

## Review Article

# A review of clinical trials: mesenchymal stem cell transplant therapy in type 1 and type 2 diabetes mellitus

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**Abstract:** Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are widely prevalent metabolic diseases with differing pathologies. T1DM manifests due to autoimmune destruction of the pancreatic beta cells, resulting in a diminished secretion of insulin. T2DM originates from a state of insulin resistance, resulting in hyperglycemia and reduction in beta cell mass. Both diseases can cause severe health consequences. Despite the globally increasing prevalence of both T1DM and T2DM there remains to be a medically defined cure for either of these diseases. Recently, mesenchymal stem cells (MSCs) have been proposed as a possible curative treatment method. In this review, we explain the molecular mechanisms underlying MSCs and their potential ability to treat T1DM and T2DM. We describe the capability of MSCs to differentiate into insulin-producing cells and regenerate pancreatic beta cells, as well as assess their role in modulating the immune system. Lastly, we evaluate the current literature focusing on the clinical application of MSC transplantation in T1DM and T2DM. Despite the favorable results, study designs and analyses cast doubt on the effectiveness of MSCs for the management of T1DM. Conversely, the positive metabolic effects consistently demonstrated in the literature offer hope for MSCs as a treatment for T2DM, at least in the short-term.

**Keywords:** Diabetes mellitus, type I diabetes, type II diabetes, mesenchymal stem cells, clinical trials, clinical application, transplantation, stem cell therapy, insulin sensitivity

## Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, the sequelae of defects in insulin secretion, insulin action, or both [1]. In the last few decades, DM has emerged as one of the major public health concerns worldwide. Millions of people around the world currently have diabetes or are at a high risk of developing diabetes in the future [2]. Most cases of DM fall into two broad pathogenetic categories. In one category, type 1 diabetes mellitus (T1DM), the cause of hyperglycemia is a deficiency in insulin secretion as a consequence of the organ-specific autoimmune destruction of insulin-producing beta cells in the pancreatic islets of Langerhans. In the other, more pervasive category - type 2 diabetes mellitus (T2DM) - the cause of hyperglycemia is a combination of cellular-level resistance

to insulin action and an inadequate compensatory insulin secretory response. Outside of these two prominent categories, classifications of DM also include gestational DM, genetic defects of the beta cell and insulin action, diseases of the exocrine pancreas, endocrinopathies, infections, as well as drug- or chemical-induced diabetes [1]. In this paper, our focus will be on T1DM and T2DM due to their significantly higher prevalence in the world population [1].

The chronic hyperglycemia that occurs as a consequence of DM is a major risk factor for long-term damage to different organs, including the eyes, kidneys, nerves, heart and blood vessels. The degree of hyperglycemia may change over time with the disease process. For diabetic patients with residual endogenous insulin production, common treatment options for DM include weight reduction, exercise, and

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oral antidiabetic agents. For patients with extensive beta cell destruction and, therefore, no residual insulin secretion, exogenous insulin is required for adequate glycemic control and survival [1]. Although these available therapeutic regimens can ameliorate hyperglycemia, they are less effective in reversing insulin resistance, and do not prevent the progressive beta islet cell degeneration, and therefore are ineffective in altering the progression of DM [2, 3]. Recently, mesenchymal stem cells (MSCs) derived from different adult tissues have attracted great attention as a more promising treatment of DM. MSCs are defined as a fibroblast-like cell population capable of differentiating into multiple mesenchymal lineages in vitro, including bone, fat, and Wharton's jelly [4, 5]. MSCs possess the capacity to differentiate into islet-like insulin producing cells (IPCs), to promote the regeneration of pancreatic islet beta cells, and to protect endogenous pancreatic islet beta cells from apoptosis through immunomodulatory mechanisms [4, 6-8]. However, to date most of these beneficial effects have only been observed in animal models of DM. In order to improve the treatment of DM and progress toward the implementation of stem cell therapy as the first potential curative method, clinical trials of MSC therapy are of critical importance. In this paper, we will review the current clinical applications of MSCs in the treatment of T1DM and T2DM in order to examine their therapeutic potential and posit potential future directions to improve their clinical efficacy.

### Pathophysiology of T1DM and T2DM

DM was recognized over 100 years ago, and the mechanisms by which 'insulin-resistant', or T2DM differed from the autoimmune etiology - T1DM - were demonstrated as early as 1936 [2]. Since then, numerous causative factors have been proposed. Central to these genetic, environmental, and metabolic influences is the pancreatic beta cell and its relationship to the body's insulin-sensitive tissues: liver, skeletal muscle, and adipose tissue. In a healthy state, a feedback loop exists between the pancreatic beta cell and these tissues, whereby beta cells secrete insulin, and insulin then augments glucose, amino acid, and fatty acid uptake in adipose tissue and skeletal muscle, while simultaneously suppressing gluconeogenesis in the

liver [2]. These insulin-sensitive tissues then feedback on the beta cells to prevent excessive insulin secretion, although the mechanisms by which this occurs remain to be fully elucidated.

In T2DM, a combination of agents - encompassing both lifestyle behaviors (i.e. diet, physical activity) and genetic elements (such as genes involved with fat metabolism and beta cell function) - results in a disruption of this feedback loop [2]. Often referred to as a state of 'insulin resistance,' these tissues demonstrate a diminished capacity for glucose uptake, predisposing one to hyperglycemia. Moreover, because of this failure to adequately uptake glucose, adipose tissue, skeletal muscle, and hepatocytes are unable to exert negative feedback on the pancreatic beta cells, resulting in sustained insulin secretion and a potential state of hyperinsulinemia. Finally, as these pathological states continue, beta cell mass decreases due to a variety of potential, and not yet completely identified factors. This loss in beta cell number accompanied by an inability to replace them results in an imbalance in beta cell turnover. This decreased capacity to secrete insulin in the presence of glucose is thus believed to account for the progression from impaired glucose tolerance to T2DM [2].

Dysfunction in the aforementioned feedback loop between insulin-sensitive tissues and pancreatic beta cells is not the biological basis for T1DM. Rather, the inciting cause is an inability of the pancreas to secrete insulin [3]. T1DM is an autoimmune disease, resulting from a multitude of genetic and environmental factors that leads to a lack of self-tolerance, and, ultimately, the destruction of the pancreatic beta cells. Of the numerous immunological mediators believed to influence this disease, regulatory T (Treg) cells have been studied as an essential contributory agent [3]. In a healthy state, Treg cells suppress the immune system, preventing overactivation and autoimmune damage. Mutations to Treg genes can thus result in a diminished capacity for these cells to perform their usual regulatory function, and are at least partially responsible for the immune-mediated damage in T1DM. Hence, it is this dysfunction of the immune system - when combined with environmental factors and other stochastic events - that is thought to result in the destruction of the pancreatic beta cells, and, consequently,

the body's capability to secrete insulin and/or monitor glucose homeostasis [3].

### **Current treatment options for T1DM and T2DM**

Due to these distinctions between the pathogenesis of T1DM and T2DM, the two metabolic disorders have different approaches to treatment. As previously stated, a progressively worsening state of insulin resistance and impaired glucose tolerance typically precedes T2DM. As such, great emphasis is placed on preventing this deterioration by implementing lifestyle changes in those individuals identified with elevated fasting plasma glucose (FPG) levels or with common comorbidities such as obesity [2]. Depending on the patient profile - signs/symptoms, contraindications, and cost, for example - different pharmacological agents are often combined with the therapeutic lifestyle interventions. Some medications targeting the gastrointestinal system aim to slow gastric emptying, thereby decreasing the rate of glucose absorption, while others augment duodenal hormones in an effort to facilitate glucose uptake [2]. The kidney is another therapeutic target in the treatment of T2DM because of its ability to conduct gluconeogenesis and its role in glucose reabsorption [9]. In severely insulin-resistant individuals, exogenous insulins and medications aimed at enhancing insulin sensitivity have demonstrated their efficacy as treatment options [2]. Because the metabolic impairment in T1DM is not the resistance of cells to insulin but rather the absence of insulin, pharmacological interventions are necessary and always involve the administration of exogenous insulin. Other medications (such as immunosuppressive agents) have been used as adjunctive treatment options in the research setting [3].

Concerningly, none of the aforementioned therapies and medications are capable of completely halting the pancreatic beta cell destruction observed in both T1DM and T2DM. In addition, these medications may be associated with significant adverse effects, including acute pancreatitis and cardiovascular events [2]. Therefore, a safe and effective treatment option that is capable of preventing this reduction in beta cell mass is of great clinical relevance. Recently, studies in mouse models have demonstrated an ability for MSC derived from

bone marrow to effectively lower blood glucose levels and increase pancreatic beta cell mass [10]. If confirmed, such a treatment option would offer tremendous therapeutic potential to individuals with DM. Due to their ability to not only stop the destruction of pancreatic beta cells, but also to regenerate these cells, MSCs may provide a possible curative treatment to both T1DM and T2DM.

### **Molecular mechanisms of MSC actions in DM treatment**

#### *Differentiation into insulin producing cells*

MSCs possess a developmental plasticity to adopt a pancreatic endocrine phenotype. Naturally, this was suggested to be the primary mechanism by which MSCs could be utilized in the treatment of diabetes. Studies have demonstrated that MSCs from various tissues and organs, such as bone marrow, adipose tissue, and Wharton's jelly within umbilical cords, are capable of differentiating into islet-like cells, or functional IPCs. The first incompletely differentiated IPCs expressing insulin and nestin were obtained by culturing rat bone marrow-derived MSCs (BM-MSCs) in medium with a high glucose, nicotinamide and beta mercaptoethanol concentration [8]. Comparative studies have also shown that Wharton's jelly-derived MSCs (WJ-MSCs) are easier to source and culture, in addition to their superior differentiation potential towards a mature pancreatic beta cell as compared to BM-MSCs. The higher expression of pancreatic and duodenal homeobox 1 (Pdx-1), insulin secretion, and mRNA expression of C-peptide in differentiated WJ-MSCs, as compared to BM-MSCs, also demonstrates superior insulin secretion properties of IPCs induced by WJ-MSCs [11].

However, some experimental evidence counters the efficacy of this mechanism in restoring islet functionality. These studies posit that differentiated IPCs are not the source of the regenerated pancreatic beta cells. *Ianus et al.* discovered significant regeneration of adult beta cells in diabetic mice after BM-MSCs transplantation, yet only 1.7 to 3% of regenerated islet beta cells were of bone marrow origin, indicating another source of these beta cells [12]. Another study discovered that donor green fluorescent protein positive cells in the islets did not express insulin after bone marrow

transplantation [13]. Together, these similar findings imply that the transdifferentiation from bone marrow-derived cells to pancreatic beta cells is rarely observed. Therefore, it remains controversial as to whether the reduction of hyperglycemia and restoration of normoglycemia in MSC transplanted diabetic models is due to MSC differentiation into IPCs, or the result of entirely distinct mechanisms.

### *Regeneration of islet beta cells*

In addition to IPC differentiation, MSCs also help to regenerate endogenous pancreatic islet beta cells by secreting various cytokines and growth factors. Si *et al.* discovered that infusion of MSCs resulted in significant endogenous beta cell regeneration in a diabetic rat model. Additionally, MSC infusion significantly improved insulin sensitivity as evidenced by elevations in phosphorylated insulin receptor substrate 1 (IRS-1), protein kinase B (Akt), and GLUT4 within insulin target tissues [7]. Deficiencies in expression of each of these are believed to be involved in the development of insulin resistance [7].

Lee *et al.* also demonstrated that, following the transfer of BM-MSCs into diabetic mice, there was an increase in regenerated mouse pancreatic islet beta cells that produce murine insulin [14]. However, only a few human cells appeared, further strengthening the theory that the insulin-producing effect of MSCs is not a result of MSC differentiation into IPCs, but rather is due to the capability of MSCs to induce regeneration of endogenous islet beta cells.

### *Immunomodulatory & antiapoptotic effect: protection of endogenous cells*

In addition to regenerating endogenous pancreatic islet beta cells, MSCs are also capable of protecting these cells through immunoregulation. This immunoregulation is believed to be the main mechanism through which MSCs exert their antidiabetic effects-through immunomodulation, MSCs can prevent the autoimmune destruction of insulin producing pancreatic beta cells in T1DM [15]. Immunoregulatory properties of MSCs include the abilities to: (1) suppress T cell responses to mitogenic and antigenic stimulation [6], (2) inhibit dendritic cell differentiation [16] and (3) inhibit B cell proliferation in a dose-dependent manner [17]. Ta-

ken together, these studies demonstrate how the immunosuppressive effects of MSCs can effectively reduce the autoimmune responses that would otherwise lead to the destruction of pancreatic beta cells.

The protection of endogenous pancreatic islet cells is further enhanced by MSCs' antioxidative and antiapoptotic effects. A cytokine analysis of ICAs derived from WJ-MSCs revealed an amplification of anti-inflammatory cytokines such as TGF- $\beta$  and TNF- $\alpha$  and a depletion of pro-inflammatory cytokines. In the same analysis, MSCs also displayed a capability to reduce total reactive oxygen species, nitric oxide, and superoxide ions; to downregulate Caspase3, Caspase8, p53; and to upregulate Bcl2, thereby confirming MSCs' antiapoptotic properties [4]. Diabetic hyperglycemia often leads to oxidative stress injury which further exacerbates the progression of diabetes. Therefore the antioxidative and antiapoptotic capacity of MSCs may further promote pancreatic islet cell survival, and thus prevent -- or at least slow -- the deterioration from impaired glucose tolerance to T2DM. The immunoregulatory properties of MSCs are therefore believed to be vital to the restorative effects observed in both T1DM and T2DM patients treated with MSCs.

### **Clinical application of MSC transplantation for the treatment of T1DM**

Here we summarize the results of four clinical trials that utilized autologous human stem cells for treating T1DM (**Table 1**). Different patient ethnicities, selection criteria for the trials, and treatment durations may have contributed to the heterogeneity of the results. Nevertheless, some methods of treatment produced more therapeutic results than others based on four major criteria - fasting plasma glucose level (FPG), C-peptide, glycated hemoglobin (HbA<sub>1c</sub>), and daily insulin injection dosage. In general, the use of MSCs or other forms of stem cells appear to be well tolerated, although a few studies showed less optimal results.

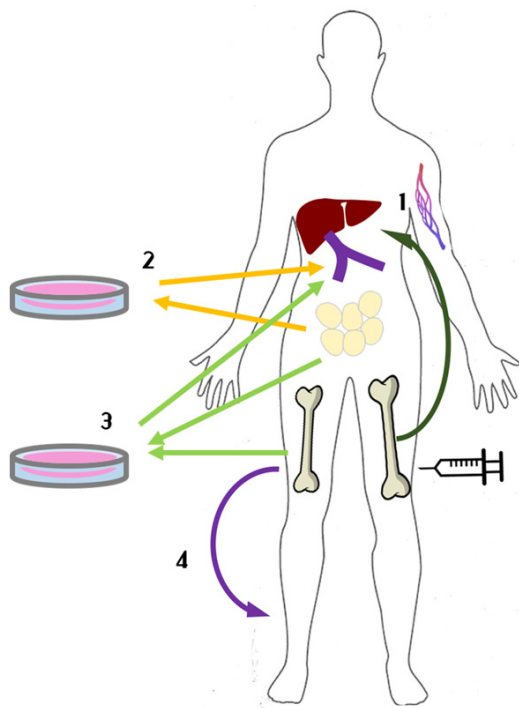
In a study conducted by Mesplez *et al.* [18], the patients under treatment were injected with colony stimulating factor (G-CSF) (**Figure 1**), and their bone marrow was extracted and implanted to the sixth segment of the liver on the fifth day. In the two patients treated, negative values in islet cell antibodies (ICA), glutam-

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**Table 1.** Summary of clinical trials using MSCs to treat patients with T1DM

Study	Treatment (See Figure 1)	n	Duration	FPG (mg/dl)	C-peptide (ng/ml)	HbA1c (%)	Insulin Dose (IU/day)
Mesples <i>et al.</i> 2013 <sup>a</sup>	1	3	Baseline	334 ± 171	0.53 ± 0.09	11.85 ± 1.84	6 ± 1
			12 months	122.5 ± 12.5	0.95 ± 0.36	7.5 ± 0.5	8 ± 2
Thakkar <i>et al.</i> 2015 <sup>b</sup>	2	10	Baseline (autologous IPCs)	269.6 ± 93.04	0.22 ± 0.21	10.9 ± 2.15	63.9 ± 20.9
			24 months (autologous IPCs)	218.0 ± 72.45	0.93 ± 0.24	7.5 ± 1.05	39.66 ± 9.37
	10	Baseline (allogeneic IPCs)	309.5 ± 67.01	0.02 ± 0.01	11.39 ± 1.9	57.5 ± 21.8	
		24 months (allogeneic IPCs)	202.5 ± 51.34	0.46 ± 0.29	8.01 ± 1.04	38.5 ± 13.34	
Dave <i>et al.</i> 2015 <sup>b,c</sup>	4	10	Baseline	269.6	0.22	10.99	64
			27 months	197.2	0.92	6.72	39
Carlsson <i>et al.</i> 2015	5	20	Baseline	N/A	0.29 ± 0.05	6.5 ± 0.4	0.43 ± 0.05 (IU/kg/day)
			12 months	N/A	0.32 ± 0.05	6.3 ± 0.2	0.39 ± 0.05 (IU/kg/day)

Most studies did not conduct statistical significance tests to compare follow-up values to baseline. However, overall values showed improvement at follow up for each of these studies. Carlsson *et al.* 2015 did not find any statistically significant differences between baseline and 12 month follow up. Zhao *et al.* found significant improvements in values including an increase in C-peptide, decrease in HbA1c, and lowering of daily insulin dose. <sup>a</sup>Due to small sample size, no statistical significance tests were performed. <sup>b</sup>Statistical significance tests comparing to baseline were not conducted. <sup>c</sup>No data on standard errors reported.



**Figure 1.** Schematic drawing of T1DM clinical trials treatment methods. 1) *In vivo* stimulation of bone marrow with G-CSF<sup>a</sup> and BMSCs implanted into the liver. 2) MSCs from adipose tissue (autologous or allogeneic) differentiated to IPCs and implanted into portal/thyroid circulation. 3) Mix of MSC-derived IPCs from adipose tissue and HSCs from BM injected to portal circulation. 4) BM-MSCs intravenously infused. <sup>a</sup>G-CSF, granulocyte colony-stimulating factor.

ic acid decarboxylase autoantibodies (GAD), and anti-insulin antibody levels were observed, along with increased levels of C-peptide and decreased blood glucose and HbA1c, thereby indicating increased insulin secretion and ame-

lioration of hyperglycemia in these patients. The success of this study suggests that treatment with autologous bone marrow stem cells (BMSCs) is effective. As demonstrated by their findings, BMSCs reverse the production and action of anti-pancreatic islet antibodies and significantly increase C-peptide concentrations [18]. However, the study may not be generalizable, as the small sample size limited the researchers' abilities to adequately evaluate the data and demonstrate statistical significance (Table 1).

Research presented by Thakker *et al.* [19] showed that isolated MSCs from human adipose tissue also displayed an ability to mitigate DM-induced hyperglycemia (Figure 1). MSCs were selected based on CD45<sup>+</sup>/73<sup>+</sup>/90<sup>+</sup> markers and were further differentiated to IPCs, which was confirmed by immunofluorescent markers such as Pax6, Isl-1, and Ipf-1. IPCs derived from MSCs were injected into the portal/thyroid circulation. There was a significant reduction in plasma glucose and HbA1c levels, augmented C-peptide levels, and decreased exogenous insulin requirement following the administration of the MSCs [19]. 0.81% of the infused cells were CD34<sup>+</sup>, much higher than Mesples *et al.* study (>0.22%) [18, 19]. Given that the Thakker *et al.* study [19] showed a better therapeutic result, it is reasonable to suggest that the higher percentage of CD34<sup>+</sup> IPCs, or greater hematopoietic stemness, may have contributed to the alleviation of T1DM prognosis. Although this study showed significant differences between the two treatment methods, they lacked statistical significance to demon-

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strate the efficacy of MSC therapy as they did not compare the results to baseline values (**Table 1**).

Using a methodology similar to that presented by Thakker *et al.* [19], another group of researchers showed significant improvement in DM clinical markers after MSC-derived IPC and autologous hematopoietic stem cell (HSC) co-infusion into the portal circulation (**Figure 1**) [20]. After 27 months of treatment, each of the ten patients showed significant improvement in all four of the criteria (FPG, C-peptide, HbA1c, and daily insulin dose) with no significant side effects [20]. Notably, the reduction of GAD antibodies from 331.1 IU/mL to 123 IU/mL indicates reduced beta cell damage, further supporting the idea that HSCs can slow down the destruction of beta cells, allowing increased insulin secretion. In summary, one group showed seemingly positive results with the use of IPCs and BMSCs in the treatment of T1DM [20]. However, the authors did not conduct statistical tests to demonstrate their results were significantly better than the baseline or report data on standard errors (**Table 1**).

Likewise, a randomized pilot study in which BM-MSCs collected from the iliac crest were transferred to T1DM patients showed only transient improvement in insulin secretion and no significant long-term improvement (**Figure 1**) [21]. CD 14<sup>-</sup>/31<sup>-</sup>/34<sup>-</sup>/45<sup>-</sup>/73<sup>+</sup>/90<sup>+</sup>/105<sup>+</sup> MSCs were cryopreserved and then intravenously infused four weeks later. C-peptide levels in these patients were rescued at ten weeks, but the therapeutic effect quickly diminished. One year following the intravenous infusion, there was no significant improvement in C-peptide levels, HbA1c values, or required insulin dose (**Table 1**) [21]. Along with the aforementioned clinical trials, it appears to be difficult to compare outcomes and obtain statistically significant results, thereby questioning the efficacy of MSC therapy in treating T1DM.

### Clinical application of MSC transplantation for the treatment of T2DM

As previously described, the primary insult in T1DM is the autoimmune destruction of pancreatic beta cells, making MSC transplantation and differentiation into IPCs a logical treatment. Conversely, the primary insult in T2DM is believed to be a result of insulin-sensitive tis-

ues failing to properly respond to insulin. At first glance, this seems to infer that MSC transplantation and differentiation into IPCs would have little clinical relevance for this population. However, as previously described, T2DM often results in decreased pancreatic beta cell mass and function [2]. How or when this destruction occurs exactly remains a topic of debate. Consequently, utilizing MSC transplantation for the treatment and possible cure of T2DM has become not only reasonable, but within the realm of possibility.

Clinical demonstrations of the efficacy of stem cell transplantations for the treatment of T2DM began with a published clinical trial by Bhansali *et al.* in 2009. Their group utilized autologous BMSCs, transplanting them into the pancreases of ten patients with T2DM with measurements taken at baseline and again at 6-months follow-up. They found 7/10 patients were able to decrease their insulin requirement by  $\geq 50\%$  and of those seven patients, two were able to discontinue insulin completely (7 and 41 days post-BMSC therapy, respectively). As a group, all patients demonstrated significant reductions in daily insulin requirements and HbA1c, while simultaneously displaying significant increases in fasting C-peptide levels (**Table 2**) [22].

In 2011, Jiang *et al.* [23] attempted a similar approach to T2DM in a pilot phase I clinical trial, utilizing placental-derived MSCs (PD-MSCs) to treat a total of ten patients with T2DM. Instead of a single dose, they administered three separate intravenous infusions of PD-MSCs to patients at 1 month intervals, with follow up testing conducted 3 months after the last infusion. Their findings corroborated those demonstrated by Bhansali *et al.* [22]: all patients treated with the PD-MSCs displayed significant reductions in daily insulin and HbA1c values, and significant increases in C-peptide levels (**Table 2**) [23].

Bhansali and colleagues performed another clinical trial in 2014. They designed a prospective, randomized, single-blinded and placebo-controlled study in order to evaluate both the efficacy and safety of BMSC therapy in patients with T2DM. A total of 21 patients with T2DM (11 cases, 10 controls) participated in the study and cases received an injection of autologous BMSCs while controls received a placebo

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**Table 2.** Summary of clinical trials using MSCs to treat patients with T2DM

Study	Treatment Method	n	Duration	FPG (mg/dl)	C-peptide (ng/ml)	HbA1c (%)	Insulin Dose (IU/day)
Bhansali et al. 2009	Autologous BMSCs injected into gastroduodenal artery	10	Baseline	136.5 ± 25.1	0.6 ± 0.1	8.4 ± 0.6	69.4 ± 6.6
			6 months	119.1 ± 21.3	1.1 ± 0.2 <sup>a</sup>	7.3 ± 0.8 <sup>a</sup>	28.2 ± 7.4 <sup>a</sup>
Jiang et al. 2011	Three IV infusions of PD-MSCs at 1 mo intervals	10	Baseline	N/A	2.6 ± 2.1	9.8 ± 2.2	63.7 ± 18.7
			6 months	N/A	3.5 ± 2.3 <sup>a</sup>	6.7 ± 1.2 <sup>a</sup>	34.7 ± 13.4 <sup>a</sup>
Bhansali et al. 2014	Two autologous BMSCs injections into superior pancreaticoduodenal artery at 12 wk interval	11	Baseline (Cases)	94.5 (87.7-103.4)	0.7 (0.3-1.2)	6.9 (6.4-7.1)	42.0 (31.0-64.0)
			12 months (Cases)	104.0 (98.5-118.5)	N/A	7.1 (6.6-7.5)	14.0 (0.0-30.0) <sup>a,b</sup>
		10	Baseline (Controls)	103.0 (95.0-112.3)	1.2 (0.7-1.6)	6.9 (6.2-7.0)	40.5 (31.8-44.3)
			12 months (Controls)	104.0 (101.0-113.0)	N/A	7.0 (6.9-7.5)	27.5 (23.5-33.3) <sup>a</sup>
Bhansali et al. 2017	Autologous BM-MSCs and BM-MNCs were injected into the superior pancreaticoduodenal artery	10	Baseline (BM-MSC)	104.4 (95.4-111.6)	0.4 (0.3-0.4)	6.9 (6.6-7.0)	47.5 (34.0-52.3)
			12 months (BM-MSC)	120.6 (115.2-120.6)	0.4 (0.4-0.5)	6.4 (6.0-7.1)	24.0 (12.0-33.0) <sup>a</sup>
		10	Baseline (BM-MNC)	113.4 (99.0-115.2)	0.5 (0.4-0.6)	6.7 (6.4-7.3)	68.0 (50.5-84.3)
			12 months (BM-MNC)	117.0 (111.6-120.6) <sup>a</sup>	0.7 (0.4-1.1)	7.0 (6.7-7.5)	38.0 (24.3-41.5) <sup>a,b</sup>
		10	Baseline (Controls)	108.0 (111.6-122.4)	0.5 (0.4-0.7)	6.5 (6.2-6.8)	48.5 (29.5-76.0)
			12 months (Controls)	113.4 (99.0-120.6)	0.7 (0.4-0.9)	6.1 (6.0-6.8)	45.0 (26.3-67.5)

Studies reviewed showed significant improvement in in T2DM patients treated with MSCs, especially in significantly decreasing daily required insulin doses. Bhansali et al. 2009 and Jiang et al. 2011 showed significant improvements in C-peptides, HbA1c levels, and insulin dosages using their distinct methods of MSC treatment. Bhansali et al. 2014 showed significant improvement in daily insulin requirements. Bhansali et al. 2017 showed significant decreases in daily insulin requirements for patients treated with BM-MSCs and BM-MNCs in addition to a significant increase in FPG levels in patients receiving BM-MNCs (negative result). <sup>a</sup>Statistically significant difference from baseline (P < 0.05). <sup>b</sup>Statistically significant difference between Cases and Controls (P < 0.05).

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injection. Follow-up measurements were taken at 3, 6, and 12 months, and all 21 patients maintained 12-month follow-up. Significant improvement between cases and controls was noted for insulin dosage and C-peptide levels. Furthermore, despite a reduction in insulin requirements by  $\geq 50\%$  in cases, HbA1c values were maintained below 7% (**Table 2**) [24].

More recently, in 2017 another randomized placebo-controlled study emerged, this time examining the efficacy of autologous BM-MSCs compared to bone-marrow derived mononuclear cells (BM-MNCs, effectively HSCs along with other mononuclear lymphoid cells). Ten patients received BM-MSCs, ten received BM-MNCs, and ten served as a control group and underwent a sham procedure. All patients were followed for 12 months. At 12 months follow-up, the BM-MSC group showed only a significant reduction in daily insulin requirements. Conversely, the BM-MNC group showed a significant reduction in insulin requirement and a significant increase in stimulated C-peptide at 12-months follow-up. The decrease in daily insulin requirement was greater in the BM-MNC group compared to the BM-MSC group. In addition, the BM-MSC group showed augmented insulin sensitivity post-treatment (**Table 2**) [25].

These studies clearly establish that MSC transplantation does indeed help to alleviate some of the metabolic burden T2DM places on the body. These studies have demonstrated the ability of these treatments to significantly lower the daily insulin requirements for patients [22-25], to lower HbA1c levels [22, 23], and to increase levels of circulating C-peptide [22]. Moving forward, future studies are required to further elucidate the extent of the efficacy of such therapy, as well as to better understand the mechanisms through which MSC transplantation can improve the T2DM population.

In T2DM rat models, MSC therapy has been demonstrated to reduce circulating blood glucose levels, effectively ameliorating hyperglycemia [7, 26-29]. Multiple mechanisms were found to be involved in this reduction, including the promotion of beta cell function and an improved insulin sensitivity in peripheral tissues. Insulin sensitivity is believed to improve due to upregulation of GLUT-4 expression and increased levels of phosphorylated IRS-1 and Akt in target tissues (both IRS-1 and Akt are

part of insulin signal transduction pathway to promote GLUT-4 translocation and glucose uptake). However, the ability of MSCs to ameliorate hyperglycemia may be short-lasting; thus far, reduced circulating glucose levels have been observed only transiently following MSC treatment. Si *et al.* found that a single MSC infusion led to alleviation of hyperglycemia for only four weeks. After that time, hyperglycemia steadily returned to pre-treatment values [7]. Hao and colleagues had similar findings, concluding that the same effects only lasted for approximately 2-3 weeks, even after administering serial infusions of MSCs [26]. Consequently, additional studies in animal models are needed to ascertain the sustainability of this treatment in the long term in addition to further elucidate the precise mechanisms of MSC action. One downside of animal studies such as these, however, is that the animal models used do not truly reflect a true T2DM pathogenic state in humans [24].

Additionally, more clinical studies in humans are required to determine potential adverse effects of such treatments. Bhansali *et al.* noted instances of nausea in 6/10 patients and vomiting in 1/10. Two patients developed a transient drop in hemoglobin that spontaneously corrected within a month [22]. Jiang and colleagues recorded no obvious side effects, and in fact noted improved renal and cardiac function to various degrees after MSC therapy [23]. Two episodes of nausea and vomiting were recognized in patients by Bhansali *et al.* in 2017, however this was post-glucagon administration for the assessment of beta cell function and was not an actual part of the MSC treatment regimen [25]. Even with minimal adverse effects, clinical studies to date have been small, and follow up has only been for 12 months, at maximum. Future studies with larger patient populations in addition to longer periods of follow up are necessary to further establish both the safety and efficacy of this treatment method. The true potential of stem cell therapy in the treatment of T2DM remains to be fully explored. Still, these and previously mentioned studies have a number of limitations including the lack of placebo arms, small sample sizes, and inconsistent methods for deriving MSCs-some used incompletely purified BM-MSCs, others further purified and used MSCs, and Jiang *et al.* [23] even utilized PD-MSCs.



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Additionally, routes of administration varied widely, even among animal studies. While the individual outcomes of these studies are promising, such inconsistencies and discrepancies between study design and methodology make the study outcomes as a whole difficult to compare.

### Conclusion

MSCs have been studied extensively for their therapeutic potential in the treatment of T1DM and T2DM. As a result of their endogenous pancreatic beta cell restorative capabilities in addition to their immunomodulatory properties, MSC therapy has been shown to ameliorate hyperglycemia in rat, murine, and human trials. Here, we focused on human clinical trials that used MSCs to treat patients with T1DM and T2DM in order to assess the efficacy of such therapy.

The studies reviewed demonstrated questionable benefits of MSC therapy for treating T1DM despite the ability of MSCs to differentiate into IPCs and induce Treg cell differentiation. These studies largely lacked statistical analysis for assessing differences between baseline and follow-up levels of FPG, C-peptide, HbA1c, and daily insulin dose. Nevertheless, three out of the four studies examined showed general improvements in each of these diabetic measures after MSC-based therapy. The other study did show, however, that there was no significant difference in baseline and follow-up measures 12 months after treatment.

Conversely, given the research presented here, T2DM patients did show improvements after treatment with MSCs. Each of the four reviewed studies demonstrated significant decreases in patients' daily insulin requirements, though significant changes in FPG, HbA1c, and C-peptide levels were not as consistent. Nonetheless, the decrease in insulin dosages accompanied by either maintenance of HbA1c levels below 7% or continually declining HbA1c levels indicates an improvement in the patients' T2DM status. The discrepancies in the strength and consistency of the effects on FPG and C-peptide highlight the potential differences in mechanisms through which MSCs may act to treat T1DM as compared to T2DM. Due to a lack of significance testing conducted in the available T1DM studies, it is difficult to compare outcomes, and

without further clinical trials it is impossible to definitively state the value of MSC therapy in treating T1DM. Therapies that utilize other non-MSC sources of stem cells, such as cord blood-SCs, however, have shown significant improvements in T1DM status [30]. Therefore, any benefits of MSC therapy in T1DM have yet to be elucidated, while it appears MSC administration to patients with T2DM does have therapeutic benefits.

Despite the robust improvements in the biomarkers for diabetes in both of these populations, many of the clinical trials examined have similar limitations. These include small sample sizes, lack of control arms in some cases, and inconsistent methods for deriving and administering MSCs. Although improvements in T1DM and T2DM were observed in many of the trials, the clinical measures assessed rapidly reverted to baseline and were not sustained longitudinally. A significant, sustained therapeutic effect from MSC therapy in T1 and T2DM has yet to be observed. Larger studies with more patients and longer follow-up times need to be constructed. Moreover, studies to determine the ideal source of MSCs and route of administration should be conducted for the purposes of consistency. These additional studies would also allow for a more accurate comparison between clinical trials. The studies presented reported no significant adverse effects of MSC therapy.

**Table 3** summarizes completed or ongoing clinical trials using MSCs as therapy for T1DM and T2DM. Given the number of such studies, this field is growing and it is our hope that greater progress will be made towards treating and potentially curing these high-morbidity diseases. In addition to these clinical trials, further animal studies are needed to continue to elucidate the specific mechanisms through which MSCs exert their anti-diabetic effects and to further study the gap between the positive outcomes seen in T1DM animal models treated with MSCs and the clinical trials presented here. Enhanced knowledge of these mechanisms will allow for more specific improvements to MSC transplantation for the treatment of DM. Additionally, with the advent of new technologies such as CRISPR, precise modifications and manipulations of stem cells may now be made which -- in combination with improved compre-

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**Table 3.** Summary of active/completed clinical trials around the world for the treatment of T1 and T2DM using MSCs

NCT Number	Study Title	Status	Treatment Method	T1 or T2DM
NCT00690066	PROCHYMAL® (Human Adult Stem Cells) for the Treatment of Recently Diagnosed Type 1 Diabetes Mellitus (T1DM)	Completed (12/2011, results not posted)	IV infusion of <i>ex vivo</i> cultured human MSCs	T1DM
NCT01068951	Treatment of Patients with Newly Onset of Type 1 Diabetes With Mesenchymal Stem Cells	Completed (9/2013) <sup>a</sup>	IV infusion of autologous MSCs	T1DM
NCT02940418	Use of Stem Cells in Diabetes Mellitus Type 1	Recruiting	Two IV infusions of donor-derived ASCs 6 months apart	T1DM
NCT03484741	Mesenchymal Stem Cell Therapy for Type 1 Diabetes Mellitus Patients	Recruiting	IV infusion of autologous BM-MSCs, allogeneic UC-MSCs <sup>b</sup> , and PRP <sup>c</sup>	T1DM
NCT02893306	MSC Administration for the Management of Type 1 Diabetic Patients	Active, not recruiting	IV infusion of allogeneic, <i>ex vivo</i> expanded MSCs	T1DM
NCT01576328	Safety Study of Mesenchymal Precursor Cells in Type 2 Diabetes	Completed (10/2015)	IV infusion of human MSCs, 3 different doses	T2DM
NCT03343782	Outcomes of Expanded Autologous Bone Marrow-derived Mesenchymal Stem Cells Therapy in Type II Diabetes	Recruiting	IV infusion vs. dorsal pancreatic a. Infusion of autologous BM-MSCs	T2DM
NCT01719640	MSC and BM-MNC in Type 2 Diabetes Mellitus	Completed (1/2015, results not posted)	IV infusion of BM-MSC+BM-MNCs+insulin vs. BM-MNCs+insulin	T2DM
NCT01759823	Bone Marrow Derived Stem Cell Transplantation in T2DM	Completed (10/2015) <sup>d</sup>	Autologous BM-MSCs and BM-MNCs injected into the superior pancreaticoduodenal artery	T2DM

Clinical trials were found using the search tool on [clinicaltrials.gov](http://clinicaltrials.gov) and the criteria "Diabetes Mellitus" (Condition or Disease) and "Mesenchymal Cells" (Other terms). <sup>a</sup>See **Table 1** for the study published by Carlsson *et al.* in 2015. <sup>b</sup>Umbilical cord-derived MSCs (UC-MSCs). <sup>c</sup>Platelet-rich plasma (PRP). <sup>d</sup>See **Table 2** for the study published by Bhansali *et al.* in 2017.

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hension of MSC therapeutic mechanisms in DM treatment -- may also enhance clinical outcomes and provide a potential cure for T1DM and T2DM.

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### Disclosure of conflict of interest

None.

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