*Cytotherapy*, 2015; 0: 1–8

International Society for Cellular Therapy ISCT®

# Predictive factors for prolonged remission after autologous hematopoietic stem cell transplantation in young patients with type 1 diabetes mellitus

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**Abstract**

*Background aims.* Autologous hematopoietic stem cell transplantation (auto-HSCT) followed by immunoablation is a promising therapy for type 1 diabetes mellitus (T1DM) treatment due to the immunosuppression and immunomodulation mechanisms. Indeed, a considerable number of patients have been able to discontinue insulin use with this treatment. However, nonresponse and relapse occur after auto-HSCT. It is important to select the patients who can potentially benefit from this treatment, but the factors that might influence the therapeutic outcome are unclear. The objective of this study was to explore the predictors for prolonged remission after auto-HSCT therapy. *Methods.* The data for this study were extracted from an open-label prospective study, which was performed to treat new-onset T1DM patients with auto-HSCT. The 128 patients were categorized into insulin-free (IF) or insulin-dependent (ID) groups according to their response to treatment during the follow-up. We compared the baseline data of the two groups and explored possible prognostic factors and their odd ratios (ORs) with univariate analysis and multivariate logistic regression. Receiver operating characteristic curves (ROC) were performed to test the model discrimination function. *Results.* During a follow-up of 28.5 ± 8.3 months, 71 of 128 patients in the IF group discontinued insulin use, whereas 57 of 128 patients in the ID group did not decrease their insulin dose or resumed insulin treatment after a transient remission. Multivariate logistic regression analysis demonstrated that prolonged remission was positively correlated with fasting C-peptide level (OR = 2.60, 95% confidence interval [CI]: 1.16–5.85) but negatively correlated with onset age (OR = 0.36, 95% CI: 0.14–0.88) and tumor necrosis factor-α levels (OR = 0.32, 95% CI: 0.14–0.73). ROC analysis confirmed the combined predictive function of these three variables (AUC = 0.739, 95% CI: 0.655–0.824). *Conclusions.* Age and fasting C-peptide and tumor necrosis factor-α levels were identified as possible predictors for prolonged remission following auto-HSCT therapy.

**Key Words:** *autologous hematopoietic stem cell transplantation, predictive factors, remission, type 1 diabetes mellitus*

## Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by insulitis, resulting in β-cell destruction and decreased insulin production [1]. Patients will eventually take exogenous insulin to regulate their glucose metabolism, which relieves the symptoms or delays complications but does not address β-cell destruction [1]. Scientists have been searching for new methods to stop autoimmune attacks in the early stages of disease to delay or reverse T1DM progression [2]. Allogenic hematopoietic stem cell transplantation (HSCT) has been applied for autoimmune disease for the last two decades [3], and it was gradually introduced into T1DM treatment with the objective of eliminating autoreactive T-cell clones and "resetting" the immune system with infused HSCs [4]. However, the clinical application of allogenic HSCT was impeded by high mortality and related complications, such as GVHD [5], cardiovascular disease [6], hypertension [7], hyperglycemia [8], endocrine complications [9], and secondary autoimmune diseases [10,11].

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*(Received 7 April 2015; accepted 4 July 2015)*

ISSN 1465-3249 
http://dx.doi.org/10.1016/j.jcyt.2015.07.006

Over the past 10 years, autologous (auto)-HSCT has emerged as a new therapy to cure T1DM. During this process, hematopoietic stem cells were derived from peripheral blood stem cells, which were mobilized from bone marrow by granulocyte colony-stimulating factor. Auto-HSCT was then performed in patients who were conditioned by immunoablation. The first report on the treatment of new-onset T1DM with auto-HSCT was published by Voltarelli *et al.* [12], which had an encouraging result of 86.95% (20 of 23) remission for an average of 29.8 months [13]. Auto-HSCT was demonstrated to reset the disturbed immune system through immunomodulation [14] and anti-inflammatory mechanisms [12,15,16]. By now, several clinical trials have been performed to treat T1DM with auto-HSCTs [14,15,17], most of which have achieved beneficial effects, except that nonresponse and relapse occurred in some patients [18–20]. Even though diabetic ketoacidosis and high-dose steroid use have been identified as adverse factors, and exclusions were made from the enrollment [21,22], whereas other factors influencing or predicting remission were still unknown. Identification of these prognostic factors may facilitate the selection of the most appropriate candidates and increase remission rates. It may also be useful for predicting the outcomes of individual patients after auto-HSCT.

## Methods

### *Study design and population*

This study was a retrospective analytical study of auto-HSCT treatment for T1DM patients. The open-label prospective clinical trial was conducted from January 2008 through July 2010 at the Biotherapy Department of PLA 455 Hospital (Shanghai, China). With oversight from a planning committee, the principal investigators designed the protocol and received ethical approval from the Institutional Ethics Committee of PLA 455 Hospital. Informed consent according to the Declaration of Helsinki was signed by all of the patients or by the parents of patients younger than 18 years old. All patients were diagnosed by the 2006 diagnostic standards of the American Diabetes Association [23]. The inclusion criteria were as follows [12]: aged 12 to 35 years, no longer than 6 weeks from initial T1DM diagnosis to the beginning of treatment, presence of anti-glutamic acid decarboxylase (anti-GAD) antibodies and detectable C-peptide levels in serum. The exclusion criteria were a history of ketoacidosis coma or high-dose steroid use before transplantation, active viral and bacterial infections, liver disease (cirrhosis or hepatitis), kidney disease (glomerular filtration rate <60 mL/min), severe allergic diseases, pregnancy, and other diseases or conditions that were not suitable for immunosuppressive treatment. Among the enrolled patients (n = 150), 8 were excluded for incomplete clinical or laboratory records, and 14 lost contact after the treatment. A total of 128 patients (n = 128) were included in our retrospective study.

### *Stem cell mobilization*

Bone marrow hematopoietic stem cells were mobilized with cyclophosphamide and granulocyte colony stimulating factor (Filgrastim, Kirin, Tokyo, Japan) [12,24]. Cyclophosphamide (2.0 g/m$^2$) was infused in two divided doses daily, and its metabolites were bound by Mesna (sodium 2-mercaptoethanesulfonate, 4 g/m$^2$ in 24-h infusion). A daily dose of granulocyte colony-stimulating factor (10 μg/kg) was administered the day after cyclophosphamide infusion until the cell collection was completed. Leukapheresis was performed according to the CD34+ cell concentration, initiating at 10 cells/μL and ending when the CD34+ cell counts were no less than 3.0 × 10$^6$ cells/kg body weight. The stem cells were then frozen in 10% dimethyl sulfoxide and stored in liquid nitrogen until use [12].

### *Conditioning regimen*

The patients were conditioned before cell transplantation. Cyclophosphamide was administered intravenously over 1 hour at a daily dose of 50 mg/kg on days 5, 4, 3 and 2 before the stem cell infusion. Rabbit antithymocyte globulin (thymoglobulin, IMTIX Sangstat) was administered at a daily dose of 0.5 mg/kg on day 5 before transplantation and at a daily dose of 1 mg/kg on days 4, 3, 2 and 1 before stem cell infusion [12,13,24].

### *Auto-HSCT*

Stem cell infusion was performed on day 0. Cell viability was calculated using the ADAM MC Auto Cell Counter (NanoEnTek). After transplantation, a daily dose of granulocyte colony-stimulating factor (5 μg/kg) was administered to stimulate HSC proliferation until the neutrophil count was greater than 1000/μL [12]. The patients were hospitalized after auto-HSCT in the Hematology Department Intensive Care Unit. When discharged, the patients were informed that they should comply with general guidelines and lifestyle modifications (diet, physical activity and avoidance of infection risk) at home.