Author's response to reviews

**Title:** Reversal of type 1 diabetes via islet beta cell regeneration following immune modulation by cord blood-derived multipotent stem cells

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**Version:** 3  **Date:** 17 November 2011

**Author's response to reviews:** see over
**Answers to reviewer’s comments:**

We appreciate the reviewers’ very constructive critiques of our manuscript. We found them to be extremely useful in guiding our revisions to improve the manuscript. We have followed each of the suggestions and comments.

**Answers to reviewer #1:**

1. **Abstract:**

   a) **Please clarify the abbreviation by changing the order of the words (CB-SCs).**

   We have changed the initial description of CB-SCs to indicate that they are cord blood-derived stem cells.

   b) **Please explain the “Stem Cell Educator”**.

   We have updated the **Methods** section to include a description of the design of the Stem Cell Educator.

2. **Materials and Methods:**

   The question arises from which donor or recipient source the CB-SCs in the “Stem Cell Educator” came from.

   We have clarified that CB-SCs are generated from allogeneic cord blood donors (see Page 8). Our previous work has demonstrated that CB-SCs display very low immunogenicity and fail to stimulate the proliferation of allogeneic T lymphocytes (Zhao Y, et al. Experimental Cell Research, 2006, 312: 2454-2464; Zhao Y, et al. Immunology Letters, 2007, 108: 78-87).

3. **Results:**
a) Please explain the comparative view of the non-treated and treated group (A or B) starting with Fig. 2.

We have added this information and clarified the difference between control and treatment groups. The composition of groups A and B is further clarified in the text (see page 10, first paragraph under Results).

b) Please include a table 2 after treatment like table 1 before treatment to see the important clinical changes.

We appreciate your suggestion. We think the improvement of fasting C-peptide levels is an important outcome for monitoring the efficacy of Stem Cell Educator therapy. Therefore, we list the C-peptide levels from each subject and calculate their changes in comparison with the baseline levels of C-peptide in a new Table 2.

4. Discussion:

a) Please include ref. for the immune cell infiltrate of human pancreatic islets after diabetes manifestation (Richardson SJ et al., 2010). There are clear differences between the infiltration pattern between the NOD mice and the human situation.

We appreciate your providing this important reference regarding immunopathology of pancreata in human T1D. We have quoted this reference in the text.

b) Are there other possible explanations for the improvement of beta cell function measures by C-peptide than the regeneration?
Yes. Other mechanisms may contribute to the improvement of islet β cell function. Specifically, glucotoxicity, autoimmune destruction, and chronic inflammation in pancreatic islets may contribute to impaired function in subjects having moderate T1D with some residual β cell function (Group A), and a reduction in inflammation or other factors may produce relatively rapid recovery of function. Notably, we found that some individuals in Group A exhibited hypoglycemia after their usually daily dose of insulin as early as the second day following Stem Cell Educator therapy, and an adjustment in the dosage resolved the situation on subsequent days. This suggests there is some measure of functional recovery of existing islet β cells after receiving Stem Cell Educator therapy.

Quality of written English: Needs some language corrections before being published

Our manuscript has been improved by a professional medical science writer.

Answers to reviewer #2:

As curing diabetes could hardly be a job for just a few weeks, I consider the report as too preliminary and believe that showing data from later stages, especially for the c-peptide levels (9-12 months, or longer, that the authors probably already have in hand), will make the findings much stronger.

We agree with the reviewer that it is important to examine longer-term outcomes. Thus, we now show plasma C-peptide levels from longer periods following Stem Cell Educator therapy, specifically for Group B severe T1D subjects with no residual pancreatic islet β cell function at baseline. To date, we have examined their plasma C-peptide levels after receiving Stem Cell Educatory therapy for 9 months. Notably, we found that fasting C-
peptide levels were continually improved at 40 weeks post treatment (see revised Figure 2C, \( p = 0.026 \)). We have added this information in the text and revised the figure 2C and figure legend.