

## CASE REPORT

# Human embryonic stem cell therapy for aplastic anemia

Geeta Shroff<sup>1</sup> , Rakesh Gupta<sup>2</sup> & Lalduhawmi Zadeng<sup>1</sup>

<sup>1</sup>Nutech Mediworld, New Delhi, India

<sup>2</sup>Internal Medicine, Indraprastha Apollo Hospital, New Delhi, India

### Correspondence

Geeta Shroff, Nutech Mediworld, H-8, Green Park Extension, New Delhi 110016, India. Tel: +91 11 26180039; Fax: +91 11 26560089; E-mail: geetashroff@hotmail.com

### Funding Information

No sources of funding were declared for this study.

Received: 1 July 2016; Revised: 14 February 2017; Accepted: 15 March 2017

*Clinical Case Reports* 2017; 5(6): 919–922

doi: 10.1002/ccr3.950

## Introduction

Aplastic anemia (AA) is a rare hematologic condition [1]. The incidence of AA is reported to range between 1.4 and 14 cases per 1,000,000 people [2, 3]. Environmental factors lead to variation in the incidence in Asian countries (higher) and the Western countries [4–7].

The AA is classified as nonsevere AA (nSAA), severe AA (SAA), and very severe AA (vSAA). The threshold values of blood cells for nSAA are as follows: neutrophils – <1.0 g/L, platelets – <50 g/L, reticulocytes – <20 g/L; for SAA, neutrophils – <0.5 g/L, platelets – <20 g/L, reticulocytes – <20 g/L; and for vSAA, neutrophils – <0.2 g/L (especially granulocytes), platelets – <20 g/L, reticulocytes – <20 g/L, respectively [8].

Till date, the only immunosuppressive therapy approved by the Food and Drug Administration for the treatment of AA is the horse antithymocyte globulin (ATGAM (R); h-ATG) drug [9]. Other immunosuppressive medications include cyclosporine (CsA), granulocyte colony-stimulating factor (G-CSF), alemtuzumab, and methylprednisolone (6MPred) [10–12].

For more than a decade, the treatment of AA involves stem cell transplantation from different sources such as peripheral blood stem cells, bone marrow stem cells (BMSC), hematopoietic stem cells (HSCs), and umbilical

## Key Clinical Message

Human embryonic stem cell (hESC) therapy is the potential therapeutic option for the treatment of patients with aplastic anemia (AA). The study showed a remarkable improvement in the AA patient subsequent to hESC administration. No adverse events occurred in the patient. hESC therapy is safe and effective for AA patients.

## Keywords

Aplastic anemia, human embryonic stem cells, stem cells, transplantation.

cord stem cells [13–16]. However, the use of human embryonic stem cells (hESCs) for blood disorders is still in in vitro study stage [17]. The current study is the very first to present a case of AA treated with hESC therapy. Previously, hESC therapy has been employed for the treatment of several terminal conditions including spinal cord injury, cerebral palsy, diabetes, Duchenne muscular dystrophy, and Friedreich's ataxia [18–23].

## Methodology

### Material and methods

Human embryonic stem cell line is cultured and maintained in accordance with our in-house patented technology in a Good Laboratory Practices, Good Manufacturing Practices, and Good Tissue Practices compliant laboratory at our facility (Patent-WO 2007/141657A PCT/1B 2007 Published 13 December 2007). The details of the technique used to isolate, culture, and store hESCs have been described elsewhere [24]. The cell lines are free of xeno-product and are chromosomally stable even after >4000 passages [24].

The hESC therapy consisted of one treatment session. The patient was administered with 1 mL hESCs on 23 September 2015 *via* an intravenous route (*i.v.*) to allow

the cells to reach at the injured site and result in repair and regeneration.

The study was approved by an independent Institutional Ethics Committee (IEC). The patient gave written and video-informed consent prior to start of the treatment. In-house physicians and nurses monitored any antigenic or anaphylactic response. The principles laid down by “Declaration of Helsinki” were followed while conducting the entire study [25].

## Case history

A 38-year-old female patient presented to our facility for the treatment of AA. In the starting of 2015, the patient started getting bruises over body easily under normal pressure. The patient history revealed that she had received methotrexate (1 g) *i.v.* for three cycles. She had undergone multiple platelet transfusions at another hospital and was diagnosed as a case of pancytopenia. Her hemogram showed no improvements even after the transfusion. The patient was then referred to another facility where she was diagnosed with AA and received steroid therapy, but the symptoms did not improve. Although advised antithymocyte globulin (ATG) treatment, she chose stem cell therapy.

She presented to our facility with the following symptoms: tendency to bruise easily, inability to do routine work with ease, excessive lethargy, and fatigue and breathlessness on climbing stairs.

On 23 September 2015, the patient underwent laboratory investigations at our facility. The total blood count before therapy is presented in Table 1. Peripheral smear showed predominantly macrocytic with few normocytic, normochromic red cells with anisopoikilocytosis.

The patient was treated with hESC therapy along with the medication including Clip-MF (500 g × BD; FDC LIMITED, B-8, MIDC Area Waluj - 431 136, Dist. Aurangabad, Maharashtra), Primolut N (BD; Zydus Cadila Healthcare Ltd. Majitar, East Sikkim 737136), and Graftin (100 mg × BD; Ranbaxy Laboratories Ltd. (Croslands) Croslands House, Plot No. 89, Road No. 15, MIDC, Marol, Andheri (East), MUMBAI 400 093 India). These

medicines were prescribed during previous treatment regimens wherein Clip-MF is a hemostatic agent, tranexamic acid and Primolut N (hormone) help to stop menstrual bleeding, and Graftin was prescribed to increase white blood cell (WBC) counts.

After undergoing a single treatment session, she showed a remarkable improvement in her symptoms. The tendency to bruise had reduced; she was able to carry out her daily routine with ease and did not feel lethargic or fatigue. Blood glucose level was normal and she no longer had breathlessness while climbing stairs. Her blood count improved (differential leukocyte count, DLC: 52/34/0.3/10.5; platelet count: 0.59; and hemoglobin, Hb: 12.3) (Table 1), and the medication was also reduced at the end of the treatment. Follow-up of the patient revealed that she is off to medications since March 2016. Patient is now with platelet count of 1.07 and Hb of 13.4. She is fit and has no problems.

## Discussion

The use of in-house cultured hESCs for the treatment of AA resulted in a remarkable improvement in the patient's condition. The cell line (mixture of neuronal and non-neuronal cells) was isolated from a fertilized ovum at two-celled stage in contrast to the 64-celled stage or inner cell mass of the blastocyst used by most of the researchers as the cells do not have developed antigens at the former stage, thus cannot lead to immunosuppression [24].

Several complications are associated with the use of traditional therapies for AA such as immunosuppressive therapy and cell transplantation (HSC and BMSC). Immunosuppressive therapy is reported to be associated with partial responses, delayed responses, failure to respond, relapses, and early deaths [15]. Stem cell transplantation is used as a first-line therapy in younger patients with related donor, but results in lesser survival rates and increased risk of graft-versus-host disease (GVHD) in patients with unrelated donors and older patients [13–16]. Thus, there is a need of a therapy that can improve the survival rates of older patients and does not pose the risk of GVHD. Our hESC line could be presumed to prevent the risk of GVHD because these cells do not have antigens on

**Table 1.** Blood count of the patient before, during, and at the end of human embryonic stem cell therapy.

Duration of treatment (Date)	Initiation of treatment (23 September 2015)	After 1 week (30 September 2015)	After 1 month (21 October 2015)	After 50 days (18 January 2016)	At the end of treatment (20 October 2016)
Hb (g%)	8.10	9.0	7.7	11.2	13.4
TLC (cells/mm <sup>3</sup> )	4100	4600	3490	3500	5400
Platelet Count (10 <sup>5</sup> /mm <sup>3</sup> )	24,000	52,000	62,000	72,000	107,000
ANC (cells/mm <sup>3</sup> )	2460	1656	1221	1820	–

Hb, hemoglobin; TLC, total leukocyte count; ANC, absolute neutrophil count.

their surface that could lead to any immune response or rejection of the transplanted cells. In vitro studies have reported the effect of several extrinsic factors that stimulate the differentiation of hESCs into hematopoietic progenitor cells. Cytokines and growth factors including vascular endothelial growth factor (VEGF) and bone morphogenetic protein 4 (BMP4) are found to play a synergistic role in the formation of early hematopoietic progenitors derived from embryonic stem cells (ESCs) [17, 26, 27]. It has also been established that hemangioblasts derived from hESCs are capable of migration to the site of injury and restore the vascular system [17]. Thus, we could hypothesize that hESCs in our study are also differentiated into hematopoietic progenitor cells by these cytokines and growth factors. No adverse event was reported in the study patient, and no teratoma formation occurred. Our previous studies on other terminal conditions have also not reported any serious adverse events [18–23].

We conclude that hESC therapy might serve as a potential therapeutic option for AA. However, this is a first patient report assessing the efficacy and safety of hESCs on AA. The bone marrow aspiration and study is pending. Several parameters need to be assessed before the use of this therapy clinically, such as the effect of therapy on patients of different ages, severity of the disease, long-term effect.

## Authorship

GS: is responsible for conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published. RG: is responsible for patient care. LZ: is responsible for patient care and acquisition of clinical history.

## Acknowledgments

The authors acknowledge all the doctors, staff, and patients of the Nutech Mediworld. The authors also acknowledge Knowledge Isotopes Pvt. Ltd. (<http://www.knowledgeisotopes.com>) for the medical writing assistance.

## Conflict of Interest

The authors have no conflict of interest.

## References

- Segel, G. B., and M. A. Lichtman. 2010. Chapter 34: Aplastic anemia: acquired and inherited. Pp. 463–483.

- Maluf, E., N. Hamerschlak, A. B. Cavalcanti, A. A. Junior, J. Eluf-Neto, R. P. Falcao, et al. 2009. Incidence and risk factors of aplastic anemia in Latin American countries: the LATIN case-control study. *Haematologica* 94:1220–1226.
- Hamerschlak, N., E. Maluf, R. Pasquini, J. Eluf-Neto, F. R. Moreira, A. B. Cavalcanti, et al. 2005. Incidence of aplastic anemia and agranulocytosis in Latin America—the LATIN study. *Sao Paulo Med J* 123:101–104.
- Mary, J. Y., E. Baumelou, and M. Guiguet. 1990. Epidemiology of aplastic anemia in France: a prospective multicentric study. The French Cooperative Group for Epidemiological Study of Aplastic Anemia. *Blood* 75:1646–1653.
- Kojima, S. 2002. Aplastic anemia in the Orient. *Int. J. Hematol.* 76(Suppl. 2):173–174.
- Issaragrisil, S., K. Chansung, D. W. Kaufman, J. Sirijirachai, T. Thamprasit, and N. S. Young. 1997. Aplastic anemia in rural Thailand: its association with grain farming and agricultural pesticide exposure. *Aplastic Anemia Study Group. Am. J. Public Health* 87:1551–1554.
- Benitez-Aranda, H., M. A. Velez-Ruelas, S. Diaz-Cardenas, E. Sanchez-Valle, M. Xolotl-Castillo, M. T. Duenas-Gonzalez, et al. 2002. Incidence of aplastic anemia in a defined subpopulation from Mexico City. *Hematology* 7:229–232.
- Aplastic Anemia - Diagnostics and Therapy of Acquired Aplastic Anemia. 2012. Available at <https://www.onkopedia-guidelines.info/en/onkopedia/guidelines/aplastic-anemia-diagnostics-and-therapy-of-acquired-aplastic-anemia/@view/html/index.html> (accessed October 20, 2016).
- Young, N. S., P. Scheinberg, and R. T. Calado. 2008. Aplastic anemia. *Curr. Opin. Hematol.* 15:162–168.
- Frickhofen, N., J. P. Kaltwasser, H. Schrezenmeier, A. Raghavachar, H. G. Vogt, F. Herrmann, et al. 1991. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German Aplastic Anemia Study Group. *N. Engl. J. Med.* 324:1297–1304.
- Frickhofen, N., and S. J. Rosenfeld. 2000. Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine. *Semin. Hematol.* 37:56–68.
- Bacigalupo, A., B. Bruno, P. Saracco, E. Di Bona, A. Locasciulli, F. Locatelli, et al. 2000. Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midollo Osseo (GITMO). *Blood* 95:1931–1934.
- Passweg, J. R., and J. C. W. Marsh. 2010. Aplastic anemia: first-line treatment by immunosuppression and sibling

- marrow transplantation. *Hematology Am. Soc. Hematol. Educ. Program* 1:36–42.
14. Gupta, V., M. Eapen, R. Brazauskas, J. Carreras, M. Aljurf, R. P. Gale, et al. 2010. Impact of age on outcomes after bone marrow transplantation for acquired aplastic anemia using HLA-matched sibling donors. *Haematologica* 95:2119–2125.
  15. Scheinberg, P., and N. S. Young. 2012. How I treat acquired aplastic anemia. *Blood* 120:1185–1196.
  16. Kim, H., B. S. Kim, D. H. Kim, M. S. Hyun, S. H. Kim, S. H. Bae, et al. 2011. Comparison between matched related and alternative donors of allogeneic hematopoietic stem cells transplanted into adult patients with acquired aplastic anemia: multivariate and propensity score-matched analysis. *Biol. Blood Marrow Transplant.* 30:1289–1298.
  17. Tian, X., and D. S. Kaufman. 2008. Differentiation of embryonic stem cells towards hematopoietic cells: progress and pitfalls. *Curr. Opin. Hematol.* 15:312–318.
  18. Shroff, G. 2015. Human embryonic stem cell for the treatment of multiple sclerosis: a case report. *Case Report Int.* 4:38–42.
  19. Shroff, G. 2015. Treatment of lyme disease with human embryonic stem cells: a case series. *J. Neuroinfect. Dis.* 6:167.
  20. Shroff, G. 2015. A novel approach of human embryonic stem cells therapy in treatment of Friedrich's Ataxia. *Int. J. Case Rep. Imag.* 6:261–266.
  21. Shroff, G. 2015. Human embryonic stem cells in the treatment of spinocerebellar ataxia: a case series. *J. Clin. Case Rep.* 5:1.
  22. Shroff, G., A. Gupta, and J. K. Barthakur. 2014. Therapeutic potential of human embryonic stem cell transplantation in patients with cerebral palsy. *J. Transl. Med.* 12:318.
  23. Shroff, G., and P. Hopf-Seidel. 2015. Use of human embryonic stem cells in the treatment of Parkinson's disease: a case report. *Int. J. Emerg. Ment. Health* 17:661–663.
  24. Shroff, G. 2015. Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. *Int. J. Recent Sci. Res.* 6:3730–3738.
  25. World Medical Association. 2002. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J. Postgrad. Med.* 48:206–208.
  26. Srivastava, A. S., E. Nedelcu, B. Esmali-Azad, R. Mishra, and E. Carrier. 2007. Thrombopoietin enhances generation of CD34 + cells from human embryonic stem cells. *Stem Cells* 25:1456–1461.
  27. Chadwick, K., L. Wang, L. Li, P. Menendez, B. Murdoch, A. Rouleau, et al. 2003. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood* 102:906–915.