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Review Article

A review of the emerging potential therapy for neurological disorders: human embryonic stem cell therapy

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Abstract: The first human embryonic stem cell (hESC) line was developed in the late nineties. hESCs are capable of proliferating indefinitely and differentiate into all the three embryonic germ layers. Further, the differentiation of hESC lines into neural precursor cells and neurons, astrocytes and oligodendrocytes showed their potential in treating several incurable neurological disorders such as spinal cord injury (SCI), cerebral palsy (CP), Parkinson's disease (PD). In this review, we will discuss the global scenario of research and therapeutic use of hESCs in the treatment of neurological disorders. Following this, we will discuss the development of a unique hESC line, how it differs from the other available hESC lines and its use in the treatment of neurological disorders. hESCs were isolated from mixture of neuronal and non-neuronal progenitor cells in their pre progenitor state in a Good Laboratory Practices, Good Tissue Practices and Good Manufacturing Practices compliant laboratory. Blastomere cells have served as a source to derive the hESCs and the xeno-free culture was demonstrated to be more safe and effective in clinical therapeutic application of hESCs. All the patients showed a remarkable improvement in their conditions and no serious adverse events were reported. This study concluded that hESC lines could be scalable and used in the treatment of various neurological disorders such as SCI, CP, and PD.

Keywords: Stem cell lines, spinal cord injury, cerebral palsy, Parkinson's disease, transplantation, stem cell therapy

Introduction

The establishment of the first human embryonic stem cell (hESC) line in the year 1998 provided a path for the development of other hESC lines globally [1]. hESCs are empowered with a unique capability and a property of proliferating indefinitely and differentiating into all three embryonic germ layers and all tissue cell types. Differentiation of hESC lines into neural precursor cells and neurons, astrocytes, and oligodendrocytes [2], shows their potential in treating several incurable neurological disorders like spinal cord injury (SCI), cerebral palsy (CP), Parkinson's disease (PD) and many more. Viewing the potential of hESC therapy in treating various terminal conditions both *in vitro* and *in vivo*, this therapy could be used as the first line therapy in the future. The research on the transplantation of hESC into humans is currently in progress. There are several limitations that need to be overcome prior to the use of hESC therapy in humans. This paper will present an overview of hESC research and transplantation globally. Subsequently, we will discuss the development of hESC line at our institute that has been able to treat various terminal diseases.

Global scenario of hESC research and transplantation

Stage of isolation

Primitively, the inner cell mass (ICM) of preimplantation stage embryos (blastocysts) and embryonic germ (EG) cells derived from primordial germ cells (PGCs) served as the source of hESCs [3, 4]. Later, the scientists targeted single blastomeres (4-cell and 5-cell stage) and morula embryos (8-cell stage) as a source to derive hESCs [5-7]. But, the chromosomal stability of these hESC lines is limited to 66 passages making them unfit for clinical use [8-10].

Xeno-free culture medium

Researchers are continuously striving to develop a standard culture medium to maintain hESCs in undifferentiated state for their long-term use. Traditionally, mouse embryonic fibroblasts (MEFs) were used as feeder layers for culturing stem cells [11, 12]. However, several concerns were reported that precludes the use of MEF for culturing hESCs; they included the graft rejection, transmission of animal-derived infectious pathogens and viral particles [13, 14]. These culture systems also used bovine serum which is associated with risk of graft rejection, and prion and bovine virus transmission to the cell lines [15, 16]. Further studies investigated the alternative approach of using human feeder cells derived from human fallopian tube cells, fetal muscle and skin, fetal foreskin, transgenic fetal liver stromal cells, umbilical cord, bone marrow, endometrial cells and placental cells [17]. Use of autogenic feeder layer from the stem cells being cultured was another approach that even eliminated the risk of allogeneic pathogen that is associated with donor feeder layer cells [18]. However, the use of feeder layers in hESC culturing is still recognized to be cumbersome. Intensive labour requirement and inconsistencies between feeder populations troubles the maintenance of feeder [17]. The development of feeder free and xeno-free cultures was a significant development; of which xeno-free culture was demonstrated to be more practical in clinical therapeutic application of hESCs. Thus, chemically defined xeno-free mediums are being developed including Hillex[10] microcarrier suspension culture, Alkanethiol with heparin binding proteins, Poly [2-(methacryloyloxy) ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide]-coated plates (PMEDSAH) and synthetic acrylate surfaces with peptides (PAS): Vitronectin, bone sialoprotein. But, yet several limitations are associated with their commercial use such as cell passages and fold-expansion. Daily replenishing of chemically defined media is suggested but it is labour intensive and expensive [17].

Although 2D culture represents an advancement in the development of xeno-free cultures, they are limited in mimicking the *in vivo* stem cells physiologically [17]. Researchers have developed 3D culture mediums which are indicated to be better in mimicking the behaviour of stem cells *in vitro* close to that of *in vivo* [19]. However, the culture design should be such that it allows for large-scale propagation and should be cost-effective [17]. Data on the use of these culture systems for long-term propagation of hESC needs to be gathered.

Evidences of clinical application of hESCs

Several studies have been conducted to assess the efficacy and safety of hESCs in animal models of neurological disorders including SCI, CP and PD. These studies have provided a hope for the conduct of clinical trials using hESC therapy. Though there is a long way to confirm the therapeutic potential of hESC therapy in humans; few clinical trials have been conducted.

Spinal cord injury

The first phase 1 trial of hESC derived oligodendrocyte progenitor cells transplantation approved by FDA began in 2009. However, the company Geron Corporation that launched the trial terminated it due to financial constraints. In 2013, Bioplasma acquired the stem cell unit of Geron including the phase I trial of hESC and named the subsidiary, Asterias. The 3 year follow-up results of the five patients transplanted were announced in 2014, according to which no patient has reported any serious adverse event (SAE) till date [20, 21]. Recently in 2016 at Keck Medical Center of University of Southern California (USC), neuroscientists have treated a total quadriplegic, 21 year old patient with stem cells (AST-OPC1), as a part of a multi-center clinical trial. The patient had substantially recovered the functions of his upper body within two months of treatment [22].

Parkinson's disease

In 2001, a randomized clinical trial by Freed *et al* was conducted in 40 patients with severe PD, aged 34-75 years. Patients were randomized to receive hESC derived dopamine (DA) neurons or sham surgery. The study results demonstrated that transplanted DA neurons were able to survive in patients and provided more benefits to younger patients [23].

hESC line developed at our institute

History and development

We isolated our first hESC line in the year 1999, a mixture of neuronal and non-neuronal progenitor cells in their pre progenitor state. The

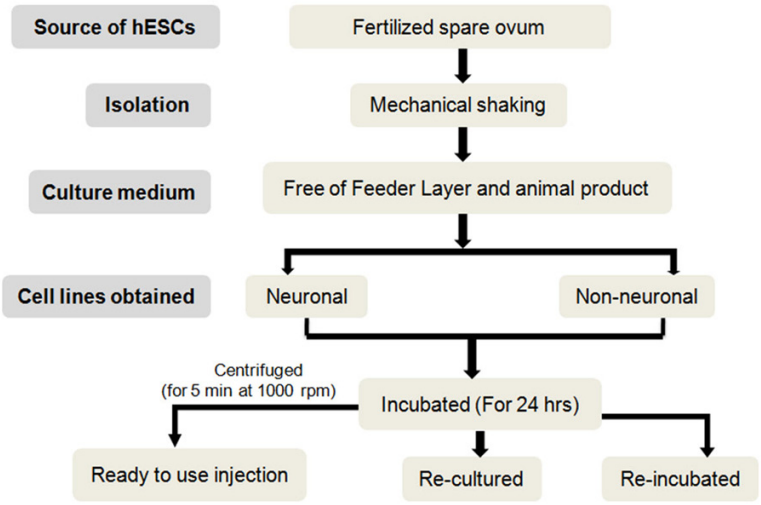

Figure 1. Steps in development of human embryonic stem cell lines.

cell line is developed in Good Laboratory Practices (GLP), Good Tissue Practices (GTP) and Good Manufacturing Practices (GMP) compliant laboratory at our institute. With due consent from the donor during routine *in vitro* fertilization (IVF) procedure, a single, spare, expendable 2-day old fertilized ovum was obtained. The cells were isolated with mechanical shaking [24]. Subsequently, media [Roswell Park Memorial Institute medium (RPMI) and Dulbecco's Modified Eagle's Medium (DMEM; Himedia Labs, Mumbai, India)] was added to the cells and β-human chorionic gonadotropin (HCG) agonist (16-64 µl of 500 IU/ml, Serum Institute of India, Pune, India) and progesterone (16-64 µl of 250 mg/ml, Sun Pharma, Mumbai, India) were also added. And, the suspended cells were incubated at 37°C in a horizontal position at an ambient temperature in the carbon dioxide and water jacketed incubator.

Following an incubation period of 24 hrs, the cell suspension was divided into two different flasks and media (RPMI and DMEM) were added to the cells. The cells obtained were re-incubated (37°C) for 24 hrs in a water jacketed incubator with an atmosphere of 5% CO_2. The flasks with the cell suspension and media were filled to the brim and placed in a vertical position during reincubation. After 24 hrs, the cell suspension was taken out and divided into three aliquots, first for re-culturing, second for storage at freezing temperature and third was made ready to inject (RTI). For making RTI, the cells were centrifuged for 5 minutes at 1000 rpm and the pellet was suspended in normal saline (Nirlife, Nirma Ltd. Ahmedabad, India) (**Figure 1**). Before injecting into a patient, the cell containing syringes are thawed by placing the syringes in between palms of the hands so that they reach the body temperature. Our patent document gives the complete details of cell culture and differentiation techniques [17, 24]. The evidence for the use of hESCs at our facility were submitted to and accepted at the House of Lords, Regenerative Medicine, Science and Technology Committee [25]. Till date, our hESC line is patented in 77 countries (including Australia, U.S., Japan and Israel). The culture medium used for our cell line can be used for large scale production of hESCs. Our culture medium is free of xeno-products and contaminants, thus making the therapeutic use of our cells hassle free. We have not observed graft rejection in any of our patients and neither used immunosuppressants.

Ethical issues

The cell lines used in our study were obtained from a single fertilized ovum. The other advantage is that a single embryo is able to provide therapeutic amounts of hESCs and/or their derivatives to treat multitudes of patients. Thus, there is no repeated exploitation of human embryos, and the number of ethical issues associated with the use of hESC therapy could be avoided.

Guidelines on hESC research and transplantation

Several international and national guidelines have been developed and are regularly revised to ensure that hESC research is conducted within the legal and ethical boundaries. The guidelines aim at ensuring that the embryo donated for the purpose of research is with the due consent of the donor [26-28]. Recently, the International Society for Stem Cell Research (ISSCR) released updated guidelines which provide guidance on the stem cell re-

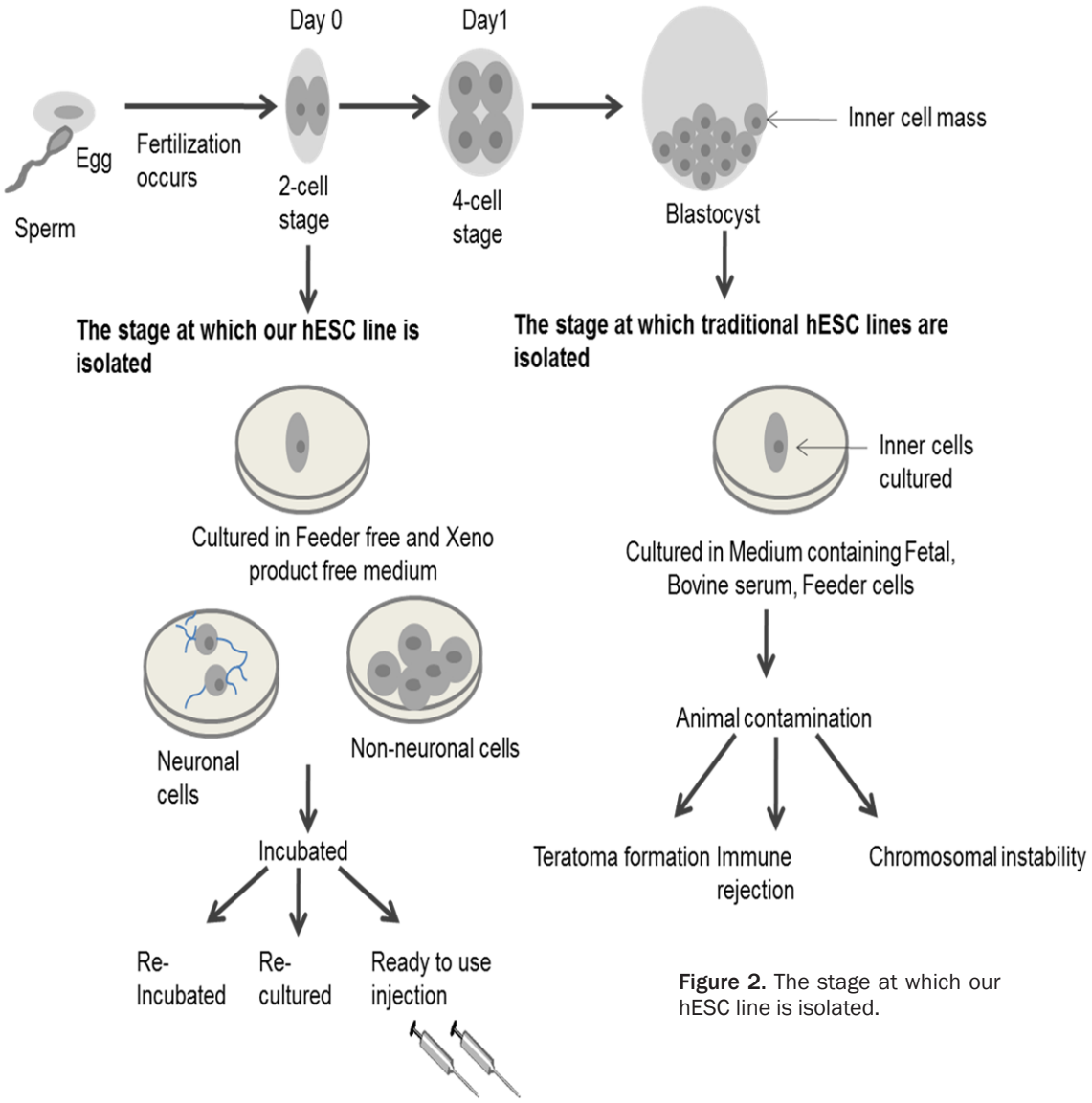

Figure 2. The stage at which our hESC line is isolated.

search and clinical translation. These guidelines provide recommendations on sourcing material (donor consent, donor screening) and manufacturing of stem cell products (quality control, processing, components in culture or preservation of cells and release criteria). These guidelines also recommend conducting animal studies prior to research in humans to ensure safety and efficacy of the stem cells. As per guidelines, results of preclinical studies should be published prior to the first clinical trial report [28].

In India, updated guidelines on stem cell research and translation were issued by the Indian Council of Medical research in 2013. The use of stem cell as therapy (except haematopoietic stem cells) is not yet approved and these guidelines only provide recommendations on preclinical and clinical research of the stem cells [28].

When we started working on hESC therapy, the guidelines on the Ethics of Biomedical Research on Human Participants were followed. These guidelines have been revised from time to time. Following the release of updated gui delines in 2013, Institutional Committee for Stem Cell Research and Therapy (IC-SCRT) was formed at our institute. All our studies conducted after the release of these guidelines have been reviewed and approved by IC-SCRT. There

is also an institutional ethics committee since 2003.

Stage of harvesting

Our hESCs have not shown immune rejection in any of the patients. This is because of the embryonic stage at which we harvested the cells, the embryonic immunogenic gene is not activated. The hESC lines have been obtained from a single fertilized ovum stage, post pronuclear fertilization and first cell division, a stage at which cells initiate differentiating or rather are in the transition phase and have not acquired any antigenic property. We could hypothesize that this is the reason why hESCs do not result in immune reaction upon transplantation. **Figure 2** presents the stage of isolation of our hESC line and how it differs from the primitive hESC lines.

Genomic stability

The genomic instability is one of the biggest challenges associated with prolonged culturing. However, our cell lines overcome this challenge as our hESC lines are karyotypically stable even after > 4000 passages. The hESCs that we prepared have also been confirmed for the expression of genomic integrity marker telomerase at mRNA level [29].

The other factors elucidated to affect genomic integrity of cell lines are choice of nutrient medium, culturing methodology, passaging number and technique [30]. We have retained the stable conditions of culture media and substrates. Our culture media is free of any antioxidants, growth factor, insulin or insulin substitutes, collagen precursors or collagen precursor substitutes, residues or "conditioned media", trace elements, animal products and feeder cells. We use non-enzymatic passaging method and have conducted regular checks on spontaneous differentiation. All these considerations have resulted in maintenance of stable cell lines over the years on repeated passaging.

Ready to use form

The hESCs can be made available in the ready to use form with a viability of 90% [31]. The composition is prepared in compliance with the international regulatory standards of safety and quality in a form that can be injected into human patients for therapeutic purposes. These ready to inject compositions of hESCs and/or their derivatives are stored in conditions of storage which make them suitable for direct transplantation on thawing.

Mode of action of the hESCs

For the therapeutic action of the cells, it is necessary that the transplanted cells reach the target site or the site of injury also referred to as homing. Several studies have assessed the mode of action of other stem cells including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), human amniotic fluid stem cells and endogenous stem cells and have observed them to reach the target site.

The studies also observed that the transplanted stem cells act combined with local stem cells in the injured tissue to accomplish the healing process. Liu *et al* reported that the immune system of the patient releases signals (interferon (IFN)-γ and tumor necrosis factor (TNF)-α) to communicate with the transplanted MSCs during the repair of the injured tissue. Thus, the stem cells get attracted to the site of injury [32]. Another study identifying the homing of MSCs to the target site *via* the stromal cell-derived factor-1 (SDF-1)/CXC receptor 4 (CXCR4) pathway reported that SDF-1, a chemoattractant, is released by the injured tissue which attracts the transplanted stem cells [33, 34]. Vascular endothelial growth factor (VEGF) is another key mediator of mobilization [35]. We could hypothesize that hESCs might follow the same pattern to migrate to the target site and initiate the regeneration and repair process (**Figure 3**).

Another point to be noted is that these cells are very small sized (< 1 μm) and have very high multiplication rate. Their small size makes it possible for them to cross all the barriers of the diseased body and reach the target sites easily. These cells resemble and behave like previously very small embryonic stem cells [36]. The small size of cells is clinically beneficial as it permits cells to cross the blood brain barrier *via* the parenchyma and reach the affected site.

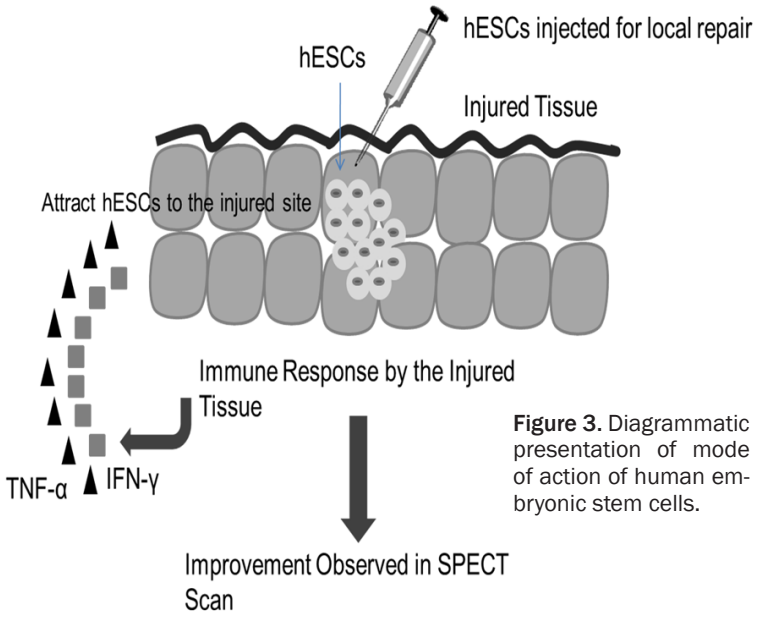

Figure 3. Diagrammatic presentation of mode of action of human embryonic stem cells.

diagrammatic presentation of routes of injecting hESCs.

The dosage of the hESCs administered varies depending upon the type of disease, clinical condition of the patient and severity of the symptoms. It has been observed from previous studies that in progressive disorders like Friedreich's ataxia, brain injury and amyotrophic lateral sclerosis, the cells have to be transplanted continuously throughout the life, as on discontinuation of therapy there might be chances of degeneration, however in static neurological diseases *viz*; SCI, CP and cerebrovascular accident, the hESC therapy treatment is required at periodic intervals only, the cells once transplanted provide lifelong benefit without worsening the condition further [38-41].

Treatment schedule

First of all, the patients are assessed for hypersensitivity reactions to hESCs by a subcutaneous (s.*c.*) administration of 0.25 mL hESCs. If the patient does not develop any hypersensitivity reaction, then further procedure is initiated. The dosage of the therapy varies for different conditions. Following it, the first step is "Priming". In this step, the patients are injected with hESCs *via* intramuscular (*i.m.*) route so as to allow the recipients' system to accept the transplanted stem cells. Then, hESCs are administered *via* intravenous (*i.v.*) route after every 10 days which helps in "homing" to the required area (injured tissue). For the local action of the hESCs, different supplemental routes including epidural infusion or injection/caudal injection; deep spinal injection and subarachnoid injection were used to allow the cells to be placed in close proximity to the brain and spinal cord. Nasal sprays were also used in brain disorder.

The treatment protocol comprises of "treatment phases (T1, T2, T3)" wherein hESCs are injected. There is a "gap phase" of 4-6 months between the subsequent treatment phases which provide enough time for the transplanted hESCs to grow, repair and regenerate the injured tissue. The duration of gap phase was decided on the basis that in human embryo, all organs are developed within 14-16 weeks of gestation [37]. **Figures 2** and **4** represents the

Clinical application

The first study was conducted for the duration of three years (2000-2002) during which the efficacy and safety of these cell lines was established in 33 patients suffering from both neurological and non-neurological disorders [42]. All the patients showed a remarkable improvement in their conditions. No SAE were reported in the patients [43]. Following the success in this study, treatment protocols were developed for the conditions like CP, SCI, lyme disease (LD) and multiple sclerosis (MS) where lifelong treatments can be continued and no deleterious effects were seen. These hESC lines have been used to treat several terminal/incurable conditions in the last 14 years which included CP, SCI, PD, MS, visual impairment, Friedreich's ataxia, spinocerebellar ataxia and many more [24, 25, 38, 40, 41, 44]. The data for some of the patients treated has been validated by international bodies including the Moody's International (Document number NH-heSC-10-1), the Quality of Austria Central Asia Pvt. Ltd. Accreditation Company (Document number QACA/OCT/2013/26) and GVK Biosciences (NM-Hesc-10-1, 18 November 2010). These companies examined the medical and

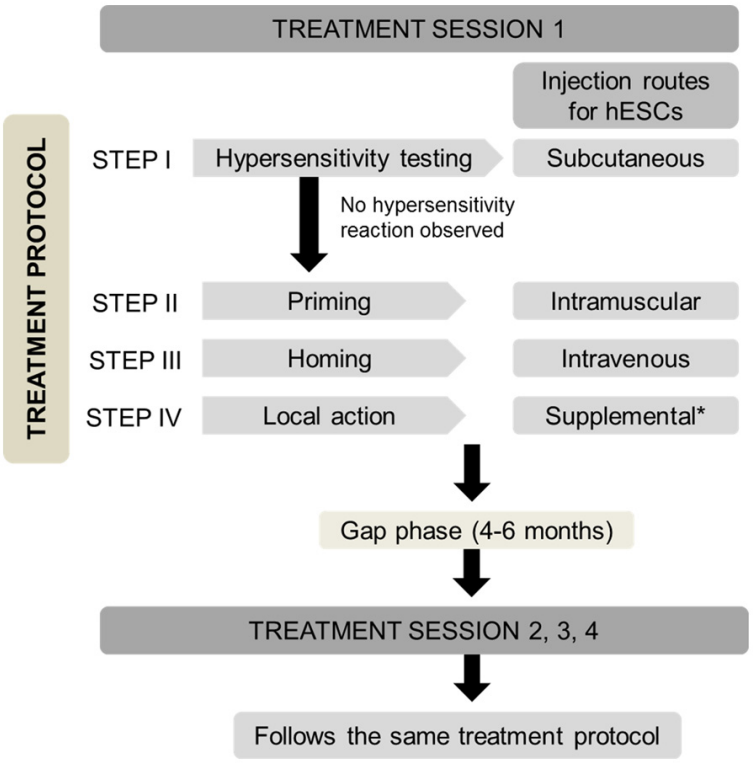

Figure 4. Treatment protocol for hESC therapy. *Supplemental routes used depending on the target organ: epidural infusion/caudal injection, deep spinal injection, subarachnoid injection, retro-bulbular; The dosage of the cells vary upon the type of disease, clinical condition of the patient and severity of the symptoms.

statistical data of the treatment and also met the patients [39, 40].

Table 1 present the results of studies published on the use of hESCs in various terminal/incurable conditions. We have also statistically validated the efficacy of hESC in treating various disorders including CP, SCI, PD, LD (Papers under preparation/Submitted to the journals).

Safety

A total of 33 patients were treated with hESC therapy from the year 2002 to 2004 to assess the safety of hESC line. The patients were suffering from various terminal conditions including SCI, PD, acute cauda equina lesions, autosomal recessive disorders, motor sensory neuropathy with diplopia (vasculitis), mild diffuse cerebral atrophy, chronic renal failure secondary to lupus nephritis, Huntington's chorea, mental retardation with microcephaly, liver metastasis, diabetic foot (amputation), diabetes mellitus, psoriasis, systemic lupus erythematosus (SLE), Duchenne muscular dystrophy (DMD), cirrhosis, developmental delay, hypothalamic astrocytoma, post traumatic paraplegia and colitis. Only mild adverse events were reported including fever, rash/erythema, mild pain in the abdomen, headache, urinary tract infection (UTI), swelling of legs (edema), body ache and pain at the lower back and limbs [43]. Till date, none of our studies have reported any SAE [24, 25, 38-41, 43, 44]. Transplantation of hESCs derived from the inner cell mass of the blastocyst stage embryos have been reported to be associated with teratoma formation [45, 46]. However, no teratoma formation has been observed in any of our treated patients. This is because; our hESC line is derived from a fertilized ovum after 24-48 hrs of fertilization, the stage at which the hESC has no antigen development. Thus, the hESC line developed from this stage also has no antigens on their surface and do not lead to teratoma formation.

Conclusion

There is a need of more hESC lines that could be scalable and used in the treatment of terminal conditions. Till date, we have treated more than 1400 patients with various terminal conditions using these novel hESC lines. Since they are scalable, free of xeno-products and can be prepared in ready to use form, these characteristics envision that they can be made available on a large scale to treat variety of incurable and/or terminal diseases.

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Table 1. Published studies on the use of human embryonic stem cells in various terminal conditions

Disease	Type of study (Year)	Number of patients	Symptoms before therapy	SPECT scan/MRI/CEST	Symptoms after therapy	Follow up status
Cortical visual impairment	Case series (2014)	40	Blind/had no perception of light, n = 8 Had perception of light, n = 16 Could identify blurred images, n = 10 Could see objects up to a distance of 25 cm from the eye, n = 6	Had normal perfusion, n = 2, Had significant improvement, n = 18 Had moderate improvement, n = 3	Gained normal vision, n = 27; Could see objects 25 cm from the eye, n = 10 Could see blurred images, n = 2 Had perception of light, n = 1	NA
Cerebral palsy	Retrospective study (2014)	91	Impaired hearing and cognitive skills; seizures	SPECT scan showed improved perfusion in all patients	Cognitive skills improved in 69% of the patients; No seizures, n = 90 and hearing improvement, n = 8	NA
Friedreich's ataxia	Case series (2015)	3	Case 1: Walking with help of crutches, Weakness in LLs (left > right) Case 2: Weakness of limbs (LL > UL), Coordination difficulties in UL; Difficulty in standing, walking and climbing stairs Case 3: Unable to stand, walk or sit; Used wheel chair; Unable to do his day to day activities; Managed to have food and dress by himself; Lost coordination and balance; Had slurred speech; Hearing loss; Mild difficulty in swallowing with aspiration of liquids infrequently	NA	Case 1: Able to stand and walk, Exercise endurance during physiotherapy increased and could do static cycling more effectively Case 2: Better trunk control and coordination, abdominal, Increased muscle strength, Standing up from sitting position and walking with support, reduced Dysarthria Case 3: Spine curvature improved, Neck was more erect, Leg movement increased significantly, Had reduced LLs spasticity and better endurance, Able to walk 5-7 steps forward and backward with full calipers	NA
Emphysematous COPD	Case report (2015)	1	Shortness of breath, Pain radiating to the neck, Increased cough and wheezing especially at night and after getting up from sleep for the past 9 months	CEST showed paraseptal emphysematous changes in bilateral upper lobes and rest of parenchyma appeared normal	Absence of cough and phlegm, Improved sleeping and overall stamina	No cough, phlegm, or wheezing, Was able to work full time and to walk long distances
Spinal cord injury	Case series (2015)	5	Movement of UL and LL absent, Loss of sensation, Sitting balance, the plantar reflex and the abdominal reflex absent, Bladder and bowel control/sensation absent	NA	Significant improvement in sitting balance, control and sensation of bowel and bladder, power and movement of limbs (UL and LL)	NA
Glaucoma	Case series (2015)	2	Poor vision; Macular degeneration; Diminished peripheral vision; Increased IOP, Light sensitivity; Cognitive abilities absent such as concentration, aggressive behavior and no social interaction and Poor balance	NA	Signs of improvement in their sight; such as peripheral vision, Improved cognitive abilities like concentration and speech, Aggression reduced, and Improved balance (could climb stairs)	NA
Cerebrovascular accident	Case series (2015)	22	Leg maintain position, n = 22; Leg flexion, n = 22; Gait, n = 22; Arm outstretched position, n = 19; Arm raising, n = 21; Fingers, n = 12; Foot dorsiflexion, n = 16; Wrist extension, n = 14; Speech, n = 15; Facial movements, n = 10; Level of consciousness, n = 2; Comprehension, n = 1; Gaze, n = 1, Sitting and standing balance, n = 21	NA	Leg maintain position, n = 22; Leg flexion, n = 22; Gait, n = 22; Arm outstretched position, n = 19, Arm raising, n = 20, Fingers, n = 12; Foot dorsiflexion, n = 16; Wrist extension, n = 14; Speech, n = 15, Facial movements, n = 10; Level of consciousness, n = 2; Comprehension, n = 1, Gaze, n = 1, Sitting and standing balance, n = 20	NA

Lyme disease	Case series (2015)	5	Unable to walk straight or maintain balance while sitting and standing, Affected speech and chewing, Poor bladder and bowel control, Disturbed sleep, Impaired memory and concentration, Mood swings with depression, Decreased levels of energy and appetite, Mild hearing loss, Blurred vision and Stiffness	NA	Regained balance and able to perform regular activities with less effort, Improvement in blurred vision, tremors, Had higher energy levels, Improved stamina and appetite, Decreased numbness in the UL, Decreased stiffness and no slurring of speech	NA
Spinocerebellar ataxia	Case series (2015)	3	Increased frequency of micturition, Tremors, Difficulty in writing, walking and swallowing, Affected speech, Difficulty in writing since last 14 years, Memory loss, Sleeplessness, Gait and Walking imbalance	SPECT scan showed decreased area of hypoperfusion	Improvement in overall stamina, endurance, coordination, sitting balance, standing and walking ability, speech and flexibility, Reduction in tremors and No head nodding	Two patients have been followed up and are keeping well
Duchenne muscular dystrophy	Case series (2015)	5	Loss of balance; Scoliosis; Breathlessness; Inability to stand, walk, or sit; Difficulty in climbing stairs; Flexion deformity and Elevated CPK levels	NA	Regained active standing and trunk balance; Improvement in ability to walk, stand, sit, breathing capacity, hand functions and muscle strength; Increased body weight and Reduction in CPK levels	Four patients are well and one patient died of anaphylactic reaction to an antibiotic
Multiple sclerosis	Case report (2015)	1	Sensory stretching on left forearm, Spasticity in left leg; Heaviness while walking; Burning sensation in both legs (more on left side) and Weight loss	Tractography showed a mild reduction in the size of lesions in bilateral periventricular white matter and in the right occipital white matter	Improvement in muscle bulk, tone and power; Had increased energy level and power of ULs and Gained weight	NA
Non-healing wounds	Case series (2015)	6	Non-healing Ulcers; Wound due to injury and Deep bed sore	NA	Reduction in the size of wounds and granulation	NA
Parkinson's disease	Case report (2015)	1	Resting tremors; Bradykinesia; Muscle Rigidity; Back and neck stiffness; Unclear speech, Micrographia, Imbalanced walking and Urinary urgency with incomplete voiding	SPECT scan showed normal hypoperfusion in the cerebral region and a significant improvement (> 60%) occurred in the degree of perfusion in the cerebellar regions	Reduction in tremors, bradykinesia, muscle rigidity and numbness; Reduction in pain and stiffness in the neck, shoulder and low back; Improvement in neck movements, walking balance, and writing skills and Able to speak louder	Is off to all anti-Parkinson medications and has a mild resting tremor in his right hand as the only persisting symptom
Diabetes mellitus	Case series (2015)	3	Taking hypoglycemic drugs; Low energy levels; Poor eye sight; Allergic to wheat and dairy products; Weakness and numbness of LLs; Pain in LLs on walking; Discomfort in LLs on supine position; Unable to sit for a long time; Weight loss; Bladder sphincter	NA	Stopped taking hypoglycemic drugs; Reduced blood glucose levels; Improvement in eye sight, stamina, gait pattern endurance, mental focus ability and muscle strength; No pain in LLs; Reduction in secondary side effects of high blood sugar such as affectation of cardiac, kidneys, polyneuropathy and vision and Weight regain	NA

SPECT: Single-photon emission computed tomography; MRI: Magnetic resonance imaging; CEST: Chemical exchange saturation transfer; NA: Not applicable; UL: Upper limb; LL: Lower limb; IOP: Intraocular pressure; CPK: Creatine phosphokinase.

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Disclosure of conflict of interest

None.

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