donated umbilical cord these cells can be expanded and grown in the laboratory so that one MSC can give rise to millions of MSCs. In contrast mature human islets cannot be grown in a laboratory.

The properties of these MSCs were then tested with state-of-the-art techniques including examination of gene expression and proteins from these cells. Gene expression is a process by which a gene gets turned on in a cell to make RNA and proteins. The aim here was to investigate more about the workings of the MSCs and what makes a "good" one in the context of islet transplantation. Importantly these cells were manufactured to Good Manufacturing Practice (GMP). This means that not only were these exact cells tested in our mouse model – manufacturing can potentially induce changes in the properties of the cells - but that these cells consistency and quality were high and can be injected into humans.

In the second phase of the study a small number of islets which we knew if transplanted alone would not cure diabetes, were transplanted with and without MSCs into two groups of diabetic mice (8 per group).

Blood glucose control was observed for over 100 days and compared. Studies were also set up to compare the degree of vascularisation of islets (creation of blood vessels between islets and liver) within the organs in those receiving MSCs with islets versus islets alone. Further studies were designed where islets were transplanted into specific diabetic mice where it was known that the islets would be rejected, based on their genetic background, within a few days. It was then determined if the co-transplants with the MSCs decreased the rejection of islets.

The public was involved in the planning of the project: patients from the islet transplant clinic were approached with respect to their views on the project and the prospect of such a treatment in humans. Updates with the lay panel were held to inform them of the project progress and what this could mean for patients in the future.

## WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

These MSCs derived from umbilical cord when first tested in the laboratory showed increased expression of specific genes and proteins that firstly confirmed they were not inflammatory (eg. CCL23, CCL26) and secondly that they could dampen down inflammation (eg. CD274, CFH) and thirdly that they could potentially improve the blood vessel formation of islets into the liver (eg. CXCL2, CXCL1; Figure 1). These results gave some confidence to next test whether following transplantation of islets plus MSCs into a mouse, this could reduce the loss of islets from inflammation and death. Secondly we also wished to test if it could lead to better vascularisation of islets in the mice.
When these MSCs were transplanted with low numbers of islets in mice, 6 out of 8 mice were cured from their diabetes by the end of the study whereas those receiving islets alone did not achieve a cure (Figure 2). Greater levels of insulin secretion were also seen in the mice receiving MSCs plus islets. Mice that were transplanted with islets plus MSCs, showed greater blood vessel formation around the islets versus those transplanted with islets alone. In the mouse models that were predicted to reject islets, in the islet alone group, rejection took place 3 days post transplant, whereas in the group receiving the co-transplants (co-transplants refers to the transplantation of islets plus MSCs), rejection of the islets took place 8 days later (Table 1).

In summary co-transplantation of islets with MSCs when compared to transplants with islets alone resulted in better blood glucose control. This is driven by decreased inflammation at the site of transplantation in the liver coupled with better vascularisation of the islets in the liver and less rejection of islets.

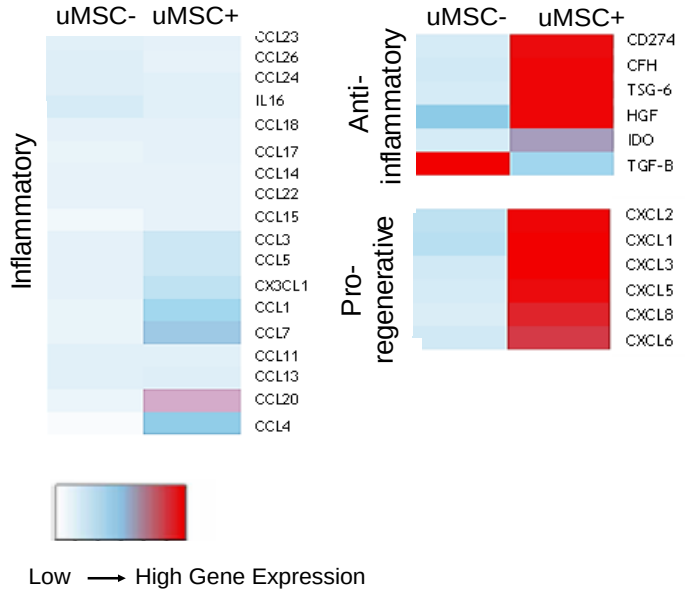
This supported the concept that fewer islets may be needed to potentially cure diabetes and, or, impact on blood glucose control thereby improving islet transplant outcomes. Furthermore, the fact that the MSCs could be manufactured as an off-the-shelf product meant that there is no reliance on obtaining donor tissue to improve these outcomes.

**Table 1**

| | Islets alone | Islets plus MSCs |
|---|---|---|
| Type 1 cure ? | 0/8 mice cured | 6/8 mice cured |
| Insulin secretion | Lower | Higher |
| Blood vessel formation around islet | Less | More |
| Rejection of islets | 3 days after transplant | 8 days after transplant |

Legend. Summary of outcomes comparing transplanting islets alone versus islets plus MSCs into diabetic mice.

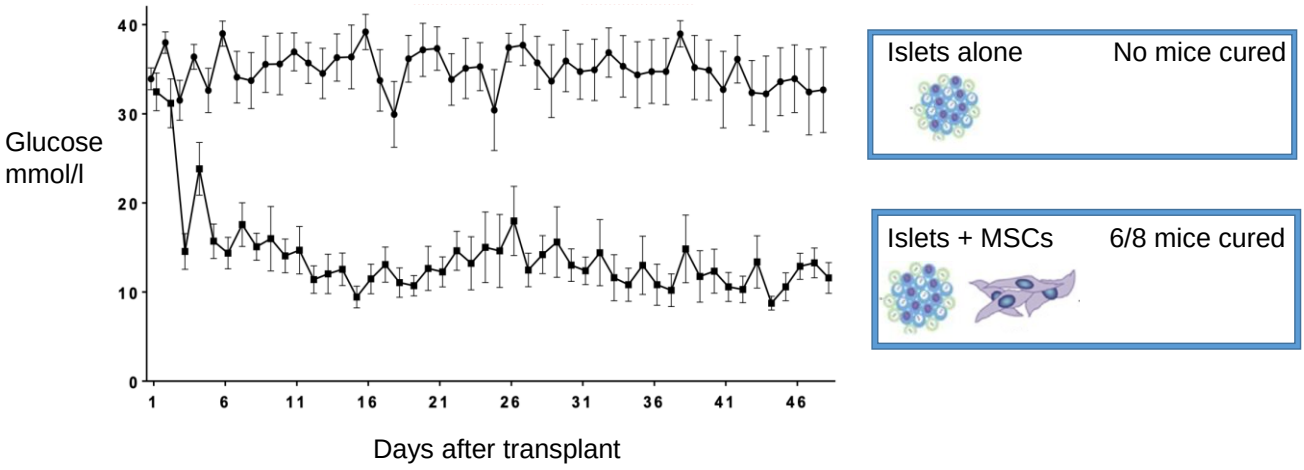
**Figure 1**




uMSCs – human umbilical cell MSCs.
- Refers to experiments where MSCs were not stimulated; + Refers to experiments where MSCs were exposed to an inflammatory challenge.

Legend. uMSCs were taken and genes that are known to have inflammatory, anti-inflammatory and pro-regenerative (blood vessel forming) properties were run. Individual genes are shown (eg. CCL23, CCl26 etc). High gene expression is denoted by red and indicates that the genes that have an anti-inflammatory and pro-regenerative role are increased.

**Figure 2**




Legend. Improved blood glucose control in mice transplanted with MSCs and islets with the majority of mice cured from their diabetes.

## WHAT IMPACT COULD THE FINDINGS HAVE?

These results mean that the strategy of co-transplanting islets with MSCs could be adopted in human islet transplantation potentially to improve blood glucose control, decrease loss of transplanted islets and by improving vascularisation of islets. Taken together this would mean that insulin secretion is increased from the transplanted islets. Furthermore, their anti-rejection properties could be exploited to decrease the anti-rejection medication required which would decrease the side effects from these drugs. The next step would be to conduct clinical trials in humans focussed on safety of such cells when transplanted with islets (Phase 1 clinical trials).

Such a strategy would also mean that donor pancreases that have lower numbers of islets isolated (eg. below 300,000 islets) may be used effectively for transplant purposes with the MSCs to treat someone with Type 1 diabetes. This would ease the pressure off the islet transplant waiting list and make best use of donated pancreases – currently only 11% that are offered are used for transplant purposes.
Uniquely these MSCs could provide an "off-the-shelf solution" for everyone that comes in for islet transplantation and tissue typing is not required. Furthermore it is not necessary for the patient's own tissues to be used to manufacture MSCs which would be time consuming, costly and would pose numerous logistical problems.

To summarise the implications could be as follows:

- Patients

Improved islet transplant function for a longer period of time

Glucose controlled better in the MSC treated individuals, as insulin is produced more effectively from the islet cells in those individuals with the possibility of greater insulin independence rates from the islet plus MSC transplant

Potentially islets from one pancreas could meet the requirements for one person

Better use of donated pancreases

Decreased time for patient on the transplant waiting list and more patients transplanted

Decreased doses of anti-rejection drugs with decreased complications

- Policy - Introduction of MSCs as an off-the-shelf solution for co-transplantation with islets into all human islet transplant units

- Practice - Co-transplantation of MSCs with islets in all transplant units

## HOW WILL THE OUTCOMES BE DISSEMINATED?

Our results have been published in the Journal Science Translational Medicine (Forbes S..Campbell JDM, 2020) and Cytotherapy (Thirlwell K, Forbes S and Campbell JDM, 2020). We have disseminated our findings via public forums including national radio programmes including The Naked Scientist (https://www.thenakedscientists.com/articles/interviews/new-cell-transplant-diabetes), other public forums eg. 100 years of insulin diabetes events (October 2021 - shorturl.at/houH9), Science Film Club and at research conferences including Diabetes UK, Immunology conferences, Royal College of Physician events and talks with Industry.

## CONCLUSION

This study demonstrated that MSCs could be manufactured from human umbilical cord rapidly and in large quantities and then "banked" that is stored in a "bank" of biological material, for future use. These MSCs have already been produced to GMP standards. These cells had properties which dampened down inflammation and encouraged blood vessel formation. When MSCs were transplanted with islets, the transplant controlled glucose to a greater extent compared to islets alone due to better insulin secretion and cured diabetes in the majority of animals transplanted. Less rejection of the islets and greater blood vessel formation between insulin-producing cells (islets) and the liver into which the islets were transplanted was seen. These encouraging results point at the potential benefit that such a strategy could have in a clinical trial in humans. For people living with Type 1 diabetes who meet criteria for islet transplantation, improving the current islet treatment method could improve insulin secretion from their transplanted islets and improve their quality of life and it would increase the number of patients who could be helped.

## RESEARCH TEAM & CONTACT

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