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Original Article

Evaluation of the clinical efficacy of stem cell transplantation in patients with type 1 diabetes mellitus

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Abstract: *Background:* Stem cell transplantation is an emerging therapeutic option for type 1 diabetes (T1D). Here, we explored the therapeutic efficacy of stem cell treatment for T1D by meta-analysis. *Methods:* The search strategy included the following keywords: stem cell; haematopoietic stem cell (HSC); mesenchymal stem cell (MSC); cord blood stem cell (UBSC); and T1D. These were used to search the following databases: MEDLINE; the Cochrane Controlled Trials Register; EMBASE; the Wanfang Database; the China Science and Technology Periodical Database; and China Journal Net. *Results*: 32 eligible clinical trials met our inclusion criteria with a total of 712 patients including 405 males. Glycosylated haemoglobin (HbA1c) was decreased following stem cell therapy in patients with T1D (12 month: $P<0.001$; 9 month: $P=0.0003$; 6 month: $P<0.001$; 3 month: $P<0.001$). Moreover, after the 15-, 18-, 21- and 24-months long-term follow up, HbA1c still showed significant decreases (24 month: $P=0.002$; 21 month: $P=0.002$; 18 month: $P<0.001$; 15 month: $P=0.0003$). Meanwhile, C-peptide levels increased following stem cell therapy (12 month: $P<0.001$; 9 month: $P<0.001$; 6 month: $P<0.001$; 3 month: $P<0.001$) and also demonstrated benefits after long-term follow up (24 month: $P<0.001$; 18 month: $P<0.001$). Insulin requirements were also reduced in patients receiving stem cell therapy (3 and 6 months: $P<0.001$). The glutamic acid decarboxylase antibody (GAD-Ab) titer showed no decrease after the stem cell therapy in T1D at the 6 and 12 months follow up ($P>0.05$). Lastly, we investigated the immune function of regulatory T cells (Treg) and $CD4^+/CD8^+$ after the stem cell therapy in T1D. The analysis showed no significant decrease with Treg and $CD4^+/CD8^+$ at the 6 and 12 month follow up ($P>0.05$). *Conclusions*: Our meta-analysis of clinical trials suggests that stem cell therapy for T1D provides a sustained improvement in glycaemic control, enhanced the endogenous insulin production and the regeneration of β-cells, and maintained complete or very good partial responses for a long time. Thus, stem cell therapy provides a promising cell replacement therapy for T1D.

Keywords: Stem cell, meta-analysis, type 1 diabetes

Introduction

Type 1 diabetes (T1D) is an autoimmune disorder in which the body's immune system attacks and destroys pancreatic insulin-producing cells (IPCs) and generally affects children and young adults [1]. A European study that evaluated a population across 17 countries from 1989 to 2003, and which registered 29,311 new cases of T1D, predicted a doubling of new cases of T1D in children younger than 5 years in 2020. The prevalence under age 15 years would rise from 94000 in 2005, to 160000 in 2020 [2-4]. Unfortunately, insulin administration cannot exactly mimic the physiologic secretion of insulin in the body and cannot definitively cure diabetes [3, 4]. In an effort to explore a new therapy for T1D, stem cells (SCs) have been investigated.

To date, SCs have opened up new horizons for the cure of T1D with advantages of their immunological regenerative properties, especially for a specific population of T1D patients who do not respond to conventional therapy [4]. In 2003 for the first time, an HSC transplant was performed in a T1D patient by Voltarelli *et al* [5]. A recent analysis reported on 23 patients treated with HSC transplants with a mean follow-up of 30 months. 20 of these patients gained a

period of time being free of insulin therapy [6]. Due to the unique properties of stem cell from a variety of accessible source have bring endless hope for T1D, which were significant for their ability to control the autoimmunity, restore immune homeostasis, preserve the residual β cells, reverse β-cell destruction, and protect the regenerated insulin-producing cells against the re-attacking. And the stem cell types include embryonic stem cells (ESCs), umbilical cord blood-derived MSCs (UCB-MSCs), bone marrow (BM)-HSCs and BM-MSCs, adipose tissue-derived MSCs (ADSCs), induced pluripotent stem cells (iPSCs) and pancreas-derived multipotent precursor cells for the different origin, which have been all included in our meta-analysis [7, 8].

In China, Gulou Hospital affiliated to the Medical College of Nanjing University first performed a haematopoietic stem cell transplantation for the treatment of T1D in August, 2006 [9]. In November 2010, Shanghai Ruijin Hospital and Nanjing Gulou Hospital jointly completed 28 autologous stem cell transplantations in type 1 diabetic patients (clinical trial registration number: NCT00807651). In the trial conducted at the University of Nanjing in China, autologous HSC infusion induced an insulin-free state in four patients treated less than 3 months from diagnosis but not in 11 patients treated 3-12 months after diagnosis [10]. However, several issues remain to be clarified, particularly with regard to the potential contribution of concomitant immunosuppression.

The observation of Haller *et al* showed that autologous cord blood transfusion was safe and could maintain endogenous insulin production in T1D children, mainly due to the highly functional regulatory T cells (Treg) populations in cord blood [11]. Moreover, this study also demonstrated that stem cells can protect and recover the integrity of blood vessel and cell membranes in the islets, which also play important roles in T1D treatment. HSCs are, to date, among the most often used SCs in the clinic for the therapy of autoimmune diseases. Autologous HSC transplantation associated with nonmyeloablative conditioning and immunosuppression has been shown to reverse T1D in humans [12]. Although the use of unmanipulated CB-SCs and adult HSCs appears very safe, it is important to determine whether oncogenicity is linked to a specific lineage or sublineage and which cells are more likely to generate tumours. Two studies confirmed that MSCs may bear marked oncogenic potential, which must be addressed before moving forward with any MSC-based therapy in humans [13, 14]. It was also demonstrated that the levels of C-peptide secreted by in vitro differentiated islet-like cells from iPS cells and hESCs were very low compared with that of adult human β cells [15]. However, the mechanisms by which stem cells achieve β-cell regeneration and maturation in vivo are poorly understood.

Studies demonstrate that the generation of glucose-responsive β cells from human pluripotent stem cells (hPSC) in vitro could provide a source for cell transplantation therapy in diabetes. A scalable differentiation protocol has been reported by Melton *et al*, which can generate hundreds of millions of glucose-responsive β cells from hPSC [16]. Emerging data are modifying current concepts about how pancreas cellular identity is remodelled in response to developmental and environmental factors under both homeostasis and stress situations. These data could prove to be helpful in producing more valid therapies for T1D. Stable ESC lines have been generated that express key transcription factors involved in the development of pancreatic-cells. As well, procedures have been developed to avoid harm to patients with T1D. These steps have created novel protocols for the safe clinical uses of stem cell therapies [17]. The constant advances in this field, as well as the rapid progress of science, make the possibility of an effective stem cell therapy for T1D a realistic goal in the foreseeable future. Here, we address the efficacy of the stem cell therapy in T1D through a meta-analysis.

Materials and methods

Search strategy and selection criteria

The electronic search strategies used here have been described in our previous study [18]. The search terms included the medical subject headings of “stem cells”, “type 1 diabetes”, “mesenchymal stem cell”, “cell therapy”, “haematopoietic stem cell” and “beta cell” for the full text search. The initial search was performed in Nov. 2012, with updates in Dec. 2015. We also searched http://www.ClinicalTrials.gov websi-

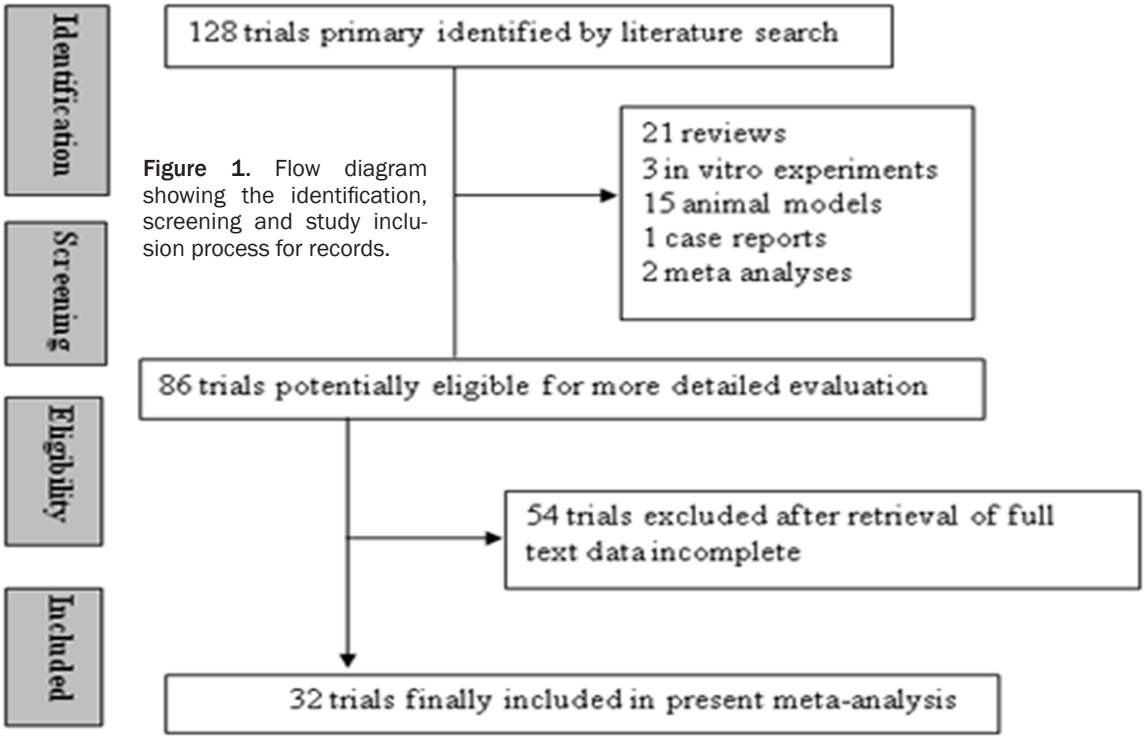


Figure 1. Flow diagram showing the identification, screening and study inclusion process for records.

te for the information of prospective and ongoing trials, which have been described in detail previously [18].

Data extraction and quality assessment

Data extraction and evaluation were independently conducted by three authors after referring back to the original publications. We collected the trial data including authors' names, journal, year of publication, sample size per arm, regimen used, median or mean age of patients, sex, history of T1D, glycosylated haemoglobin (HbA1c), C-peptide, insulin requirement, glutamic acid decarboxylase antibody (GAD-Ab) and immunity endpoints of patients and allocation of the clinical trial design in our study.

Definition of outcome measures

The first endpoint was HbA1c, which measures the degree to which haemoglobin is glycosylated in erythrocytes and reflects the exposure of erythrocytes to glucose in an irreversible time- and concentration-dependent manner. Secondary endpoints were C-peptide levels and insulin requirement, which denote the function of pancreatic islets. We also evaluated the levels of glutamic acid decarboxylase antibody (GAD-Ab) and the immune function of Treg and $CD4^+$/$CD8^+$ in T1D after the stem cell therapy.

Statistical analysis

The analysis was carried out mainly by pair-wise comparison before and after the stem cell therapy. Therapeutic effects were reflected in HbA1c, C-peptide, insulin requirement, GAD-Ab, Treg and $CD4^+$/$CD8^+$. The methods of data analysis have been described in detail in our previous literature [18, 19].

Risk of bias across studies

To maintain the availability and consistency of the analysis, we selected published results and assessable clinical trials in our systematic review. Patient information regarding the following of doctor's advice, dietary modifications and the taking of medication was not available and might lead to the overstatement of the therapeutic efficacy.

Results

Selection of the trials

The electronic search yielded 128 references. Through the review of titles and abstracts, 42

Table 1. Baseline clinical and laboratory parameters of patients

Authors and Year	Age (Mean)	No. of patients (male)	History of T1DM (years)	Regimens (per arm)	Regimens (cell number) dose	Study design
Voltarelli JC 2007 (Brazil) [4]	19.2±5.1	15 (11)	UK	Autologous peripheral blood stem cells	11.0×10^6/kg	A prospective phase 1/2 study
Mineo D 2008 (Italy) [20]	39±6.9	6 (2)	27±11.8	Hematopoietic stem cell (HSC)	4.3×10^6/kg	Prospective nonrandomized pilot study
Li 2010 (CHN) [21]	15.1±3.9	8 (8)	0.4±0.3	Autologous peripheral blood stem cells	2×10^6/kg	No control
Gu 2010 (CHN) [22]	18.8±4.4	18 (6)	UK	Autologous hematopoietic stem cell (HSC)	UK	No control
Snarski E 2011 (Poland) [23]	25.8±4.6	8 (4)	0.16±0.02	Hematopoietic stem cell (HSC)	3×10^6/kg	No control
Feng 2011 (CHN) [24]	13±3.9	16 (7)	0.82±0.73	Hematopoietic stem cell (HSC)	5.1±1.9×10^6/kg	No control
Li 2012 (CHN) [25]	14.1±4.0	13 (10)	0.41±0.26	Hematopoietic stem cell (HSC)	4.0±2.1×10^6/kg	Open-label prospective trial
Gu 2012 (CHN) [26]	17.6±3.7	28 (14)	2.8±1.10	Hematopoietic stem cell (HSC)	UK	A prospective AHSCT Phase II clinical trial
Zhao 2012 (USA) [27]	30±9 27±11 33±9	6 (4) 6 (5) 3 (3)	6±5 11±7 6±7	Cord blood stem cells (CBSC) Cord blood stem cells (CBSC) Sham therapy	UK UK UK	Open-label phase 1/2 study
Zhang 2012 (CHN) [28]	18.5±3.9 15.7±2.1	6 (2) 3 (3)	0.17±0.09 1.83±0.29	Hematopoietic stem cell (HSC)	13.2±6.2×10^6/kg 10.5±2.6×10^6/kg	Based on insulin dependent or free
Wang 2013 (CHN) [29]	18.0±4.2 19.2±3.5	22 (10) 22 (10)	1.98±0.11 1.95±0.05	Hematopoietic stem cell (HSC) Insulin therapy	UK -	Group according the subjects' willingness
Li 2013 (CHN) [30]	21±6.1	3 (2)	5.67±4.73	Cord blood stem cells (CBSC)	4~6×10^8	No control
Trivedi 2008 (USA) [31]	20.2±6.14	5 (2)	4.5±3.91	Adipose Tissue-Derived Mesenchymal Stem Cells (ADSCs) + Hematopoietic stem cell (HSC)	3.15×10^6 (ADSCs) + 16.3×10^6 (HSC)	No control
Yu 2011 (CHN) [32]	19.67±2.58 14.83±8.18	6 (3) 6 (4)	<0.4 <0.4	Umbilical cord mesenchymal stem cells (UCMSCs) Insulin therapy	1×10^7 -	1:1 control
Cai 2012 (CHN) [33]	27.5±5.9	21 (9)	9.2±4.8	Umbilical cord mesenchymal stem cells (UCMSCs)	1×10^6/kg	No control
Hu 2013 (CHN) [34]	17.6±8.7 18.2±7.9	15 (9) 14 (8)	Newly onset Newly onset	Umbilical cord mesenchymal stem cells (UCMSCs) Insulin therapy	5.2±2.4×10^7 -	Randomly
Tan 2011 (CHN) [35]	33.5	14 (7)	18.3	pancreatic islets	10200 IEQ/kg	No control
Giannopoulou EZ 2014 (Germany) [36]	3.02 6.6	7 (5) 10 (4)	101 days 139 days	Autologous cord blood Natural controls	1.27×10^6 $CD34^+$ cells	Non-randomized controlled, open label intervention trial
Alejandro Mesples 2013 (Argentina) [37]	7	3 (0)	Recently diagnosed	Autologous bone marrow stem cells	>180×10^6/kg, $CD34^+$ cells >0.22%	Preliminary trial
Francesca D'Addio (USA) 2014 [12]	20.4±5.5	65 (41)	New-onset	Autologous Nonmyeloablative Hematopoietic Stem Cell	5.8±0.8×10^6/kg	Two Chinese center and one polish center (multicenter)
Gu Yi 2014 (CHN) [38]	8.04±3.99 8.29±2.91	14 (5) 28 (10)	Newly diagnosed	Autologous hematopoietic stem cell (AHST); Conventional insulin therapy	Common AHST method	1:2 matched case-control study
Maryam Ghodsi 2012 (Germany) [39]	32.8±16.3	30 (15)	4.23±2.21	Fetal Liver-Derived Cell Suspension Allotrans-plantation	Approximately 35-55×10^6 Cells (7-11×10^6 $CD34^+$ HSCs)	Randomization

Michael J. Haller 2013 (USA) [40]	7.2 6.6	10 (8) 5 (3)	119 days 106 days	Autologous Umbilical Cord Blood Intensive diabetes management alone	1.1×10^7 cells/kg (0.4×10^7 to 3.9×10^7)	Open-label 2:1 randomized study
Voltarelli JC 2009 (Brazil) [5]	18.4±4.6	23 (17)	Newly diagnosed	Autologous nonmyeloablative hematopoietic stem cell	Mean number of CD34 cells 10.52×10^6/kg (range, 4.98-23.19×10^6/kg)	A prospective phase 1/2 study
Michael J.Haller 2011 (USA) [10]	5.1±2.59	24 (10)	0.25±0.24	Autologous umbilical cord blood	1.88×10^7/kg	Open-label phase I study
Dave SD 2015 (India) [41]	20.2	10 (9)	8.1	Mesenchymal stem cells differentiated into insulin-secreting cells (MSC, ISC) and bone marrow (BM)-dirived hematopoietic stem cells (HSC)	60.55×10^7/Kg (BW) HSC + ISC	Prospective non-randomized open-labeled clinical trial
de Oliveira GL 2012 (Brazil) [42]	19.4±5 20.9±5.1 (Control)	14 (10) 14 (10) (Control)	Newly diagnosed	High-dose immunosuppression followed by autologous haematopoietic stem cell transplantation (HDI-AHSCT)	Not avaibale	Healthy subjects paired with patients according to gender and age
Xiang H 2015 (CHN) [43]	16.7±4.83 Insulin-free (IF) 19.96±5.72 Insulin-dependent (ID)	71 (41) (IF) 57 (33) (ID)	No longer than 6 weeks from initial T1DM diagnosis to the beginning of treatment	Bone marrow hematopoietic stem cells	3.0×10^6 cells/kg BW	Open-label prospective
Snarski E 2015 (Poland) [44]	26±5	17 (12)	New onset	Autologous hematopoietic stem cell transplantation	3×10^6/kg [21]	Reference as [21]
Thakkar UG 2015 (India) [45]	20.2±6.9 19.7±9.96	10 (9) 10 (6)	8.1±3.4 9.9±7.1	Autologous stem cell therapy (SCT) Allogenic SCT SCT- bone marrow-derived hematopoietic stem cells	$2.65\pm0.8\times10^4$/kg $2.07\pm0.67\times10^4$/kg	Prospective, open-labeled, two-armed trial
Vanikar AV 2010 (India) [46]	21.1	11 (7)	8.2	Insulin-secreting mesenchymal stem cells (IS-AD-MSC) and cultured bone marrow (CBM)	2.7×10^9 1.2×10^8	Prospective open-labeled clinical trial
Haller MJ 2009 (USA) [47]	5.5	15 (7)	4.1 month	Autologous umbilical cord blood	1.50×10^7 cells/kg	A pilot study

The table summarises the basic patient information: age; the history of the diabetes; the details of the stem cell therapy, including cell type; the dose of the stem cell infusion; and the study design.

publications were excluded for different reasons (21 for being review articles, 15 for using animal models, 1 for being a case report, 3 for being in vitro experiments, 2 for being meta analyses). We selected 86 full texts articles as potentially relevant and retrieved them for more detailed assessment. Of these, 54 studies were excluded due to a lack of detailed patient clinical data, therapy response reports or the cell type for treatment. The selection procedure of the clinical trials is presented in **Figure 1**.

Characteristics of stem cell-based therapy

After the selection process, 32 eligible clinical trials with a total of 712 patients were included in the present analysis [4, 5, 10, 12, 20-40]. All of the trials were fully published. The clinical data of the trials are shown on **Table 1**.

Most of the patients in these studies had a good performance status. The patients' median age was 18.8 years. In these 32 trials of stem cell therapy in patients with T1D, 17 clinical trials used the haematopoietic stem cell therapy method [5, 12, 20, 22-26, 28, 29, 31, 38, 41-45], nine studies used cord blood stem cells (UBSC) [10, 27, 30, 32-34, 36, 40, 47], two used peripheral blood stem cells [4, 21], and the remainder of the trials used adipose tissue-derived mesenchymal stem cell (ADMSCs) [31], pancreatic islets stem cell [35], foetal liver-derived cell [39], or bone marrow stem cells [37]. In one trial, the ADMSCs and HSC were combined together for T1D treatment [31]. All of the trials were autologous transplantations, except one, which used allotransplantation [39]. Eight studies included a control group. Four of these were divided into two groups with either cell therapy or insulin therapy used in the clinical trials [29, 32, 34, 38]. In the other four studies, controls used were sham therapy, natural controls or intensive diabetes management alone [27, 36, 40, 42]. The number of stem cells transfused into patients in these studies was mainly 10^6/kg, with some using up to 10^7/kg. Most of the studies were designed as open-label phase 1/2 trials and did not include controls. The trials were performed worldwide, including USA, Poland, Brazil, Italy, Germany, Argentina, India and China. The patient information from the selected trials, such as gender, age, history of T1D, study design and stem cell types or doses, is listed in **Table 1**.

HbA1c

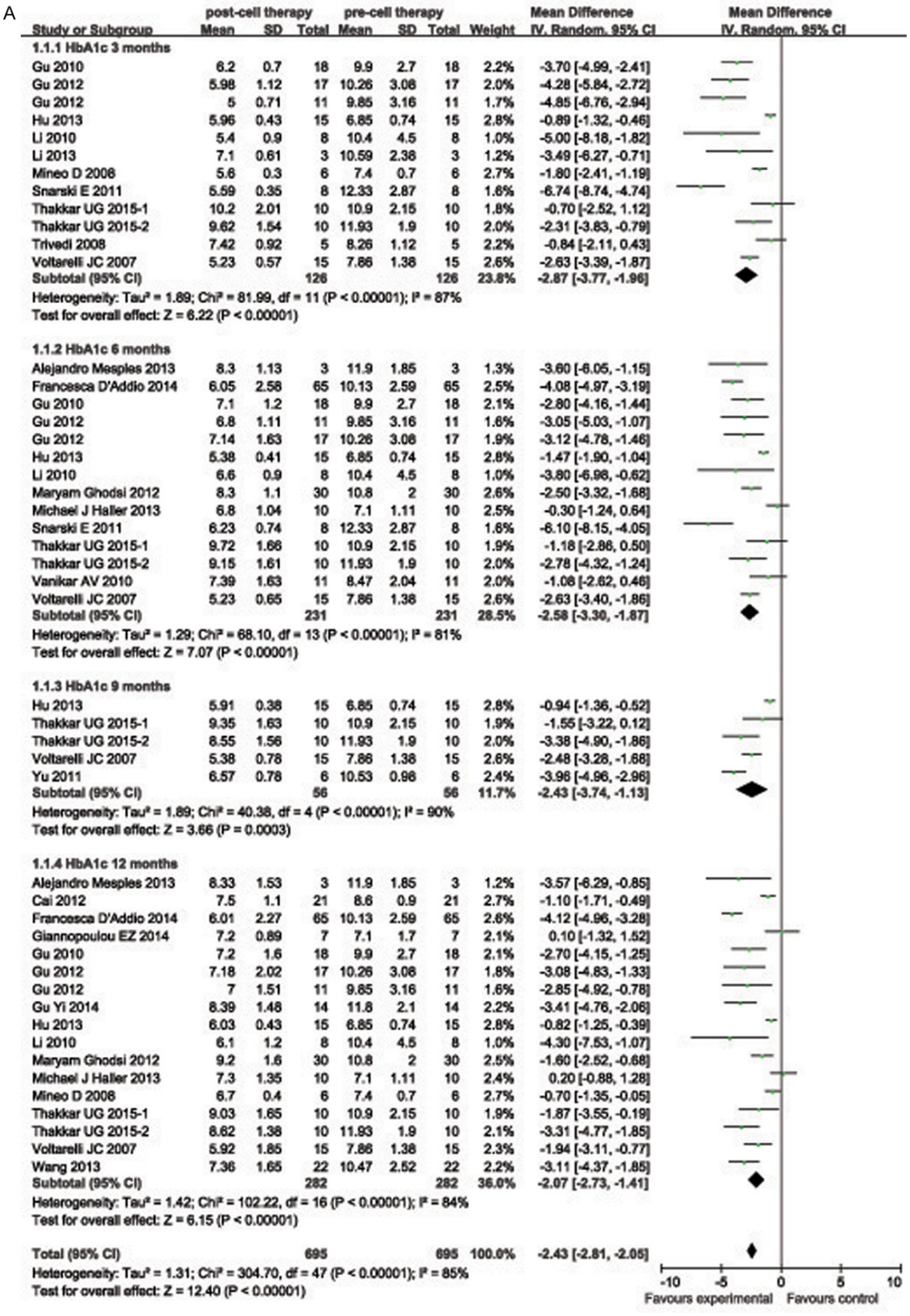
HbA1c is a stable marker of glycaemic control, and information on HbA1c was available in 23 trials [4, 12, 20-22, 26, 29-34, 36-42, 44-45] containing 448 patients (85 patients as a control group without stem cell therapy). The effect of the therapy upon HbA1c was compared with values before treatment. The estimated pooled MD for the 23 trials showed significant reductions in HbA1c in T1D after 3, 6, 9 and 12 months of follow up with the inverse variance model (MD -2.87, 95% CI -3.77 to -1.96, $P<0.00001$; MD -2.58, 95% CI -3.30 to -1.87, $P<0.00001$; MD -2.43, 95% CI -3.74 to -1.13, $P=0.0003$; MD -2.07, 95% CI -2.73 to -1.41, $P<0.00001$) (**Figure 2A**). We continued to collected the HbA1c data at the 15, 18, 21, 24 months follow up. The estimated pooled MD showed a highly significant decrease in HbA1c after 15, 18, 21 and 24 months (MD -2.13, 95% CI -3.29 to -0.97, $P=0.0003$; MD -2.39, 95% CI -3.43 to -1.34, $P<0.00001$; MD -2.21, 95% CI -3.61 to -0.81, $P=0.002$; MD -2.79, 95% CI -3.75 to -1.83, $P<0.00001$) (**Figure 2B**).

As for the efficacy of the stem cell therapy compared with control therapy, the estimated pooled MD for the four trials [29, 36, 38, 40], which included 118 patients of whom 65 were controls, showed no significant reduction in HbA1c with the inverse variance model (MD -0.27, 95% CI -0.98 to 0.45, $P=0.46$; MD 0.43, 95% CI -0.02 to 0.87, $P=0.06$) in T1D after the 6 and 12 months of follow up (**Figure 2C**).

C-peptide

C-peptide levels are the established biomarker for endogenous insulin release. Therefore, we collected the information on C-peptide levels which was available in 19 trials [4, 20, 22, 23, 26, 28, 30, 32-35, 37-39, 41, 42, 45-47]. These trials contained 308 patients (48 patients as a control group who did not receive stem cell therapy). The efficacy of the stem cell therapy, revealed by the estimated pooled MD for the selected trials, showed a significant C-peptide increase with inverse variance model (MD 0.52, 95% CI 0.30 to 0.74, $P<0.00001$; MD 0.42, 95% CI 0.26 to 0.57, $P<0.00001$; MD 0.36, 95% CI 0.22 to 0.50, $P<0.00001$; MD 0.41, 95% CI 0.25 to 0.57, $P<0.00001$) in T1D patients at 3, 6, 9 and 12 months after stem cell therapy (**Figure 3A**). We also compared the

A


post-cell therapy
pre-cell therapy
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
1.1.1 HbA1c 3 months
Gu 2010 6.2 0.7 18 9.9 2.7 18 2.2% -3.70 [-4.99, -2.41]
Gu 2012 5.98 1.12 17 10.26 3.08 17 2.0% -4.28 [-5.84, -2.72]
Gu 2012 5 0.71 11 9.85 3.16 11 1.7% -4.85 [-6.76, -2.94]
Hu 2013 5.96 0.43 15 6.85 0.74 15 2.8% -0.89 [-1.32, -0.46]
Li 2010 5.4 0.9 8 10.4 4.5 8 1.0% -5.00 [-8.18, -1.82]
Li 2013 7.1 0.61 3 10.59 2.38 3 1.2% -3.49 [-6.27, -0.71]
Mineo D 2006 5.6 0.3 6 7.4 0.7 6 2.7% -1.80 [-2.41, -1.19]
Snarski E 2011 5.59 0.35 8 12.33 2.87 8 1.6% -6.74 [-8.74, -4.74]
Thakkar UG 2015-1 10.2 2.01 10 10.9 2.15 10 1.8% -0.70 [-2.52, 1.12]
Thakkar UG 2015-2 9.62 1.54 10 11.93 1.9 10 2.0% -2.31 [-3.83, -0.79]
Trivedi 2008 7.42 0.92 5 8.26 1.12 5 2.2% -0.84 [-2.11, 0.43]
Voltarelli JC 2007 5.23 0.57 15 7.86 1.38 15 2.6% -2.63 [-3.39, -1.87]
Subtotal (95% CI) 126 126 23.8% -2.87 [-3.77, -1.96]
Heterogeneity: Tau² = 1.89; Chi² = 81.99, df = 11 (P < 0.00001); I² = 87%
Test for overall effect: Z = 6.22 (P < 0.00001)
1.1.2 HbA1c 6 months
Alejandro Mesples 2013 8.3 1.13 3 11.9 1.85 3 1.3% -3.60 [-6.05, -1.15]
Francesca D'Addio 2014 6.05 2.58 65 10.13 2.59 65 2.5% -4.08 [-4.97, -3.19]
Gu 2010 7.1 1.2 18 9.9 2.7 18 2.1% -2.80 [-4.16, -1.44]
Gu 2012 6.8 1.11 11 9.85 3.16 11 1.6% -3.05 [-5.03, -1.07]
Gu 2012 7.14 1.63 17 10.26 3.08 17 1.9% -3.12 [-4.78, -1.46]
Hu 2013 5.38 0.41 15 6.85 0.74 15 2.8% -1.47 [-1.90, -1.04]
Li 2010 6.6 0.9 8 10.4 4.5 8 1.0% -3.80 [-6.98, -0.62]
Maryam Ghodsi 2012 8.3 1.1 30 10.8 2 30 2.6% -2.50 [-3.32, -1.68]
Michael J Haller 2013 6.8 1.04 10 7.1 1.11 10 2.5% -0.30 [-1.24, 0.64]
Snarski E 2011 6.23 0.74 8 12.33 2.87 8 1.6% -6.10 [-8.15, -4.05]
Thakkar UG 2015-1 9.72 1.66 10 10.9 2.15 10 1.9% -1.18 [-2.86, 0.50]
Thakkar UG 2015-2 9.15 1.61 10 11.93 1.9 10 2.0% -2.78 [-4.32, -1.24]
Vanikar AV 2010 7.39 1.63 11 8.47 2.04 11 2.0% -1.08 [-2.62, 0.46]
Voltarelli JC 2007 5.23 0.65 15 7.86 1.38 15 2.6% -2.63 [-3.40, -1.86]
Subtotal (95% CI) 231 231 28.5% -2.58 [-3.30, -1.87]
Heterogeneity: Tau² = 1.29; Chi² = 68.10, df = 13 (P < 0.00001); I² = 81%
Test for overall effect: Z = 7.07 (P < 0.00001)
1.1.3 HbA1c 9 months
Hu 2013 5.91 0.38 15 6.85 0.74 15 2.8% -0.94 [-1.36, -0.52]
Thakkar UG 2015-1 9.35 1.63 10 10.9 2.15 10 1.9% -1.55 [-3.22, 0.12]
Thakkar UG 2015-2 8.55 1.56 10 11.93 1.9 10 2.0% -3.38 [-4.90, -1.86]
Voltarelli JC 2007 5.38 0.78 15 7.86 1.38 15 2.6% -2.48 [-3.28, -1.68]
Yu 2011 6.57 0.78 6 10.53 0.98 6 2.4% -3.96 [-4.96, -2.96]
Subtotal (95% CI) 56 56 11.7% -2.43 [-3.74, -1.13]
Heterogeneity: Tau² = 1.89; Chi² = 40.38, df = 4 (P < 0.00001); I² = 90%
Test for overall effect: Z = 3.66 (P = 0.0003)
1.1.4 HbA1c 12 months
Alejandro Mesples 2013 8.33 1.53 3 11.9 1.85 3 1.2% -3.57 [-6.29, -0.85]
Cai 2012 7.5 1.1 21 8.6 0.9 21 2.7% -1.10 [-1.71, -0.49]
Francesca D'Addio 2014 6.01 2.27 65 10.13 2.59 65 2.6% -4.12 [-4.96, -3.28]
Giannopoulou EZ 2014 7.2 0.89 7 7.1 1.7 7 2.1% 0.10 [-1.32, 1.52]
Gu 2010 7.2 1.6 18 9.9 2.7 18 2.1% -2.70 [-4.15, -1.25]
Gu 2012 7.18 2.02 17 10.26 3.08 17 1.8% -3.08 [-4.83, -1.33]
Gu 2012 7 1.51 11 9.85 3.16 11 1.6% -2.85 [-4.92, -0.78]
Gu Yi 2014 8.39 1.48 14 11.8 2.1 14 2.2% -3.41 [-4.76, -2.06]
Hu 2013 6.03 0.43 15 6.85 0.74 15 2.8% -0.82 [-1.25, -0.39]
Li 2010 6.1 1.2 8 10.4 4.5 8 1.0% -4.30 [-7.53, -1.07]
Maryam Ghodsi 2012 9.2 1.6 30 10.8 2 30 2.5% -1.60 [-2.52, -0.68]
Michael J Haller 2013 7.3 1.35 10 7.1 1.11 10 2.4% 0.20 [-0.88, 1.28]
Mineo D 2006 6.7 0.4 6 7.4 0.7 6 2.7% -0.70 [-1.35, -0.05]
Thakkar UG 2015-1 9.03 1.65 10 10.9 2.15 10 1.9% -1.87 [-3.55, -0.19]
Thakkar UG 2015-2 8.62 1.38 10 11.93 1.9 10 2.1% -3.31 [-4.77, -1.85]
Voltarelli JC 2007 5.92 1.85 15 7.86 1.38 15 2.3% -1.94 [-3.11, -0.77]
Wang 2013 7.36 1.65 22 10.47 2.52 22 2.2% -3.11 [-4.37, -1.85]
Subtotal (95% CI) 282 282 36.0% -2.07 [-2.73, -1.41]
Heterogeneity: Tau² = 1.42; Chi² = 102.22, df = 16 (P < 0.00001); I² = 84%
Test for overall effect: Z = 6.15 (P < 0.00001)
Total (95% CI) 695 695 100.0% -2.43 [-2.81, -2.05]
Heterogeneity: Tau² = 1.31; Chi² = 304.70, df = 47 (P < 0.00001); I² = 85%
Test for overall effect: Z = 12.40 (P < 0.00001)
-10 -5 0 5 10
Favours experimental Favours control

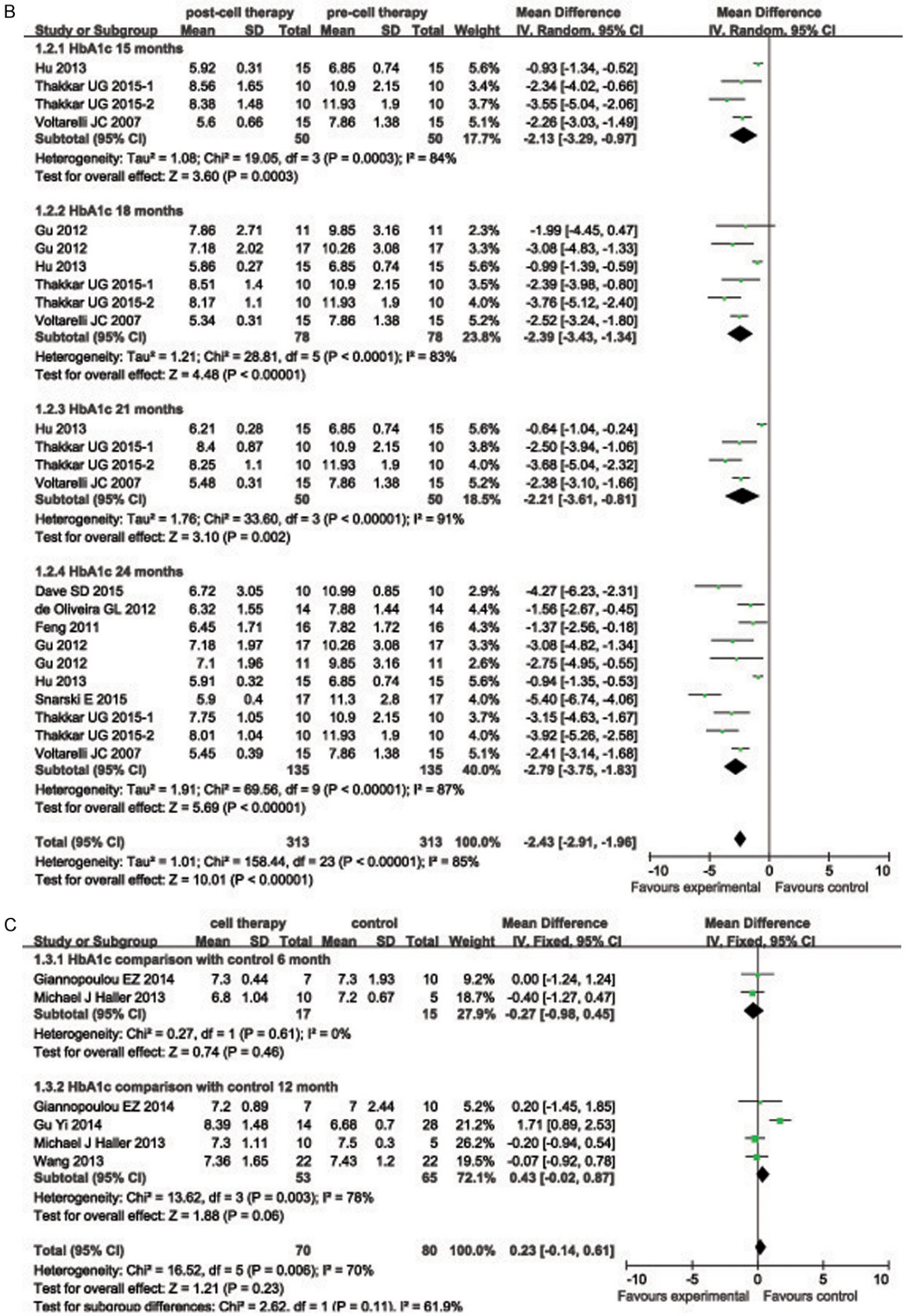
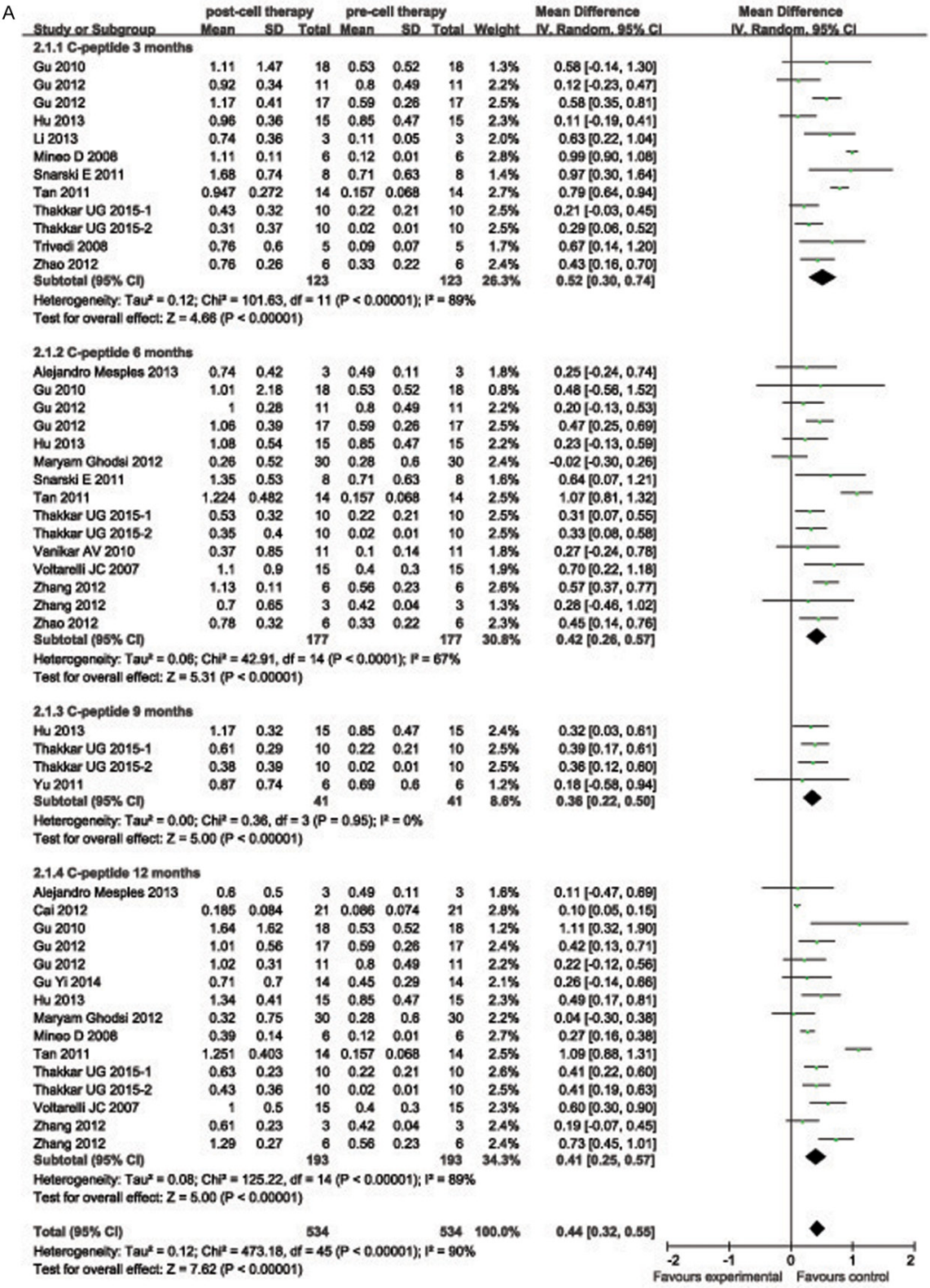


Figure 2. Comparison of glycosylated haemoglobin (HbA1c) after one year follow up (A); Comparison of HbA1c after up to two years follow up (B); Comparison of HbA1c with the control group (C). The random and fixed effects meta-analysis model (Mantel-Haenszel method) were used. MD, mean difference. Each trial is represented by a square,

the centre of which gives the MD for that trial. The size of the square is proportional to the information in that trial. The ends of the horizontal bars denote a 95% CI. The black diamond gives the overall MD for the combined results of all trials.

A



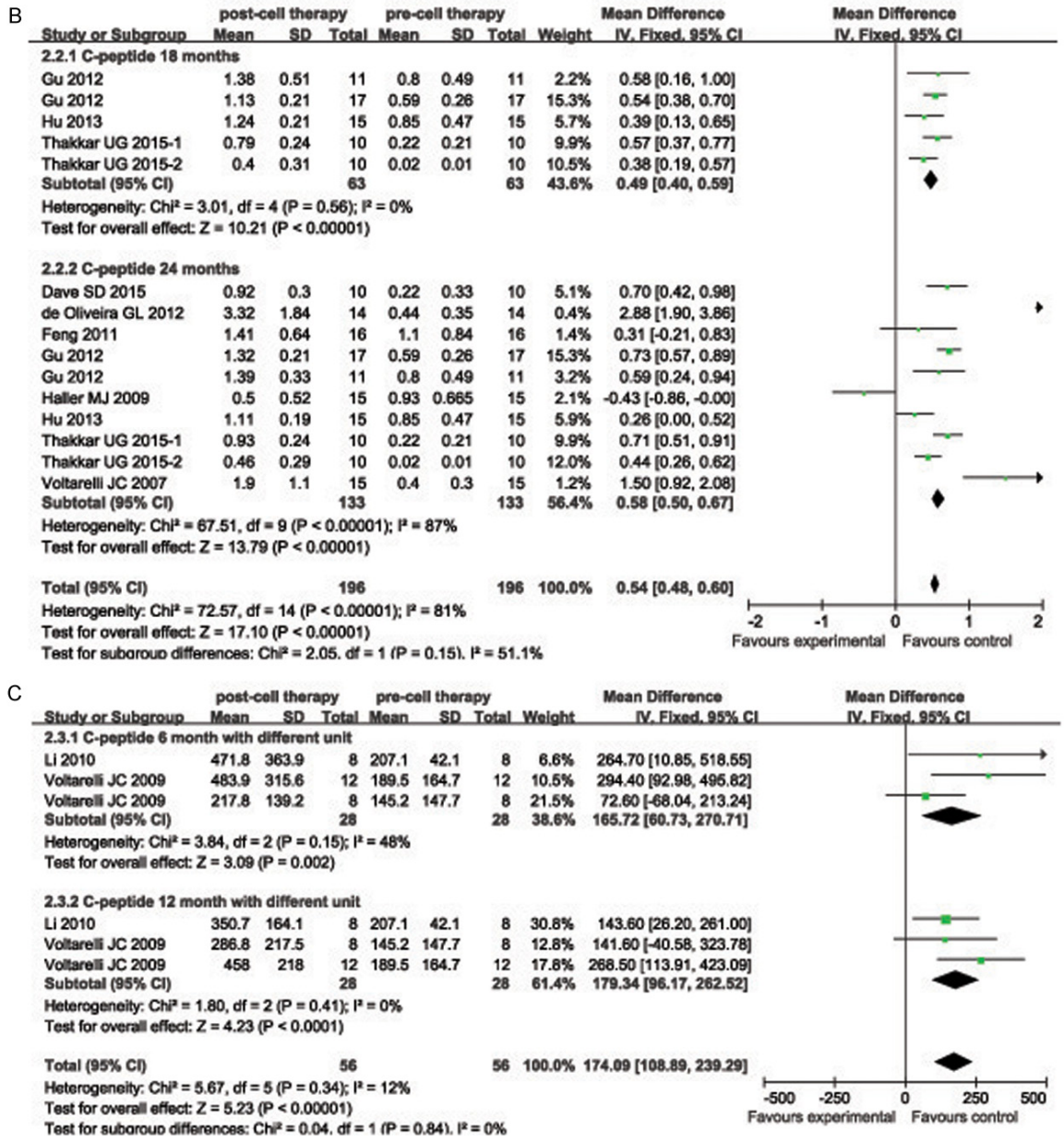


Figure 3. Comparison of C-peptide before and after stem cell treatment after one year follow up (A). The random effects meta-analysis model (Mantel-Haenszel method) was used in this analysis. Comparison of C-peptide before and after stem cell treatment with up to two years of follow up (B). Comparison of C-peptide levels with different units of measurement (C). The fixed effects meta-analysis model (Mantel-Haenszel method) was used in this analysis.

levels of C-peptide at long-term follow-up timepoints after the stem cell therapy. The estimated pooled MD showed a highly significant increase of C-peptide levels at the 18 and 24 months follow up (MD 0.49, 95% CI 0.40 to 0.59, *P*<0.00001; MD 0.58, 95% CI 0.50 to 0.67, *P*<0.00001) (**Figure 3B**). For different units of measurement, we collected another set of data to show the change in C-peptide levels from before to after the stem cell therapy. Analysis of the results showed a significant C-peptide increase at 6 and 12 months of follow up (MD 165.72, 95% CI 60.73 to 270.71, *P*=0.002; MD 179.34, 95% CI 96.17 to 262.52, *P*<0.0001) (**Figure 3C**).

Insulin requirement

Information on the insulin therapy requirement was available in 11 of the trials [20, 22, 23, 26, 28, 31, 35, 41, 45-47] containing 144 patients.

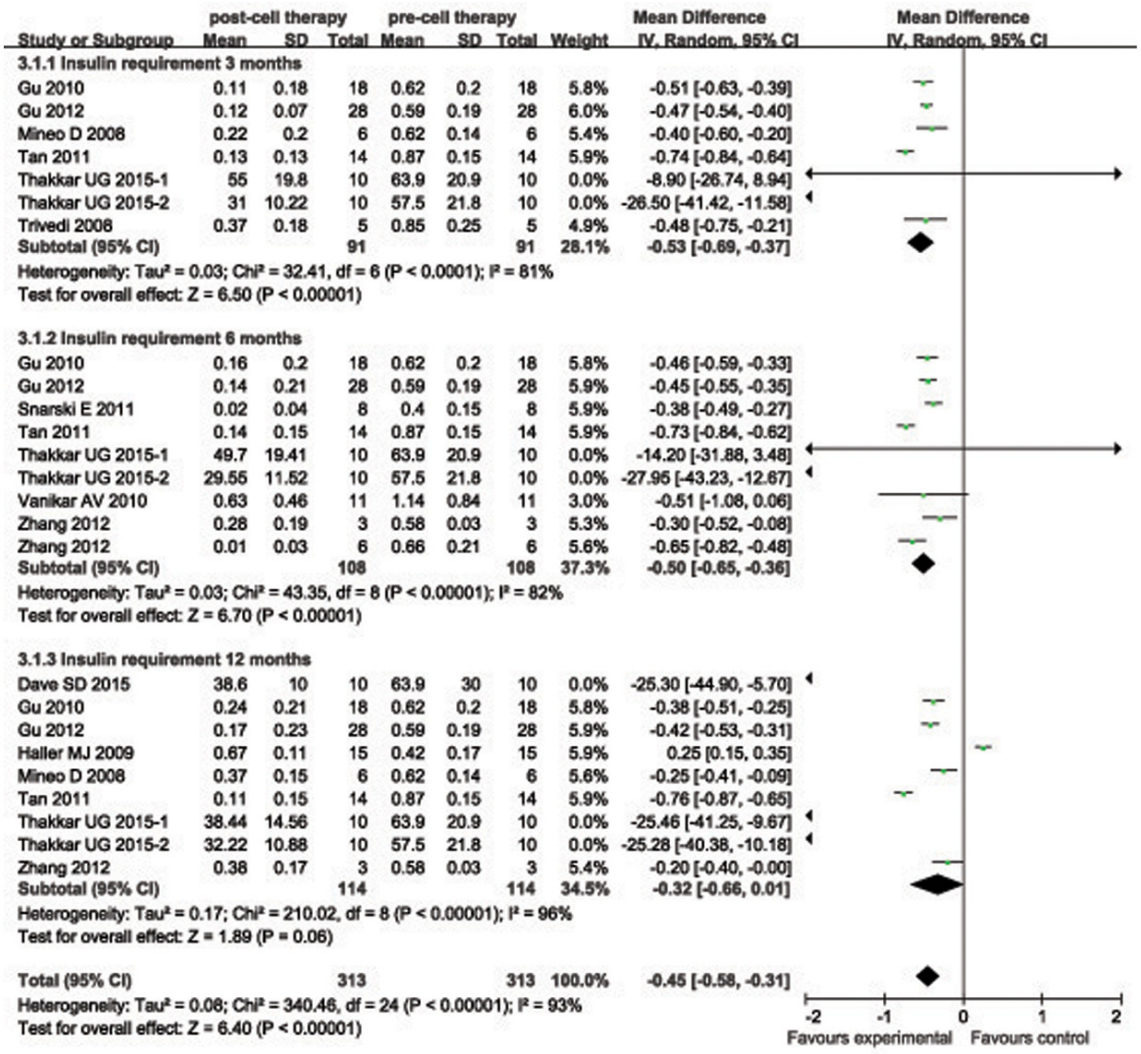


Figure 4. Forest plot for the insulin requirement assessment. The data come from the patients treated with stem cell therapy with one year of follow up. The random effects meta-analysis model (Mantel-Haenszel method) was used in this analysis.

To assess the efficacy of the stem cell therapy, we compared the levels of insulin required at different stages before and after stem cell treatment. The estimated pooled MD showed a highly significant decrease in insulin requirement at 3 and 6 months of follow up in the stem cell therapy group (MD -0.53, 95% CI -0.69 to -0.37, *P*<0.00001; MD -0.50, 95% CI -0.65 to -0.36, *P*<0.00001), but not at 12 months (MD -0.32, 95% CI -0.66 to -0.01, *P*=0.06) (**Figure 4**).

Glutamic acid decarboxylase antibody (GAD-Ab)

Glutamic acid decarboxylase is a major target of the autoimmune response that occurs in type 1 diabetes. Patients were classified by GAD antibody (GAD-Ab) in five of the clinical trials [4, 22, 41, 42, 47] containing 72 patients. To assess the efficacy of the stem cell therapy, we compared the levels of GAD-Ab before and after stem cell treatment. The estimated pooled MD showed no significant decrease in GAD-Ab at 6- and 12-months follow up in the stem cell therapy group (MD -116.39, 95% CI -436.99 to 204.21, *P*=0.48; MD -13.37, 95% CI -28.84 to 2.09, *P*=0.09) (**Figure 5**).

Immune system effect

Treg: The roles of regulatory T cells (Treg) in T1D are well known. Treg play key roles in preserving homeostasis and self-tolerance by inhibiting

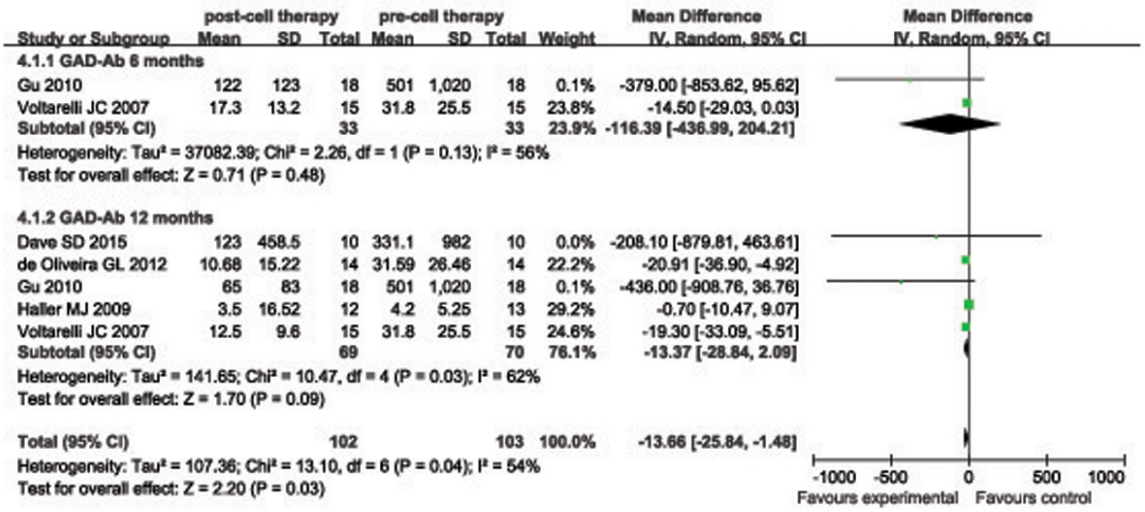


Figure 5. Forest plot for GAD-Ab. GAD-Ab is the glutamic acid decarboxylase antibody. Comparison of GAD-Ab before and after stem cell therapy. The random effects model (Mantel-Haenszel method) was used in this analysis.

auto-reactive effector T cells [6]. Evidence from both human trials and animal models has shown that the abnormalities in the quantity or function of Treg are involved in the initiation and progression of T1D [6, 7].

Thus, we collected the data of peripheral blood Treg to show the immune function of stem cell therapy in T1D. Information on Treg was available in three trials [10, 36, 40] containing 58 patients. To determine the efficacy of the cell therapy, we compared the levels of Treg before and after stem cell treatment. The estimated pooled MD showed no significant decrease in Treg at 6 and 12 months follow up in the stem cell therapy group (MD 0.23, 95% CI -0.17 to 0.64, *P*=0.26; MD -0.14, 95% CI -0.57 to 0.29, *P*=0.53) (**Figure 6A**). The Cochran's Q test had *P* values of 0.22 and 0.20, and the corresponding quantity I^2 was 34% and 38%, indicating that the degree of variability between trials was consistent with that which would be expected by chance alone.

$CD4^+/CD8^+$: For the changes in immune function, we collected the $CD4^+/CD8^+$ data before and after stem cell therapy from four clinical trials with 73 patients [10, 36, 40, 47]. The estimated pooled MD showed no significant increase of $CD4^+/CD8^+$ cell percentage after stem cell therapy at the the 6 and 12 months follow up (MD 0.07, 95% CI -0.2 to 0.34, P=0.61; MD -0.02, 95% CI -0.22 to 0.18, P=0.83) (**Figure 6B**). Cochran's Q test had *P* values of 0.63 and 0.43, and the corresponding quantity I^2 was 0%.

Discussion

Our study represents the first meta-analysis on the clinical efficacy of autologous stem cell transplantation in type 1 diabetes in populations from different countries. In our study, HbA1c and daily insulin requirement decreased significantly after stem cell transplantation and an improvement in C-peptide was demonstrated. However there was no significant change of GAD-Ab. These results are similar to our previous analysis in type 2 diabetes [48] and are equally exciting. Thus, our results suggest that stem cell therapy for T1D may provide improved glycaemic control, increased insulin biosynthesis, elevated insulin secretion from existing β-cells and might prevent islet-cell loss.

Our analysis showed significant HbA1c reduction in T1D treated with stem cells at 3, 6, 9, 12, 15, 18, 21, and 24 months compared with the control therapy. The results demonstrate that stem cells therapy may improve glycaemic control in T1D. The International Federation of Clinical Chemistry (IFCC) has provided a new reference method to obtain the actual glycosylated haemoglobin concentration expressed using the quantitative units of mmol/mol. Thus, in our analysis, we collected HbA1c data expressed as a percentage, as recommended by the American Diabetes Association (ADA) and also expressed as mmol/mol. We used the conversion formula HbA1c (mmol/mol) = [HbA1c (%)-2.15]×10.929, which could introduce the

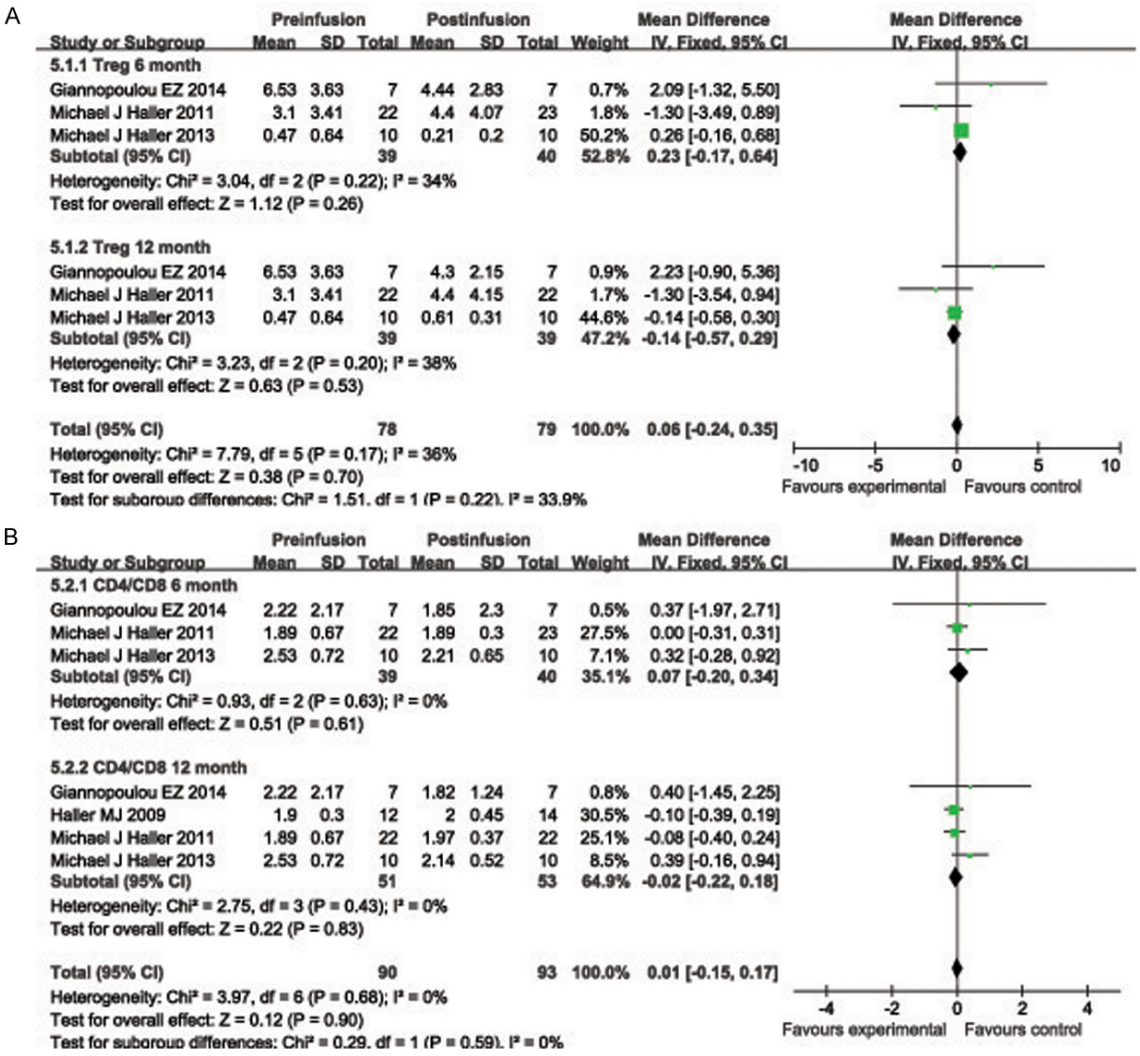


Figure 6. Comparison of immune function with Treg (A) and $CD4^+/CD8^+$ (B) before and after stem cell therapy. The fixed effects model (Mantel-Haenszel method) was used in this analysis.

errors for the analysis. Furthermore, some clinical trials used in this analysis reported the data with median value (interquartile range or ratio). For these data, we calculated the estimated standard deviation (SD) by the formula, (upper end of range-lower end of range)/1.35, and the estimated mean was equal to the median value. These calculations were included in our data acquisition process, which could introduce some bias into the analysis. In all, our results suggest that after a long-term follow up to two years, HbA1c levels were significantly decreased, and the stem cell therapies showed a clear improvement in glycaemic control. However, comparing the stem cell therapy data with the control data in the four trials for which these data were available did not show any significant reduction in HbA1c. Therefore, drawing robust conclusions might need clinical trials with larger sample sizes.

Our results also showed a significant reduction in the insulin requirement with stem cell treatment after 3, 6 and 12 months. In one study, stem cell therapy recipients were insulin free after the 12 month follow up, providing data values of zero in the analysis, which could not provide valid analysis [28]. In the same study, performed by Zhang *et al*, six patients became insulin free, while the remaining three patients still required insulin injection. These were defined as IF (insulin-free) group and ID (insu-

lin-dependent) group, respectively. The IF group showed more AHST-modified genetic events than the ID group and each group was associated with distinct patterns of top pathways, co-expression networks, and 'hub' genes (e.g., TCF7 and GZMA) [28]. In other studies, this group also reported stem cell recipients being temporarily, but not continuously, insulin free [22, 25, 26]. The achievement of exogenous insulin independent following nonmyeloablative, autologous HSC transplantation has been demonstrated in clinical trials with T1D patients [7, 26]. Thus, stem cell therapy can potentially increase insulin synthesis and decrease the requirement for insulin, which would be beneficial for T1D patients.

Our analysis showed significant C-peptide increase in T1D patients at 3, 6, 9, 12 and 24 months after stem cell therapy compared with pretreatment. The C-peptide levels and the insulin requirement demonstrate the function of the β-cells. While mechanisms by which MSCs and BMCs improve glycaemic control remain to be investigated, increased C-peptide levels suggested an increase of insulin biosynthesis in these studies. It has been reported that stem cells can serve as "tropic mediators" to support islet function in an indirect manner, such as promoting angiogenesis [49]. Recent research has shown that MSCs can be further differentiated to ISCs (insulin-secreting cells) which expressed the transcription factors ipf-1 (insulin promoter factor-1), pax-6 (paired box gene 6) and isl-1 (Islet1). All three genes centrally control reprogramming of non-pancreatic cells to acquire surrogate β-cell functions [45]. In addition, adipose-derived stem cells (ADSCs) therapy can also reduce the inflammation of cell infiltration and improve pancreatic expression of insulin and Pdx1 (pancreatic duodenal homeobox 1) [50]. Based on these studies and the results of the clinical trials, we conclude that stem cell therapy in type 1 diabetes increased C-peptide levels although this conclusion is limited by the accuracy of the C-peptide level measurements and the selection criteria. Some studies presented the C-peptide data with AUC level. We combined these data, and the results of the analysis also showed similar increases in C-peptide. Thus, the stem cell therapy improves the C-peptide levels in T1D and may involve β-cell differentiation and maturation. Although not appearing to differentiate into insulin-producing cells themselves, autologous HSCs may aid in the preservation of residual β-cells and promote an increased β-cell mass by enhancing neovascularisation, decreasing apoptosis, and/or stimulating proliferation [12].

The presence of positive GAD-Ab is a clinical/metabolic parameter of T1D that broadly comprises the inclusion criteria for every clinical trial [7]. Thus, here we compared the anti-GAD antibody titer data. After stem cell therapy in two trials that included 33 patients, GAD was not apparently decreased at 6 or 12 months. In the patients included in these studies, persistence of anti-GAD antibodies, even at low titers, showed that the conditioning regimen was not fully ablative for autoreactive B-cell clones and confirmed that the magnitude of the humoral response is not predictive of beta cell reserve or clinical response [51]. These data and the C-peptide data suggest that stem cell therapy in T1D elevates insulin secretion from existing β-cells and might prevent islet-cell loss. Randomised controlled trials that confirm and evaluate the therapeutic potential of stem cell in the treatment of T1D are needed.

Immunological tolerance via clonal exhaustion, cytokine effects, and alterations in immune cell repertoires has been suggested as outcomes of stem cell therapies for T1D. In our meta-analysis, we found no significant involvement of immune function in stem cell therapy in T1D, with no change in the Treg cells and the $CD4^+$/$CD8^+$ cells after the stem cell transplant. The receptor-mediated interactions of CD40-CD40 ligand, TNF-TNF receptor and Fas-Fas ligand impact β-cell destruction by secreting proinflammatory cytokines and ROS (reactive oxygen species), and releasing granzymes and perforin from cytotoxic effector T cells as described in detail previously [7, 52]. Research into the mechanisms of T1D have demonstrated that systemic immune alterations, which include T lymphocytes helper (Th) 1 and 2 recovery, the balance of cytokines in blood, and local regulation by the unique distribution of transforming growth factor-β1 ('a TGF-β1 ring') in pancreatic islets [37, 53], were associated with the control of diabetes. It has been demonstrated that MSCs and HSCs have immunomodulatory effects on T cells, B cells, dendritic cells (DCs), and natural killer cells (NK cells), which would

be helpful to reverse hyperglycaemia and ameliorate islet graft rejection in T1D [4, 7, 54]. The potential of stem cells from various resources have been confirmed to restore immune homeostasis [7, 55]. However, our analysis showed no positive immune effect of the stem cell therapy in T1D.

In short, the application of stem cell therapy in cure for T1D appears promising and tolerable with bona fide hope for a permanent cure without serious side effects.

Limitation

Although our meta-analysis showed that autologous stem cell implantation is safe and effective for the majority of T1D patients, it also has certain contraindications. Some studies mentioned that the therapeutic effects were influenced by the degree to which the patients followed their doctor's advice, such as regarding diet control and medication. Selection criteria for these studies included the presence of diabetes-associated complications and comorbidities, poor glucose control despite intensive insulin therapy, and symptoms such as severe hypoglycaemic episodes and hypoglycaemia unawareness that significantly incapacitate the patient. These factors might impact the quality of the meta-analysis.

Thus, the present study has several limitations. First, from a clinical trial point of view, the two groups in the study should have exactly the same background except for the intervention factor. However in some studies treatment with stem cell is a surgical operation, and as a result, patients necessarily endure more distress. Also various clinical designs such as prospective, nonrandomised, open-label and random trials were included in our meta-analysis, which may lead to distribution and implementation bias. This was the most important limitation of this study [48]. Second, subjects in each study were recruited from patients with T1D without severe complications, and the involved numbers were relatively small. Thus, these results need to be replicated in a larger cohort. In addition, follow-up studies confirming the duration of insulin independence and delineating the mechanisms of action are vital to confirm and evaluate the therapeutic potential of stem cell in the treatment of T1D.

In our analysis, stem cell therapies for T1D patients resulted in improved glycaemic control, elevated insulin biosynthesis, increased insulin secretion from existing β-cells, reduced islet-cell loss and might involve immune function. Hence, the efficacy of the application of a promising therapy for T1D could eventually benefit millions of T1D patients and alleviate the economic burden placed upon countries.

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Disclosure of conflict of interest

None.

Authors' contribution

Conceived and designed the meta-analysis: ZX Wang JX Cao. Performed the experiments: ZX Wang, JX Cao, YQ Zhao, GC Ding. Analyzed the data: JX Cao, JL Liu, JL Li, Y Liu, BL Xu. Wrote the paper: ZX Wang, JX Cao, M Wang.

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