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 Concise Review: Human Embryonic Stem Cells—What Have We Done? What Are We Doing? Where Are We Going?

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Key Words. Human embryonic stem cells • Induced pluripotent stem cells • Clinical trials • Pluripotent stem cells

\textbf{ABSTRACT}

Human pluripotent stem cells possess remarkable proliferative and developmental capacity and thus have great potential for advancement of cellular therapy, disease modeling, and drug discovery. Twelve years have passed since the first reported isolation of human embryonic stem cell lines (hESC), followed in October 2010 by the first treatment of a patient with hESC-based cellular therapy at the Shepherd Center in Atlanta. Despite seemingly insurmountable challenges and obstacles in the early days, hESC clinical potential reached application in an extraordinarily short time. Eight currently ongoing clinical trials are yielding encouraging results, and these are likely to lead to new trials for other diseases. However, with the discovery of induced pluripotent stem cells (iPSC), disease-specific hESC lines derived from patients undergoing pre-implantation genetic diagnosis for single gene disorders fell short of expectations. Lack of ethical controversy made human iPSC (hiPSC) with specific genotypes/phenotypes more appealing than hESC for drug discovery and toxicology-related studies, and in time, lines from HLA-homozygous hiPSC banks are likely to take over from hESC in clinical applications. Currently, hESC are indispensable; the results of hESC-based clinical trials will set a gold standard for future iPSC-based cellular therapy.

\textbf{SIGNIFICANCE STATEMENT}

hESC-based therapies have now become a reality. However, the development of HLA-homozygous iPSC banks, such as the one in Japan provide an ethically neutral alternative to hESC for therapeutic as well as research applications. International guidelines on screening and application of these iPSC lines will likely lead to complete redundancy of hESC lines at some point in the future.

\textbf{INTRODUCTION}

Optimism that human embryonic stem cells (hESC) would provide a virtually unlimited source of selected cell types for future cell therapy, as well as drug screening and development, has resulted in a considerable progress in stem cell biology over nearly two decades since the first hESC were derived [1]. However, the controversy over the use of hESC in research and translational medicine has not diminished over time. There is a constant clash between the obligation to protect life and the obligation to help and save those who are suffering. The very strong opinions on the moral standing of human embryos have led to the prohibition of work with hESC in some countries or, where allowed, this work is tightly regulated.

\textbf{CIRCUMVENTING ETHICAL CONTROVERSY—hESC LINES FROM SINGLE BLASTOMERES}

Ethical controversy determined the direction of early work; this focused on how to establish hESC lines without destruction of the embryo. A team from Advanced Cell Technology, a Massachusetts-based company, succeeded in deriving hESC lines from single blastomeres of cleavage stage embryos [2]. In this proof-of-principle study the embryos did not survive. To minimize the number of embryos used, the embryos were disaggregated and all blastomeres were biopsied from cleavage stage embryos, and the remaining embryo was left to develop to blastocyst stage. This strategy mimicked pre-implantation genetic diagnosis (PGD), a routine assisted reproduction procedure for selection of
healthy embryos for transfer and elimination of the embryos carrying disease-linked mutations. In spite of addressing the major ethical concerns, the technique did not become widespread. In the same year that the detailed protocol was published [4], two groups generated the first human induced pluripotent stem cell (hiPSC) [5, 6], and all the excitement around hESC started to fade—hESC were seen almost as an historical anomaly.

Instead of being celebrated as a major achievement, the technique of hESC-derivation from single blastomeres without embryo destruction became a center of controversy per se. Following nearly 50,000 public comments on the published draft Guidelines for research involving hESCs, the NIH modified the definition of hESCs [7]. hESC “are cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.” Under these guidelines, five hESC lines derived from single blastomeres by the Advanced Cell Technology team (MA09, NED1-4) [2, 3] and ten lines derived at the University of California San Francisco (UCSF81-10) [8] were ineligible for review because they were derived from a preblastocyst stage embryo and therefore, according to the definition, are not considered to be hESC lines [9]. Applications were submitted to the NIH hESC Registry in 2009 and 6 years later the decision is still pending. Paradoxically, there were no questions raised when clinical trials for macular degeneration of retina using MA09-derived retinal pigment epithelial (RPE) cells were labeled as hESC-based cellular therapy [10]. hESC generated without embryo destruction changed views on hESC patentability in the EU. Following the Directive 98/44/EC on the Legal Protection of Biotechnological Inventions [11] the European Patent Office (EPO) has refrained from granting patents for hESC on moral grounds. In 2006, the EPO’s Technical Board of Appeal decided that Chung et al. [3] provided the first disclosure of a method of establishing hESC lines without destroying a human embryo on 7 February 2008. Since then, only the hESC-related applications filed before that date were excluded from patentability [12].

**hESC Lines Carrying Disease-Specific Mutations**

hESC derived from embryos carrying monogenic inherited diseases or chromosomal aberrations were seen as tools for elucidating the etiology and pathophysiology of disorders. On that premise more than 100 hESC lines have been derived. Most of them were listed on either NIH hESC Registry or Human Pluripotent Stem Cell Registry. The spectrum of diseases was limited by the availability of PGD treatments and the frequency of the specific mutations in a given population (Table 1). The most frequently derived were hESC lines carrying specific mutations linked to Huntington disease (21 lines derived in 8 centers), Fragile X syndrome (12 lines derived in 3 centers), cystic fibrosis (12 lines derived in 6 centers), myotonic dystrophy (11 lines derived in 6 centers), and Charcot–Marie–Tooth disease (11 lines derived in 5 centers). In spite of efforts to make such lines available to the scientific community, actual interest did not match the initial enthusiasm. A relatively modest number of publications in peer-reviewed journals have described their use as research tools; in fact, the number of reviews elaborating on opportunities of using hESC lines carrying specific disease-linked mutations was several times higher than the number of actual research papers. Ethical issues, the regulatory landscape, and the limited spectrum of diseases were all drawbacks of hESC lines that hiPSC did not have and not surprisingly, disease-specific hiPSC lines took over.

**Clinical Grade hESC Lines**

Clinical grade hESC lines are lines which have been derived under current Good Manufacturing Practice (cGMP) conditions. The first clinical-grade hESC lines were the result of international efforts. Cryopreserved embryos were donated at Sydney IVF Ltd., derivation was performed at a cGMP facility in Brisbane, Australia, and the project was sponsored by the company ES Cell International, which was at that time based in Singapore [13]. The research versions of these lines were available for minimal reimbursement through the A*STAR Singapore Stem Cell Consortium (SSCC). Despite multimillion investments in these first clinical grade hESC lines, they did not gain the popularity of the H1 and H9 hESC lines derived by Thomson et al. [1], and the cells were never used in clinical trials. Since May 2010, the lines are owned by the California-based company BioTime. The company further characterized the lines at the molecular level and made the data, including copy number variation and genome sequencing, publicly available [14].

In the U.K., more than 30 clinical grade hESC lines have been derived in five centers across the country as a result of systematic investment from the Medical Research Council. The results of molecular karyotyping of 25 UK-derived clinical-grade hESC lines by whole-genome single nucleotide polymorphism array analysis was recently published [15]. Fifteen unique copy number variants greater than 100 kb and three copy-neutral regions of loss of heterozygosity greater than 1 Mb were detected in these 25 lines; none of these was associated with adaptation to cell culture. The presence of the culture artefact microduplication of chromosome 20q11.21 was, however, found at higher passages of four clinical grade hESC lines. The methodology and the results of testing cell lines for human viral pathogens has been made available for only 2 of these 25 lines, KCL033 and KCL034 [16].

Whether further investments into characterization of large numbers of hESC lines might pay off, only time will tell, especially with the expanding HLA-homzygous iPSC bank in Japan for clinical purposes [17]. The bank will contain multiple clinical grade iPSC lines homozygous for three HLA loci: HLA-A, -B, and -DR. Since autologous iPSC-based cell therapy would be financially prohibitive, the aim is to derive the lines from donors homzygous for HLA haplotypes that are found in the Japanese population at a high frequency. The cells derived from such hiPSC lines will carry a reduced risk of rejection when transplanted into recipients that are heterozygous for these haplotypes. Since Japan has a relatively homogenous ethnic population, the required size of the Japanese HLA-homzygous iPSC bank seems to be relatively small—about 50 homozygous lines will match >90% of the Japanese population [18, 19]. In the ethnically more diverse U.K., among 405 theoretical homozygous HLA combinations, a tissue bank...
| Disease                                                                 | Line           | Human pluripotent stem cell registry | NIH Institution                  | Institution         |
|------------------------------------------------------------------------|----------------|-------------------------------------|----------------------------------|---------------------|
| Adrenoleuko-dystrophy                                                 | SI-201         | RGle105-A                           | Reproductive Genetics Institute  | USA                 |
|                                                                       | UM112-1 PGD    | NIIhESC-14-0285                     | University of Michigan            | USA                 |
| Alpha thalassemia                                                      | UM112-2 PGD    | NIIhESC-15-0307                     | Australia                         | USA                 |
| Alport syndrome                                                        | GENEA073       | NIIhESC-12-0193                     | Tel Aviv Sourasky Medical Center  | USA                 |
| Amyotrophic lateral sclerosis; frontotemporal dementia                 | Lis14_Alport_3 | NIIhESC-15-0340                     | Tel Aviv Sourasky Medical Center  | Israel              |
| Androgen insensitivity                                                | Lis07_AIS_1    | NIIhESC-15-0334                     | University of Michigan            | USA                 |
| Aniridia (PAX6)                                                       | Lis08_AIS_2    | NIIhESC-15-0335                     | University of Michigan            | USA                 |
|                                                                      | UM29-2 PGD     | NIIhESC-12-0164                     | USA                               |                     |
|                                                                      | UM29-3 PGD     | NIIhESC-12-0165                     | Israel                            |                     |
| Becker muscular dystrophy                                             | SI-170         | RGle077-A                           | Reproductive Genetics Institute   | USA                 |
|                                                                       | SI-158         | RGle066-A                           | USA                               |                     |
|                                                                       | SI-164         | RGle072-A                           | Istanbul Memorial Hospital        | Turkey              |
|                                                                      | OZ-8           | IMHe011-A                           | King's College London             | U.K.                |
|                                                                         | KCL035         | NIIhESC-13-0227                     | Genea                             | Australia           |
|                                                                         | GENEAO58       | NIIhESC-12-0199                     | Australia                         |                     |
|                                                                         | GENEAO59       | NIIhESC-12-0175                     | King's College London             | U.K.                |
| Kosovo muscular dystrophy BRCA1                                       | VUB20_CMT1A    | VUBe014-A                           | Vrije Universiteit Brussel        | Belgium             |
| Charcot-Marie-tooth disease type 1                                     | STR-I-315-CMT1a| INSRMMe015-A                        | INSERM                            | France              |
| Cystic fibrosis                                                        | HUES PGD 11    | NIIhESC-11-0094                     | Harvard University                | USA                 |
|                                                                       | HUES PGD 12    | NIIhESC-11-0095                     | INSERM                            | France              |
|                                                                       | UM111-PGD      | NIIhESC-12-0153                     | University of Michigan            | USA                 |
|                                                                       | UM59-2 PGD     | NIIhESC-14-0275                     | USA                               |                     |
|                                                                       | UM59-4 PGD     | NIIhESC-16-0357                     | INSERM                            | France              |
|                                                                       | UM89-3 PGD     | NIIhESC-12-0174                     | Reproductive Genetics Institute   | USA                 |
|                                                                       | GENEAO64       | NIIhESC-12-0187                     | Vrije Universiteit Brussel        | Belgium             |
|                                                                       | GENEAO62       | NIIhESC-12-0188                     | Hadasah University Hospital       | Israel              |
|                                                                       | GENEAO63       | NIIhESC-13-0219                     | Genea                             | Australia           |
| Dystrophin dystrophy                                                  | STR-I-203-CFTR | INSRMMe008-A                        | INSeRM                            | France              |
|                                                                       | STR-I-251-CFTR | INSRMMe009-A                        | Reproductive Genetics Institute   | USA                 |
|                                                                       | Si-257         | RGle156-A                           | Reproductive Genetics Institute   | USA                 |
| Dystrophin dystrophy                                                  | VUB04_CF       | VUBe004-A                           | Vrije Universiteit Brussel        | Belgium             |
|                                                                       | VUB22_CF       | VUBe015-A                           | Hadasah University Hospital       | Israel              |
|                                                                       | HAD 2          | HADe002-A                           | Genea                             | Australia           |
| Dystrophin dystrophy                                                  | GENEAO41       | NIIhESC-12-0167                     | Reproductive Genetics Institute   | USA                 |
|                                                                       | GENEAO40       | NIIhESC-12-0171                     | Harvard University                | USA                 |
| Dystrophin dystrophy                                                  | SI-180         | RGle086-A                           | Tel Aviv Sourasky Medical Center  | USA                 |
|                                                                       | HUES PGD 3     | NIIhESC-11-0091                     | USA                               |                     |
|                                                                       | Lis48_DMD_6_N  | NIIhESC-15-0311                     | Israel                            |                     |
|                                                                       | Lis23_DMD_5    | NIIhESC-15-0328                     | Tel Aviv Sourasky Medical Center  | USA                 |
|                                                                       | Lis10_DMD_1    | NIIhESC-15-0337                     | Tel Aviv Sourasky Medical Center  | USA                 |
|                                                                       | Lis11_DMD_2    | NIIhESC-15-0338                     | Tel Aviv Sourasky Medical Center  | USA                 |
|                                                                       | Lis20_DMD_3    | NIIhESC-15-0345                     | Tel Aviv Sourasky Medical Center  | USA                 |
|                                                                       | Lis22_DMD_3    | NIIhESC-15-0347                     | Tel Aviv Sourasky Medical Center  | USA                 |
| Emery-Dreifuss muscular dystrophy                                     | Si-245         | RGle144-A                           | Reproductive Genetics Institute   | USA                 |
| Fabry disease                                                         | STR-I-171-GLA  | INSRMMe004-A                        | INSERM                            | France              |
| Facioscapulohumeral muscular dystrophy                                | VUB09_FSHD     | VUBe009-A                           | Vrije Universiteit Brussel        | Belgium             |
|                                                                       | GENEAO24       | NIIhESC-12-0170                     | Genea                             | Australia           |
|                                                                       | GENEAO49       | NIIhESC-12-0183                     | Genea                             | Australia           |
| Disease | Line | Human pluripotent stem cell registry | NIH | Institution |
|---------|------|-------------------------------------|-----|-------------|
| Factor VIII deficiency | GENE050 | NIHhESC-12-0184 | NIHhESC-14-0244 | Hadassah University Hospital |
| | GENE096 | | | Israel |
| | HAD 3 | HADe003-A | | |
| Familial adenomatous polyposis | STR-I-305-APC | INSRMe014-A | | INSERM France |
| | STR-I-355-APC | INSRMe017-A | | |
| | STR-I-359-APC | INSRMe018-A | | |
| | Lis34_FAP_3 | NIHhESC-15-0324 | | Tel Aviv Sourasky Medical Center |
| | Lis34_FAP_2 | NIHhESC-15-0325 | | Israel |
| | Lis25_FAP_1 | NIHhESC-15-0349 | | |
| Fanconi’s anemia | ST-128 | RGie040-A | | Reproductive Genetics Institute USA |
| Fragile X syndrome | WCMC-37 | NIHhESC-13-0211 | | Well Cornell Medical College USA |
| | UM139-2 PGD | NIHhESC-14-0292 | NIHhESC-15-0309 | University of Michigan USA |
| | Lis S1_FXS9_N | NIHhESC-15-0319 | NIHhESC-15-0320 | Tel Aviv Sourasky Medical Center |
| | Lis39_FXS8_N | NIHhESC-15-0326 | NIHhESC-15-0327 | Israel |
| | Lis38_FXS7_N | NIHhESC-15-0330 | NIHhESC-15-0331 | France |
| | Lis37_FXS10_N | NIHhESC-15-0348 | NIHhESC-15-0349 | |
| | Lis29_FXS7 | NIHhESC-15-0350 | | |
| | Lis01_HFX1 | | | |
| | Lis02_FXS2 | | | |
| | Lis03_FXS4 | | | |
| | Lis24_FXS5 | | | |
| | Lis26_FXS6 | | | |
| Hemophilia B | UM9-1PGD | NIHhESC-12-0154 | | University of Michigan Spain |
| Hereditary multiple exostoses | ES-11EM | ESe026-A | | Spanish Stem Cell Bank Spain |
| Huntington’s disease | GENE097 | NIHhESC-14-0248 | NIHhESC-14-0249 | Genea Australia |
| | GENE098 | | | |
| | SI-186 | RGie091-A | | Reproductive Genetics Institute USA |
| | SI-187 | RGie092-A | | |
| | SI-194 | RGie098-A | | |
| | VUB05 HD | VUBe005-A | | Vrije Universiteit Brussel Belgium |
| | VUB28 HD MFS | VUBe018-A | | |
| | STR-I-155-HD | INSRMe003-A | | |
| | KCL005 | KCl004-A | | |
| | KCL012 | KCl009-A | NIHhESC-13-0213 | King’s College London U.K. |
| | KCL013 | KCl010-A | NIHhESC-13-0214 | |
| | KCL027 | NIHhESC-13-0223 | NIHhESC-13-0241 | |
| | KCL036 | NIHhESC-13-0224 | | |
| | KCL028 | NIHhESC-13-0225 | | |
| | HUES PGD 16 | NIHhESC-12-0150 | NIHhESC-12-0151 | Harvard University USA |
| | UM17-1 PGD | NIHhESC-12-0160 | NIHhESC-12-0166 | USA |
| | GENE017 | NIHhESC-12-0169 | NIHhESC-12-0170 | Genea Australia |
| | GENE018 | NIHhESC-12-0180 | | |
| | GENE046 | HhESC-14-0245 | | |
| | GENE089 | HhESC-14-0246 | | |
| | GENE091 | HhESC-14-0247 | | |
| | GENE089 | | | |
| | HS799 | NIHhESC-13-0207 | NIHhESC-15-0308 | Tel Aviv Sourasky Medical Center Sweden |
| Hydrocephaly | Lis50_Hydrocephaly_3_N | NIHhESC-12-0150 | NIHhESC-12-0151 | Tel Aviv Sourasky Medical Center Israel |
| | Lis49_Hydrocephaly_2_N | NIHhESC-12-0160 | NIHhESC-12-0166 | |
| | Lis49_Hydrocephaly_1_N | NIHhESC-12-0169 | NIHhESC-12-0170 | | |
| | UM15-4 PGD | NIHhESC-15-0310 | NIHhESC-15-0323 | | |
| | UMK38-2 PGD | NIHhESC-12-0155 | | University of Michigan USA |
| Hypertrophic cardiomyopathy (MYBPC3) | GENE077 | NIHhESC-12-0261 | | Genea Australia |
| | GENE071 | NIHhESC-12-0191 | | |
| | GENE065 | NIHhESC-12-0200 | | |
| Disease                              | Line                   | Human pluripotent stem cell registry | NIH Institution                           | Institution          |
|--------------------------------------|------------------------|--------------------------------------|-------------------------------------------|----------------------|
| Ichthyosis                           | Lis 46Ichthyosis_2_N   | NIHhESC-15-0313                       | Tel Aviv                                 | Israel               |
| Ichthyosis                           | Lis 45Ichthyosis_1_N   | NIHhESC-15-0314                       | Sournasky Medical Center                 | Israel               |
| Juvenile retinoschisis               | GENEAO72               | NIHhESC-12-0192                       | Geneva                                    | Australia            |
| Klinefelter's syndrome               | Royan H4               | NIHhESC-15-0314                       | Royan Institute                           | Iran                 |
| Klinefelter's syndrome               | KCL008                 | NIHhESC-15-0314                       | King's College London                     | U.K.                 |
| Klinefelter's syndrome               | FC018                  | NIHhESC-15-0314                       | Cellartis                                 | Sweden               |
| Klinefelter's syndrome               | BG01V                  | NIHhESC-15-0314                       | Vicyte (Novocell)                         | USA                  |
| Klinefelter's syndrome               | WA16                   | NIHhESC-15-0317                       | University of Wisconsin                  | USA                  |
| Leuko-encephalopathy                 | Lis 41_LTBL_N          | NIHhESC-15-0317                       | Tel Aviv Sourasky Medical Center         | Israel               |
| Loey-Dietz syndrome 2                | GENEAO83               | NIHhESC-14-0256                       | Geneva                                    | Australia            |
| Marfan syndrome                      | SI-154                 | NIHhESC-14-0257                       | Reproductive Genetics Institute          | USA                  |
| Merosin deficiency 1A                | STR-I-301-MFS          | INSERM013-A                           | INSERM                                    | France               |
| Merosin deficiency 1A                | MFSS                   | NIHhESC-10-0052                       | Vrije Universiteit Brussel Stanford University | USA                  |
| Merosin deficiency 1A                | UMBR-1-PGD             | NIHhESC-14-0276                       | University of Michigan                   | USA                  |
| Merosin deficiency 1A                | GENEAO81               | NIHhESC-16-0359                       | INSERM                                    | France               |
| Merosin deficiency 1A                | STR-I-209-MEN2a        | INSERM006-A                           | University of Michigan                   | USA                  |
| Merosin deficiency 1A                | STR-I-211-MEN2a        | NIHhESC-13-0208                       | Vrije Universiteit Brussel               | Belgium              |
| Merosin deficiency 1A                | UMS5-1-PGD             | NIHhESC-14-0252                       | Hadassah University Hospital             | Israel               |
| Neurofibromatosis                    | SI-148                 | NIHhESC-14-0254                       | Reproductive Genetics Institute          | USA                  |
| Neurofibromatosis                    | SI-153                 | NIHhESC-12-018                         | Kings College London                     | U.K.                 |
| Neurofibromatosis                    | KCL018                 | NIHhESC-12-019                         | Geneva                                    | Australia            |
| Neurofibromatosis                    | GENEAO66               | NIHhESC-12-010                         | NIHhESC-15-0339                          | Tel Aviv Sourasky Medical Center | Israel               |
| Neurofibromatosis                    | GENEAO67               | NIHhESC-12-019                         | Geneva                                    | Australia            |
| Neurofibromatosis                    | Lis12_DOM_1            | NIHhESC-12-018                         | Reproductive Genetics Institute          | USA                  |
| Nemaline myopathy 2                 | GENEAO78               | NIHhESC-12-019                         | NIHhESC-15-0344                          | Geneva               |
| Nemaline myopathy 2                 | GENEAO79               | NIHhESC-12-018                         | NIHhESC-14-0252                          | USA                  |
| Nemaline myopathy 2                 | GENEAO80               | NIHhESC-12-019                         | NIHhESC-14-0253                          | USA                  |
| Neurofibromatosis                    | SI-137                 | NIHhESC-12-019                         | NIHhESC-15-0343                          | USA                  |
| Neurofibromatosis                    | SI-138                 | NIHhESC-12-019                         | NIHhESC-15-0346                          | Israel               |
| Neurofibromatosis                    | SI-140                 | NIHhESC-12-019                         | NIHhESC-15-0346                          | Israeli              |
| Neurofibromatosis                    | SI-235                 | NIHhESC-12-019                         | NIHhESC-15-0346                          | Israeli              |
| Neurofibromatosis                    | KCL024                 | NIHhESC-12-020                         | NIHhESC-15-0316                          | USA                  |
| Neurofibromatosis                    | KCL025                 | NIHhESC-15-0316                         | NIHhESC-15-0315                          | USA                  |
| Neurofibromatosis                    | Lis 47_NF1_2_N         | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | Tel Aviv Sourasky Medical Center | Israel               |
| Nonsyndromic deafness                | Lis 42_NF1_1_N         | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | Tel Aviv Sourasky Medical Center | Israel               |
| Nonsyndromic deafness                | Lis43_Connexin_3_N     | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | URLav Sourasky Medical Center | Israel               |
| Noonan syndrome                      | Lis17_Connexin_1       | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Noonan syndrome                      | Lis18_Connexin_2       | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Noonan syndrome                      | Lis21_Noonan_1         | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Osteogenesis imperfecta              | VUB23_OI               | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Osteogenesis imperfecta              | SA002                  | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Osteogenesis imperfecta              | Miz-hES13              | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Osteogenesis imperfecta              | FY-hES-S               | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Patau syndrome (trisomy 13)           | VUB23_OI               | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |
| Patau syndrome (trisomy 13)           | SA002                  | NIHhESC-15-0316                         | NIHhESC-15-0316                          | NIHhESC-15-0316      | NIHhESC-15-0316          | Tel Aviv Sourasky Medical Center | Israel               |

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from 150 selected homozygous HLA-typed volunteers could match 93% of the population [20]. However, among 10,000 HLA typed organ donors used in the study as a representative of the UK population, only 2% were identified as non-White ethnicity, whereas according to the 2011 census 12.8% of the population was non-White, which indicates the particular challenges in identifying suitable donors for the members of these communities [21]. hESC lines, such as H1, MA09, and 16 on which are based most clinical trials today, were not clinical grade lines from the start. They were derived as research grade lines and, only later were adapted to cGMP conditions. Moreover, they were derived and propagated in the presence of mouse feeder cells and/or bovine serum. Xeno-free technology was developed later [22–28] and in 2011, a team from King’s College London derived the first eight animal product-free clinical grade lines [26–28]. The lines are karyotyped at the molecular level [15]; they are also listed on the NIH hESC Registry and, therefore, eligible for use in NIH-supported research. The most recent advance is the use of a cell culture matrix containing a mixture of human recombinant laminin (LN)—521 and E-cadherin [29] to derive hESC lines from the inner cell mass of blastocysts and from single blastomere cells from cleavage stage embryos without a need to destroy the embryo. The LN-521/E-cadherin matrix allows clonal derivation, survival and long-term self-renewal of hESC under chemically defined animal product-free conditions without addition of ROCK inhibitors.

All hESC lines do not have equal developmental potential and that cannot be explained by epigenetic memory as with hiPSC lines. Some of the hESC lines have propensity toward mesodermal lineages, whereas other toward endoderm [30]. Thus, a screening of the multiple hESC lines for their differentiation propensity has become a standard approach in selection of lines for particular clinical trials. The yield of differentiated cells basically depends on propensity of the source and the efficacy of differentiation protocol. However, regardless of differentiation efficacy, unlimited supplies of hESC or hiPSC would finally give more desired cell types than any other source, and therefore make the most of invested capital.

## CLINICAL TRIALS

### Spinal Cord Injury

In spite of all the obstacles, which include a 21,000-page Investigational New Drug (IND) application with the FDA, the first patient was treated with an hESC-based cellular therapy product, oligodendrocyte progenitor cells 1 (OPC1), in a clinical trial at the Shepherd Center in Atlanta in October 2010.
only 12 years after hESC were isolated for the first time [1].

The clinical trial for spinal cord injury, sponsored by Geron, a California-based company, treated only five patients. The treatment did not cause serious adverse events, although motor or sensory neurological changes were not observed. The lack of obvious improvement in physical condition clashed with the high expectations of the public and the company’s stock dropped nearly 60% in the nine months, from January to September 2011. In order to stay in business, lack of investment and support forced the company to end the trial prematurely and to close their stem cell program [31].

All Geron’s assets were transferred to another Bay Area company, BioTime and its subsidiary Asterias Biotherapeutics, in 2013. Supported with a strategic partnership award from the California Institute for Regenerative Medicine and equity funding, Asterias reinitiated the clinical trial, and the first patient was treated in Atlanta in June 2015. The study is conducted at a total of up to eight centers in the United States. The AST-OPC1 cells will be tested with three sequential escalating doses, the highest being \(2 \times 10^6\) cells, in 13 patients with subacute, C-5 to C-7, neurologically complete cervical spinal cord injury. In February 2014, Asterias received Orphan Drug Designation from the U.S. Food and Drug Administration (FDA) for AST-OPC1, for the treatment of acute spinal cord injury. Orphan Drug Designation is granted to products that treat diseases affecting fewer than 200,000 people in the U.S., and it may provide the sponsor certain benefits and incentives, including a period of marketing exclusivity of 7 years from the first marketing application, if regulatory approval is received for the designated indication [32].

**Macular Degeneration of the Retina**

Macular degeneration of the retina is likely to be the first disease that could be, to some extent, successfully treated with hESC-based therapy. Easy accessibility with minimally invasive procedures, the subretinal space being immunoprivileged, and the fact that the stem cell transplant can be monitored regularly with noninvasive methods for structural engraftment (spectral-domain optical coherence tomography) and functional outcome (autofluorescence and visual acuity), make the eye an ideal target choice for initial hESC/iPSC-based cellular therapies. Indeed, there are currently nine clinical trials with hESC and one with iPSC-derived RPE cells [10, 31, 33–35]. The initial results and follow-up with a median time of 22 months are promising; however, we do not know how long the effects will last. Over time the hESC-derived RPE cells might succumb to the pathologically altered environment of a diseased eye and ameliorate the condition only temporarily. Nevertheless, using ocular indications as a target was an ingenious idea and it revived the field after Geron was forced to end the trial for spinal cord injury.

**Diabetes**

Clinical trials with hESC/iPSC-based therapy in type 1 diabetes have been anticipated for a long time. California company ViaCyte (formerly known as Novocell) has spent a number of years developing their glucose-responsive insulin producing PEC-01 cells as well as Encaptra, an encapsulating drug delivery system made from porous cell-impermeable membrane. They are currently tested together as VC-01, islet replacement product candidate. VC-01 is the first stem cell-based treatment for type 1 diabetes to enter clinical testing and the first patient was treated in October 2014 at the University of California San Diego [31, 36, 37].

**Heart Repair**

A clinical study of a fibrin patch embedded with hESC-derived cardiac-committed CD15+ ISL-1+ progenitors transplanted into epicardium of the infarcted area and covered with an autologous pericardial flap commenced in autumn 2014 in France [31, 38]. Following the treatment, the first patient suffering from severe heart failure New York Heart Association (NYHA) functional Class III improved to NYHA Class I and remained stable NYHA Class I 6-months after the intervention [38]. This is the first hESC-based clinical trial that originated outside of the US and that is not driven by a for-profit company.

**hESC-Derived Cancer Vaccine**

In 2011, Geron reported the development and modification of hESC-derived dendritic cells with mRNA as a potential strategy for the induction of T-cell-mediated immunity [39, 40]. With discontinuation of the stem cell program, the assets related to antigen-presenting dendritic cells GRN-VAC1 and GRN-VAC2 were transferred to Asterias. GRN-VAC2, renamed AST-VAC2, are mature hESC-derived dendritic cells that express a modified form of telomerase, which permits enhanced stimulation of immune response. In September 2014, Asterias teamed up with the UK charity Cancer Research UK and its development and commercialization arm Cancer Research Technology to bring AST-VAC2 into clinical trials in patients with non-small cell lung cancer and in January 2016 has completed the transfer of its manufacturing processes to Cancer Research UK who will produce AST-VAC2 under cGMP conditions at their Biotherapeutics Development Unit.

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**THE FUTURE—WHERE WE ARE GOING WITH hESC?**

With the development of hiPSC, free of ethical issues [5, 6], hESC started to lose their unique appeal. Within a few years, from being an indispensable research tool, hESC dropped to the level of “gold standard” demonstrating that iPSC are equally useful for addressing certain research questions [e.g., [41–44]]. Only time will show whether they will remain as a “gold standard” or they will slowly become obsolete. Most of the issues that are relevant for hESC-based therapy also apply to iPSC [45]. Therefore, it is logical that the standards set in hESC-based clinical trials should be applicable to hiPSC-based clinical trials (e.g., clinical trials in macular degeneration of retina). Since the key difference between hESC and hiPSC is the potentially modified genomic and epigenetic state of hiPSC, additional standards such as DNA methylation analysis and medium-resolution array-comparaive genomic hybridization should be applied in hiPSC-based trials.

Nonuniform epigenome transformation during reprogramming is not the only issue that may affect the quality of hiPSC [46]. hiPSC are derived from adult somatic cells, which accumulate mutations over the lifespan of the donor [47].
Specific genetic and epigenetic footprints influence the molecular and functional properties of each hiPSC clone and might lead to misinterpretation of the results in, for example, drug screening studies. This is particularly important in studies with disease-specific iPSC lines. With newly discovered relatively precise genome editing techniques such as clustered regularly-interspaced short palindromic repeats (CRISPR)/Cas9 [48, 49], it is possible to repair mutations in disease-specific hiPSC lines and in such a way generate much better controls than native non-manipulated hESC. On the other hand, using CRISPR/Cas9, disease-specific mutations can be introduced in normal healthy hESC lines, avoiding a baggage of accumulated lifetime mutations, which are typical for disease-specific hiPSC, as well as preserving the native DNA methylation footprint of hESC, which is almost never completely matched in hiPSC.

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