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Hadassah, provider of "Regulatory-Ready" pluripotent clinical-grade stem cell banks

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ABSTRACT

The Hadassah hESC Research Center's aim is to be a supplier of clinical and research-grade human embryonic stem cell (hESC) lines. In 2012, we derived the first three entirely GMP-compliant and xeno-free, fully-characterised, feeder-dependent (human umbilical cord) hESC lines developed under cleanroom conditions. In 2018, we established four new GMP and xeno-free, feeder-independent MCB hESCs under GMP conditions using commercially available reagents, media and matrix. All cell lines were derived under Israeli Ministry of Health's National Ethics Committee for Genetic Research in Humans and the ethical considerations that guided the development of the hESCs strictly followed Israeli law. Hadassah has provided its clinical-grade hESC lines to commercial entities of which two are already in clinical trials, establishing Hadassah as a key provider of clinical-grade hESC lines.

1. Introduction

Human pluripotent stem cells have been celebrated for many years for their use in basic scientific research, disease modeling, and as an unlimited renewable source of cells for transplantation therapy. The Hadassah Human Embryonic Stem Cell (hESC) Research Center was established in 2003 in order to develop technologies for the use of stem cells in regenerative medicine and cell-based therapies to treat human disease. After identifying the need for clinical-grade lines that were not available at the time, we were pioneers in deriving three GMP[1]-grade, xeno-free (XF) hESC lines on human fibroblast feeders, which are suitable for further manufacturing purposes (described in (Tannenbaum et al., 2012)) for use in preclinical studies and clinical trials. Funding for the project was provided by the Israeli Genesis Consortium for Cell Therapy, and by philanthropic gifts from the Legacy Heritage Fund Ltd. and Sidney and Judy Swartz.

Hadassah's goal is to be provider of clinical and research-grade cells. This year, we transformed our three existing clinical-grade hESC lines into a new culture system that is GMP, xeno-free, and fibroblast feeder independent. Using this new culture system, we further derived four new clinical-grade hESC lines, also fibroblast feeder independent, that were previously unreported.

The Hadassah hESC Research Center's interests include the study and development of mature hESC-derived cells for transplantation therapy in retinal and neural degenerations. Another focus of our research includes the development of novel stem cell-based therapies for the advancement of women's health and for the treatment of infertility. We utilize human pluripotent stem cells to model human diseases such as amyotrophic lateral sclerosis (ALS) towards developing innovative therapies for this devastating condition. Recently, we characterized and reported on the role of Semaphorin 3A in the ALS disease process (Birger et al., 2018).

2. Summary description of cell lines distributed and conditions of supply e.g. for collaborations only, free supply, fee scale, nature of transfer agreement

The original three Hadassah clinical-grade hESC lines (HAD-C 100, 102 and 106), that were derived on umbilical cord fibroblast feeders, were approved for inclusion in the NIH Human Embryonic Stem Cell

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[1] GMP = Good Manufacturing Practice

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Registry, are also registered in the Human Pluripotent Stem Cell Registry (hPSCreg). Currently these three lines are being used by academic and commercial entities for such medical indications as diabetes mellitus, spinal cord injuries, CNS disorders, retinal degenerations and ALS (these last two indications are already in Phase I/IIa clinical trials).

These lines, the new Seed Cell Banks (SCB) derived from them (HAD-C 100, 102, 106 SCB), their matching research-grade banks, and the new clinical-grade master cell banks propagated under new culture conditions independently of feeders, can all be obtained under the provision of Material Transfer and Cell Supply Agreements (MTA) for academia or for commercial purposes. For research-grade cells only, the cells are provided for basic research under a Material Transfer Agreement. The cost is minimal to cover the Hadassah hESC Center's expenses of expansion, and preparation of the cells. For clinical-grade cells for clinical use, the cells are provided under a Cell Supply Agreement and are separately negotiated with each entity. Prices are competitive in the field.

Also available under MTA are disease-affected hESC lines (PGD[2]-lines) from such genetic diagnoses as myotonic dystrophy type 1, hemophilia A, carrier of cystic fibrosis, Fragile X and others, as reported in our published manuscript (Turetsky et al., 2008).

The use of the cell lines is governed under the ethical guidelines of the Israeli Ministry of Health's National Ethics Committee for Genetic Research in Humans. Exact prices for the cells will be provided upon request.

3. Summary of tests for QC, characterization and safety performed on each distributed cell bank

The Quality Control (QC) and characterization profiles of the HAD-C 100, 102 and 106 primary and secondary cell banks are as described in our published manuscript (Tannenbaum et al., 2012). Since that time, HAD-C 100 primary cell bank has undergone biosafety testing (at Bioreliance, UK) for the following: RT PCR[3] for the Detection of 14 Human Viruses, Hepatitis A and B19, 28-day *In Vitro* Virus Assay, and Quantitative Transmission Electron Microscopy (TEM). The product of HAD-C 106 primary cell bank has undergone biosafety testing (Bioreliance, UK) for the following: 28-day *In Vitro* Virus Assay, RT PCR for the Detection of 14 Human Viruses, In Vitro Assay for the Presence of Bovine and Porcine Viruses, Mycoplasma, Q-PERT Assay for the Detection of Retroviruses, Test for the Presence of Inapparent Viruses, Transmission Electron Microscopy, and Sterility and Endotoxin Testing (at Lonza, Walkersville USA).

HAD-C 100, 102, and 106 SCBs were derived in 2017 and were characterized for viability, identity by STR and HLA typing, karyotyping (G-banding), morphology by microscopy, cell growth (doubling time and Ki-67 staining), flow cytometry (TRA 1-60, TRA-81, SSEA3 and PSA-NCAM) and immunostaining (Oct-4, and alkaline phosphatase activity). Pluripotency efficiency was tested by teratoma and embryoid body formation. Purity prior to cryopreservation was also assessed for sterility, mycoplasma, and LAL. In-process testing was determined for purity (sterility, LAL), and immunostaining of markers of pluripotency (alkaline phosphatase and Oct-4). For HAD-C 100 SCB, biosafety testing (DNA fingerprinting and 14-day *In Vitro* Virus Assay) was performed at Bioreliance, USA and a SNP array at the UCSF Research Cytogenetic Core Laboratory). We expect additional biosafety testing to be completed on the remaining SCBs in 2019.

The HAD-C 103, 104, 105 and 107 single-cell, feeder-free MCBs have completed QC testing. Characterization for these cell banks is nearly identical to that of the primary cell banks (as shown in (Tannenbaum et al., 2012)).

4. Information provided with cell lines

The Hadassah hESC Research Center has built the quality and regulatory documentation infrastructure necessary for clinical use for submission to regulatory entities. Dependent on individual requirements, the Hadassah hESC Research Center supplies a batch-specific Certificate-of-Analysis and QC characterization results, biosafety test results (if they exist and can be supplied), hESC line donor testing results and a brief summary of the donors' medical histories, a quality system statement for the Hadassah GMP-facility used to generate the cell line, copies of ethical approvals, lists of raw materials used for the derivation of the cell lines, and other supplementary documentation as necessary. For the feeder-independent research-grade banks, a batch-specific Certificate-of-Analysis is provided with the cells lines, and other supplementary documentation as necessary. For the feeder-independent research-grade banks, a batch-specific Certificate-of-Analysis is provided with the cells.

5. Other services and training provided to the outside community

The Hadassah hESC Research Center was established in order to develop and translate stem cell technologies and cell lines into treatments for gene therapy and regenerative medicine. The Center's main goal is to support and encourage researchers in Israel and abroad to engage in stem cell research and explore their potential for basic science and clinical applications. Therefore, Hadassah sends the cells with protocols for their thawing and expansion. We provide technical assistance and training as needed, to all who utilize the cells.

6. Any accreditation for regulatory and/or ethical issues

All cell lines were derived under Israeli Ministry of Health's National Ethics Committee for Genetic Research in Humans. The ethical considerations that guided the development of the hESCs strictly followed Israeli law and adopted the recommendations of the Bioethics Advisory Committee of the Israel National Academy of Sciences and Humanities (Revel, 2003).

The cell lines were manufactured under GMP conditions with the Hadassah hESC Research Center's Quality Assurance (QA) Unit's quality oversite. The Hadassah GMP facility has GMP-approval for manufacturing for phase 3 clinical trials that came into effect in September 2017. The GMP facility adheres to FDA and EMA[4] standards.

7. Future plans

The Hadassah hESC Research Center intends on completing the full biosafety testing of the feeder-free SCBs within 2019. We anticipate also to become a major supplier of the new clinical-grade hESC lines that we developed feeder-independently in 2018. The Hadassah hESC Research Center has plans to derive clinical-grade, universal donor, safe human ESCs as well as induced pluripotent stem cell (iPSC) lines.

Declaration of Competing Interest

Benjamin Reubinoff is the CSO and holds shares in Cell Cure Neurosciences Ltd.

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[2] Preimplantation genetic diagnosis = PGD

[3] Reverse transcription polymerase chain reaction = RT PCR

[4] EMA = European Medicines Agency

the data, and in the preparation, review, or approval of the manuscript.

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