



Article

Clinical, Radiographic, and Pathologic Improvements in a Patient with Long-Standing Crohn's Disease After Receiving Stem Cell Educator Therapy

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Abstract:

Background: Crohn's disease is a chronic inflammation affecting the gastrointestinal tract. To date, patients are commonly treated with corticosteroids or more aggressive biologics for high-risk subjects. Stem Cell Educator therapy have been successfully utilized to treatment patients with type 1 diabetes and other autoimmune conditions. **Methods:** A 78-year-old patient with long-standing Crohn's disease received one treatment with the Stem Cell Educator therapy, and followed by clinical, radiographic, pathological examinations and immune marker testing by flow cytometry. **Results:** After the treatment with Stem Cell Educator therapy, clinical symptoms were quickly improved with normal bowel movements, without abdominal pain or rectal bleeding. Flow cytometry analysis revealed marked decline of inflammatory markers such as the percentage of monocyte/macrophage-associated cytokine interleukin-1 beta (IL-1 β)⁺ cells reduced from 94.98% at the baseline to 18.21%, and down-regulation of the percentage of chemokine CXCL16⁺ cells from 91.92% at baseline to 42.58% at 2-month follow up. Pathologic examination of the biopsy specimens from colonoscopy eight weeks post-treatment showed ileal mucosa with no specific abnormality and no significant inflammation or villous atrophy; no granulomas were identified. Follow-up CT scan four and one-half months post-treatment showed no evidence of the previously seen stenosis of the ileocolonic anastomosis with proximal dilatation. **Conclusions:** Stem Cell Educator therapy markedly reduced inflammation in the subject with Crohn's disease, leading to clinical, radiographic, and pathological improvements.

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1. Introduction

Crohn's disease is one of the common inflammatory bowel diseases (IBD) that may affect any part of the gastrointestinal tract, possibly displaying extraintestinal complications in some patients. Similar to other autoimmune diseases, the potential causes of Crohn's disease remain unclear and might be associated with genetic predispositions and environmental factors. The prevalence and disease severity of Crohn's disease are increasing post COVID-19 pandemic. To date, the clinical management options for Crohn's disease are limited, commonly applying corticosteroids to treat flare-ups of symptoms (e.g., diarrhea, abdominal pain, and rectal bleeding). Additionally, the immune-suppressive biologics might be combined in severe subjects to block the inflammatory cytokines such as TNF α (e.g., Humira and Remicade) and the p40 subunit of receptors for IL-12 and IL-23

(e.g., Stelara). Therefore, the limits of conventional therapies highlight the need for novel approaches to treat Crohn's disease.

Over the last 15 years, we have developed a novel technology designated the Stem Cell Educator (SCE) therapy through the international multicenter clinical trials, which demonstrated the clinical safety and efficacy in multiple autoimmune diseases such as type 1 diabetes (T1D) [1,3], alopecia areata (AA) [5] and others[1,2]. With this technology, the patient's immune cells are co-cultured with adherent cord-blood-derived multipotent stem cells (CB-SC) in vitro. After overnight co-culture, the "educated" immune cells are re-infused into the patient's circulation. Recently, Stem Cell Educator therapy has been approved by the United States FDA for Regenerative Medicine Advanced Therapy (RMAT) designation. Here, we report the clinical safety and efficacy of Stem Cell Educator therapy to treat a patient with long-lasting established Crohn's disease.

2. Materials and Methods

2.1. Patient and treatment with Stem Cell Educator therapy

A 78-year-old man with Crohn's disease was enrolled and received treatment with Stem Cell Educator therapy via the Right-to-Try Act program at Throne's Outpatient facility according to the US FDA-approved clinical protocol for type 1 diabetes (IND 19247, ClinicalTrials.gov Identifier: NCT04011020). The consent form and clinical protocol have been approved by the central Institutional Review Board (IRB) at Advarra (Columbia, MD, USA). The signed consent form was obtained from the subject. Throne has received Blood Bank License approval for the Stem Cell Educator therapy from the Department of Health in New Jersey.

Patient received the Stem Cell Educator therapy as previously described. In brief, when the confluence of CB-SC cultures was more than 80%, they were utilized to treat patient's Peripheral blood mononuclear cells (PBMC) isolated by apheresis. After overnight co-culture, the Stem Cell Educator-treated PBMC were collected for quality control (QC) testing, and then released for infusion at our outpatient facility according to the FDA-approved protocol. Patient was scheduled to perform immune cell marker follow-up studies at 1st, 3rd, and 6th month after the treatment with Stem Cell Educator therapy.

2.2. Flow cytometry

PBMC were utilized to examine the changes of immune markers by the flow cytometry pre and post-treatment with Stem Cell Educator therapy. PBMC were isolated with Ficoll-hypaque ($\gamma = 1.077$, GE Health) and red blood cells were lysed using the ammonium-chloride-potassium (ACK) lysis buffer (Lonza, MD, USA). Flow cytometry analysis was performed as previously described by using the following antibodies including human regulatory T cell cocktail (BD Biosciences, Franklin Lakes, NJ, USA); Pacific Blue (PB)-conjugated mouse anti-human CD3, Spark Violet 538 anti-human CD4, APC/Fire 750 anti-human CD8, Spark VioletTM 538 anti-human CD14, PerCP/Cyanine5.5 anti-human CD80, PB-conjugated mouse anti-human CD86, PerCP/Cyanine5.5-conjugated mouse anti-human CD163 FITC-conjugated mouse anti-human HLA-DR mAb, PB-conjugated mouse anti-human IL-1 β mAb, PE-conjugated mouse anti-human CXCL16, PE-conjugated anti-human CD282 (TLR2), and APC-conjugated anti-human CD284 (TLR4) mAbs were purchased from (Biolegend, CA, USA). Isotype-matched immunoglobulin (IgGs) served as controls. For intra-cellular staining, cells were fixed and permeabilized according to the PerFix-nc kit (Beckman Coulter, Brea, CA, USA) manufacturer's recommended protocol. After staining, cells were collected and analyzed using a Gallios Flow Cytometer (Beckman Coulter, Brea, CA, USA) equipped with three lasers (488 nm blue, 638 red, and 405 violet lasers) for the concurrent reading of up to 10 colors. The final data were analyzed using the Kaluza Flow Cytometry Analysis Software (Kaluza Analysis 2.1, Beckman Coulter).

3. Results and Discussion

3.1. Medical history and dignosis of Crohn’s disease

A 78-year-old man had symptoms of Crohn’s Disease since his late teens with intermittent right lower quadrant abdominal pain and frequent loose stools. At age fifty-seven he presented with severe anemia with hemoglobin of 6.0 due to chronic blood loss. Barium enema was consistent with Crohn’s Disease. The terminal ileum, cecum and right colon were resected. Over the ensuing years several strictures were surgically repaired.

In early 2024, symptoms of stricture recurred. Colonoscopy showed a severe, benign-appearing, intrinsic stenosis of the colo-ileo junction. Small erosions were noted at the junction. The colonoscope was unable to pass into the small intestine. The biopsy forceps was passed and biopsies taken. Some inflammation was seen. Ulcerative colitis was noted. There was contact mucosal bleeding. Some localized Crohn's disease was seen. Contrast CT scan of the abdomen showed a loop of small bowel in the anterior pelvis that was distended to approximately 3.7 cm proximal to a short segment 1 cm region of narrowing and minimal bowel wall thickening. Similarly, there is a distended loop of small bowel in the right lower quadrant measuring up to 3.4 cm. Pathological examination of the biopsy specimens showed sections from the terminal ileum with superficial acute ileitis and increased plasma cells within the lamina propria. There was some mild villous blunting. The findings were compatible with idiopathic inflammatory bowel disease with minimal activity.

3.2. Clinical safety and improvements of symptoms after Stem Cell Educator therapy

In October 15, 2024, the subject received treatment with Stem Cell Educator therapy. The patient tolerated the procedure well without side effects and was discharged home on no medications other than vitamin supplements. The patient reported immediate improvements in bowel function which continued to improve over several weeks back to normal.

3.3. Markedly reduced the monocyte/macrophage-associated inflammatory markers IL-1β and CD163 after Stem Cell Educator therapy

IL-1β is one of major cytokines normally released by monocytes and macrophages and contribute the immune activation of T and B cells. Flow cytometry analysis demonstreated that there was a high percentage of IL-1β+ cells in patient’s PBMC at 94.98% before the treatment. Notably, the percentage of IL-1β+ cells was markedly reduced to 18.21% two months after treatment with Stem Cell Educator therapy. The activated monocyte/macrophage marker CD163 also declined from 10.24% at baseline to 0.3% post-treatment. The data indicated the down-regulation of activated monocytes/macrophages after receving Stem Cell Educator therapy.

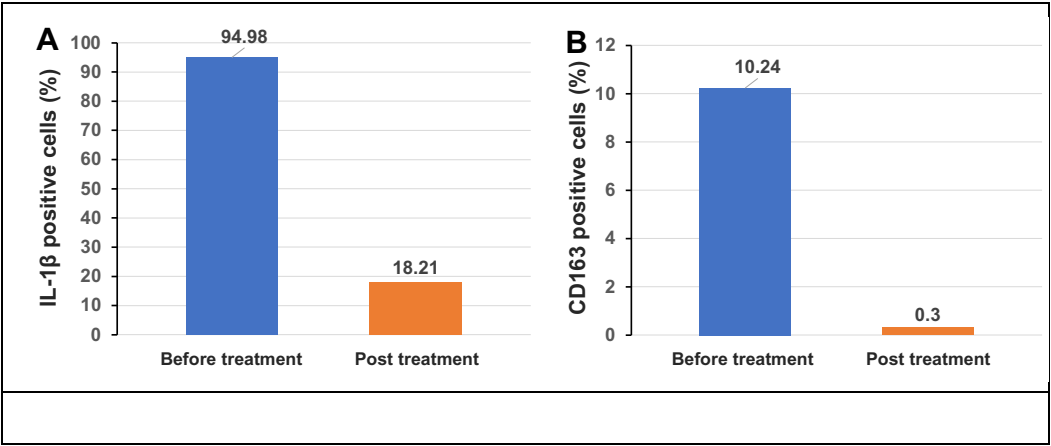
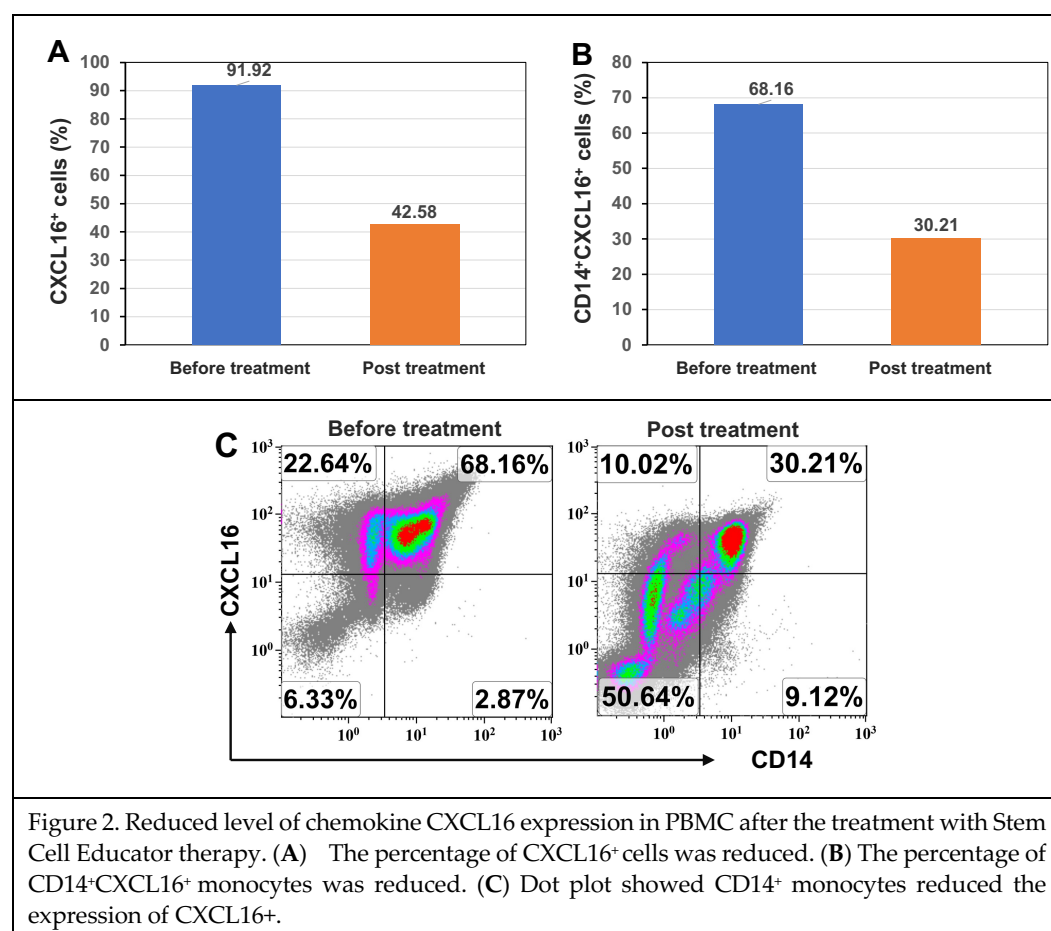


Figure 1. Decrease in monocyte/macrophage-associated inflammatory markers in PBMC after the treatment with Stem Cell Educator therapy. (A) Marked decline of the percentage of IL-1 β ⁺ cells. (B) Reduced percentage of CD163⁺ cells.

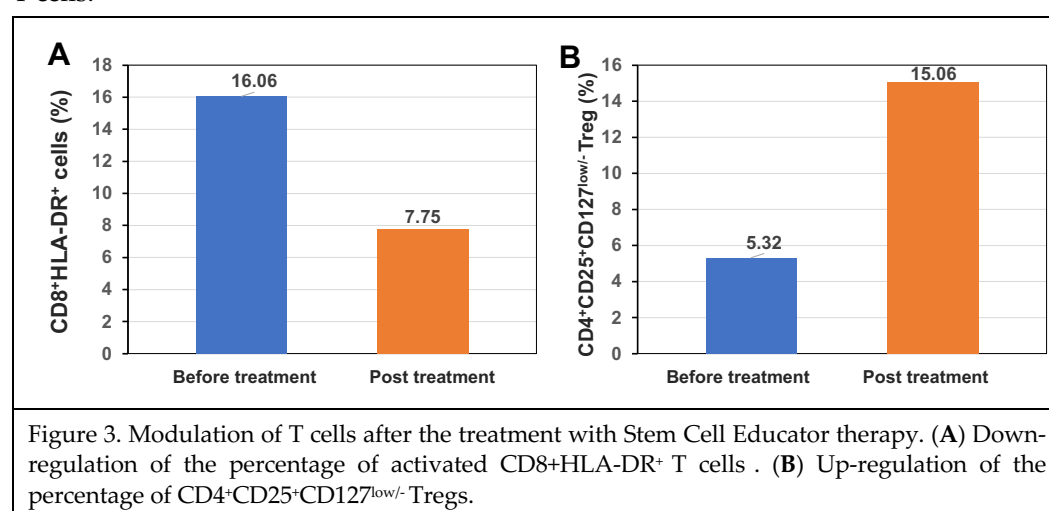
3.4. Markedly reduced the expression of inflammatory chemokine CXCL16 after Stem Cell Educator therapy

The C-X-C motif ligand 16 (CXCL16) is a chemokine and expressed by variety of immune cells such as monocytes/macrophages, dendritic cells, and B cells. Recent studies suggested the contribution of CXCL16 in the pathogenesis of autoimmune diseases such as Crohn's disease, multiple sclerosis, and rheumatoid arthritis. Flow cytometry showed that patient's PBMC displayed high percentages of CXCL16⁺ cells 91.92% at baseline. This was significantly reduced to 42.58% two months after treatment with Stem Cell Educator therapy (Figure 2A). CXCL16 is normally enriched in the monocytes/macrophages. Using the monocyte-specific marker CD14, further flow cytometry analysis demonstrated that the percentage of CD14⁺CXCL16⁺ monocytes was down-regulated from 68.16% at baseline to 30.21% at 2-month follow-up (Figure 2B and C). Additionally, the percentage of costimulating molecule CD86 (B7-2) was reduced from 3.83% at baseline to 1.66% at 2-month follow-up. There were no marked changes for costimulating molecule CD80 (B7-1, 0.00% *vs* 0.01%), CD282 (TLR-2, 1.38% *vs* 0.01%) and CD284 (TLR4, 0.01% *vs* 0.01%) between pre and post-treatment. The data implied the decline of infiltration and activation of monocytes/macrophages.



3.5. Upregulation of the percentage of regulatory T cells (Treg) after Stem Cell Educator therapy.

Using HLA-DR as a marker for activated T cells, flow cytometry showed the percentage of activated CD8⁺HLA-DR⁺ T cells was reduced from 16.06% at the baseline to 7.75% at 2-month follow up (Figure 3A). There was no changes for the percentage of activated CD4⁺HLA-DR⁺ T cells after the treatment (11.49% at baseline *vs* 12.91% at 2-month follow up). Additionally, flow cytometry demonstrated that the percentage of CD4⁺CD25⁺CD127^{low/-} Tregs was increased from 5.32% at the baseline to 15.06% at 2-month follow-up (Figure 3B), which might contribute to the down-regulation of activated CD8⁺ T cells.



3.6. Pathologic and radiographic improvements after Stem Cell Educator therapy.

On follow-up colonoscopy five weeks post-stem cell treatment the stenosis at the ileo-colonic anastomosis was improved and the colonoscope was able to pass across it 1-2 cm. Pathologic examination of the biopsy specimens showed ileal mucosa with no specific abnormality and no significant inflammation or villous atrophy. No granulomas were identified and no inflammation similar to that seen in the prior biopsy was seen in the two month post-treatment ileal biopsy. Follow-up CT scan four and one-half months post-stem cell treatment showed no evidence for small bowel obstruction. No focal segmental small bowel wall thickening was identified. Previously noted loops of small bowel in the anterior abdomen measuring up to 3.7 cm were now within normal limits. Follow-up colonoscopy six months post-Stem Cell Educator therapy the stenosis at the ileo-colonic anastomosis was further improved and the colonoscope was passed 10 cm into the terminal ileum. Pathologic examination of the biopsy specimens showed minimal non-specific chronic Inflammation providing no support for active idiopathic inflammatory bowel disease. These results show that Stem Cell Educator therapy for Crohn's Disease can provide long-term benefit up to at least six months.

Monocytes/macrophages are professional antigen-presenting cells distributed in almost every organ. Intestinal macrophages play essential roles in maintaining the gastrointestinal homeostasis, the balance of gut immune system, and the regulation of gastrointestinal motility and secretion. Clinical and animal studies have demonstrated the contribution of intestinal macrophages in the pathogenesis of Crohn's disease and colitis. Current clinical studies revealed that there were high profiles of inflammatory markers in this subject with Crohn's disease, specifically the activated monocyte/macrophage-associated markers such as IL-1 β and chemokine CXCL16. After the treatment with Stem Cell Educator therapy, these inflammatory markers were substantially reduced, as confirmed by flow cytometry analysis, leading to the clinical, pathological and radiographic improvements. IL-1 β , produced by monocytes/macrophages, is a master regulator of inflammation by controlling a variety of innate immune processes[27]. Interestingly, this subject displayed the high expression of IL-1 β similar to patients with T1D and other

autoimmune- and inflammation-associated diseases. It suggests that the activated mono-
cyte/macrophage system might be the common pathological feature in these autoimmune
conditions. Our previous studies revealed that CB-SC could release the extracellular ves-
icles (EV, exosomes), which could target monocytes and turn the differentiation of mac-
rophages from M1 (inflammatory) to M2 (anti-inflammatory) type, contributing to the re-
set of immune system. Therefore, Stem Cell Educator therapy may hold great promise in
the treatment of patients with Crohn's disease and ulcerative colitis and in improving
their quality of life.

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manuscript; Y.Z.: supervised experiments, and contributed to concepts, experimental design, data
analysis and interpretation, manuscript writing, and final approval of manuscript; B.V., patient
cares and final approval of manuscript; K.A., N.M., M. B., R.L., and D. P. performed apheresis and
patient care; H.W, Z.H., and Ye.Z performed experiments, isolate PBMC cells and flow cytometry.
T.T. provided materials; S. K., and B. S. patient care and provide materials; All authors have read
and agreed to the published version of the manuscripts.

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The consent form and clinical protocol for Stem Cell Educator therapy have been approved by the
central Institutional Review Board (IRB) at Advarra (Columbia, MD, USA).

Data Availability Statement: The data that support the findings of this study are available from the
corresponding author upon request.

Conflicts of Interest: Dr. Yong Zhao was the inventor of Stem Cell Educator technology and has a
fiducial role at Throne Biotechnologies (Throne). Drs. Boris Veysman and Richard Fox, Honglan
Wang, Zhihua Huang, and Yelu Zhao are employees at Throne. All other authors have no financial
interests that may be relevant to the submitted work.

Abbreviations: CB-SC, cord blood-derived stem cells; CXCL16, C-X-C motif ligand 16; FDA, Food
and Drug Administration; IL-1 β , interleukin-1 β ; IRB, Institutional Review Board; PBMC, peripheral
blood mononuclear cells; SCE, Stem Cell Educator; T1D: type 1 diabetes.

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