The Israeli Initiative for derivation, expansion, and biobanking of stem cells for clinical use

This proposal highlights the use of stem cells for clinical uses in regenerative medicine, organoid technology, and drug discovery for the benefit of patients. We aim to use both human pluripotent stem cells and adult human tissues stem/progenitor cells for urging clinical needs as outlined below.

1. The Israeli National Stem Cell Bank - The Pluripotent Stem Cell Arm

INTRODUCTION

Cellular therapies are one of the major advancements in current health care. The use of cellular therapies in regenerative medicines is being heralded for the curative treatment of neurodegenerative and heart diseases, diabetes, and a multitude of other serious, debilitating illnesses that impact society and affect mankind.

To effect such cures, two types of human pluripotent (can give rise to any cell type of the body) stem cells (hPSCs) may be used. Human embryonic stem cells (hESCs), which are derived from the early *in-vitro* fertilized embryo, can multiply extensively in culture and are pluripotent. An alternative type of human pluripotent stem cell is induced pluripotent stem cells (iPSCs), which are like hESCs and are developed by reprogramming of somatic (of the body) mature cells. Both pluripotent stem cell types may be directed to differentiate into a variety of functional cell types, including dopaminergic neurons, insulin producing beta cells, retinal pigmented epithelial cells, and others. Given the unique properties of pluripotent stem cells, they may serve as unlimited sources of human cells for transplantation therapy, and the replenishment of malfunctioning or dying cells. In addition, they may serve to model human diseases and develop novel therapeutic modalities and drugs.

Over the last several years, national stem cell banks have become the international standard in pluripotent stem cells. Such stem cell banks provide benefits by assuring the researchers that the cell lines they use are ethically-sourced and quality controlled. A national pluripotent stem cell bank will provide standardization that allows researchers from different laboratories to obtain comparative results, will offer structure for supply and distribution, and will provide the infrastructure for the development of safe and effective new therapies for clinical applications.

POTENTIAL IMMUNE REJECTION AND SOLUTIONS

In exploiting the enormous potential of pluripotent stem cells in transplantation therapy, one must bear in mind the probable and real-life problem of allograft immune rejection of these cells requiring systemic immunosuppression. While developing banks of autologous or HLA homozygous cell lines may be preferred, they are challenging, and are neither feasible nor practical. A likely solution to these problems is to generate a universal donor hPSC line that will not be rejected and does not require immunosuppression.

UNIVERSAL DONOR CELL LINES

The aim of this project is to generate clinical-grade hPSC lines that would be suitable for human therapeutic use and that would not be rejected after allogeneic transplantation.
The recent revolutionary breakthrough in genomic editing paves the way to two main approaches that are currently being developed worldwide to avoid immune rejection of transplanted allogeneic stem cells. In one strategy, the HLA molecules on the surface of the stem cells, which provoke their immune rejection by the recipient, are ablated (Xu, H. et al., 2019). Building on this, another strategy would be the insertion of genes that can repress immune rejection into the cells, and which shield them from the immune system (Han, X., et al, 2019).

The development of universal hPSC lines, in this project, would serve as a global "off-the-shelf" unlimited stem cell resource for the development of safe cellular products that will not be rejected after transplantation.

IPSCs VERSUS HESCs

While both iPSCs and hESCs are pluripotent and can self-renew, the use of iPSCs may avoid some of the ethical issues surrounding the use of hESCs today. Published reports list iPSC banks available worldwide, although relatively few offer GMP-grade lines suitable for clinical applications. iPSCs use have inherent drawbacks that challenge their safety, such as low reprogramming rates, genomic instability due to reprogramming methods, and others. The hESC phenotype, on the other hand, and differentiation potential is relatively more stable after clonal derivation or prolonged undifferentiated proliferation. Therefore, deriving a universal donor cell line from hESC is probably advantageous for our purpose of supplying safe product for regenerative medicine that will not require immunosuppression treatment for life.

RELYING ON EXISTING PLATFORMS -HADASSAH GMP-GRADE, XENO-FREE HESCS

Israel pioneered the development of clinical-grade hESCs. The Hadassah hESC Research Center was the first to develop hESC lines under cGMP and xeno-free (XF) conditions, in the Hadassah GMP Facility (Tannenbaum, S., et al., 2012). These clinical-grade cell lines that are suitable to serve as starting material for the development of differentiated cells for

transplantation therapy for multiple conditions, are today being distributed worldwide to academic and commercial groups that are developing cell therapies. The lines are currently being used both in Israel and the USA in two ongoing clinical trials of transplantation of differentiated retinal pigment epithelial cells in Age-related Macular Degeneration (AMD), and differentiated neural cells in Amyotrophic Lateral Sclerosis (ALS). Clinical trials in additional conditions are expected to start soon.

The Hadassah hESC Research Center can contribute:

1) Clinical-Grade hESC lines, including the accompanying regulatory, quality and ethical platform;
2) Proven QA, regulatory, and methodological experience in the development of clinical-grade pluripotent hESC banks; Knowledge in translating research technology into GMP-XF-standards;
3) GMP facility- Hadassah will provide access to Hadassah's GMP suite that is approved by the Israeli Ministry of Health for manufacturing of products for phase III clinical trials, including all relevant manufacturing documentation, etc. Hadassah will supply personnel experienced in establishing clinical-grade hESC banks in a GMP-environment.

DEVELOPMENT OF IMMUNE TOLERANCE

To enable use of the cell lines in clinical applications on a broad scale without rejection, the development of a universal donor stem cell line that can be used in regenerative medicine is needed. While PSCs and their clinical development are readily available to us, techniques in overcoming immune rejection will be developed (Ye, Q., Sung., TZ., Yang, JM., et al., 2020) (Deuse, T. et al., 2019) (Gornalusse, G., et al., 2017). The project will explore the feasibility applicability of developing HLA homozygous new clinical-grade lines (Nakajima, F., Tokunaga, K., & Nakatsuji, N. ,2007). Alternatively, we will explore the route of genome editing to ablate HLA molecules and/or introduce other factors that will make transplanted cells shielded from the immune system. Alternately, the project could license such technologies from third parties.

PLURIPOTENT STEM CELLS FOR DISEASE MODELLING AND DRUG DISCOVERY

For multiple human diseases, a reliable model for studying their cause and for the discovery of novel drugs is not available. The platform of ESCs and iPSCs provides the opportunity to develop authentic human models for both monogenic and complex polygenic disorders (Avior et al., 2016). In this process, ESCs can be derived from sick embryos, or generate models for hereditary disorders by genetic manipulations, alternatively, mature cells such as skin or blood cells from patients are reprogrammed to become iPSCs. These patient-specific iPSCs may be induced to mature into the specific cells that are affected in the patient's disease process. They may also give rise to organoids mimicking tissues that are involved in the patient's disease. The patient-derived specific mature cells and organoids

that are affected by the disease may serve to study the disease processes in culture and for the discovery of novel drugs that will have a curative potential.
Initial biobanks for human genetic disorder, based on pluripotent stem cells, already exist in different facilities in Israel but many other research groups in Israel that are studying various disorders do not have the resources, the infrastructure, or the experience to develop iPSC-based authentic models that may greatly advance their efforts to discover novel therapies. The Israeli National Bank of Stem Cells, will provide guidance and support to such groups towards developing iPSCs-based models. The bank will derive the iPSCs from patients' mature cells in collaboration with the research groups, providing them the knowledge, methodologies, specific reagents and laboratory infrastructure to develop the iPSCs. The bank will also develop a repository of the patient-derived iPSC lines that will serve the national and international scientific community.

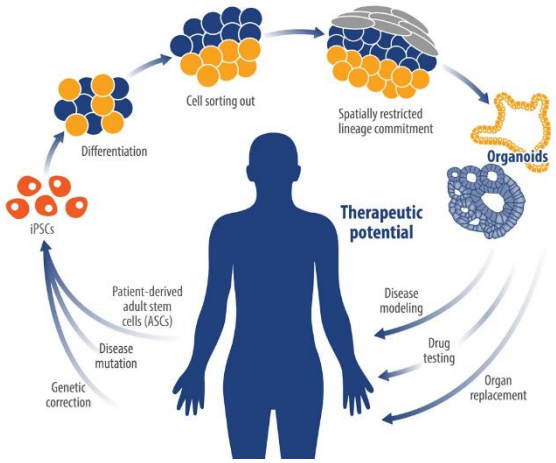
ESTABLISHMENT OF THE NATIONAL PLURIPOTENT STEM CELL BANK

The key project goal is to establish a national bank of universal donor Israeli clinical-grade pluripotent stem cell lines for the development of safe cellular products suitable for human therapeutic use. The bank will also provide training in the derivation, culture, regulation and quality assurance of pluripotent stem cells and enhance collaborations in the field of stem cells between researchers from multiple disciplines. Within this scope of the project, the bank will support the derivation of iPSCs for modeling monogenic and polygenic disorders in collaboration with researchers and establish a repository of such pluripotent cell lines. The bank will be established at Hadassah, relying on Hadassah's GMP, regulatory platforms, vast experience in derivation of clinical-grade hESCs and research-grade iPSCs. The bank will provide the clinical and research-grade cell lines worldwide, placing Israel on the global map in scientific and medical communities.

2. The Israeli National Stem Cell Bank - The Tissue Stem Cell and organoids Arm

INTRODUCTION:

Tissue stem cells are cells that reside in each of our organs and have the capacity to replace lost cells of a specific organ. For instance: blood stem cells manufacture and replace lost blood cells, kidney stem cells generate and replace lost kidney cells, the cornea maintain its special optical properties by the continuous replacement of the cells from the corneal stem cells and so forth. Tissue stem cells can generate the cells of only the specific organ in which they reside. Another major aim of the scientific community involved in regenerative medicine is to isolate tissue stem cells of all organs, expand them and use them for regeneration. Importantly tissue stem cells are adult cells and can be used for autologous therapy, meaning cells that are taken from one's own organ and are given back to heal it.



These characteristics of tissue stem cells make them transplantable and very safe to use. We aim to generate banks of expanded tissue stem cells from individuals that have just become ill with various diseases. For instance – in the kidney – collection of kidney stem cells from the urine is one elegant non-invasive solution for derivation, expansion, and bio banking. This method is now being established at the Sheba Medical Center.

UTILIZATION OF TISSUE STEM CELLS FOR REGENERATIVE MEDICINE AND ORGANOID TECHNOLOGY: CREATING THE ISRAEL ORGANOID BIOBANK AT THE SHEBA MEDICAL CENTER

Recently, hospitals in Israel have created biobanks of adult human tissue derived from surgical specimens. These biobanks cash on the wealth of numerous human tissues that are removed in clinical departments. The tissue banks are currently used for retrieval of human tissues for molecular and phenotypical analysis including all Omics that exist (Genomics, transcriptomics, metabolomics, etc.). Our aim is to take this initiative a step forward. Our initiative calls for the generation of expanded ready to use adult stem cell lines derived from a variety of human adult tissues affording cell replacement by like-to-like cells. These lines will be used upon demand for cell therapies, tissue engineering and bio-convergence technologies (combination of stem cell biology and bio-medical engineering). The adult stem cell center will be initiated at the Sheba Medical Center and then exported to other major hospitals. The center also trail blazes and leads the manufacturing and biobanking of organoids grown from tissue stem cells. Importantly, organoids grown from variety of diseased tissues will allow the generation of a one's own organoids for precision medicine and specific drug screens on one's own disease. This in turn may enable the discovery of disease modulating novel therapeutics. Organoid technology utilizing human adult cells is already established at the Sheba Medical Center for a variety of tissues including malignant ones. Human tumor organoids derived from human cancer cells represent cancer heterogeneity and are thus very relevant for cancer research; therefore, they can be an essential part of the adult organoid biobank.

Aims of the Center for Organoid Medicine at the Sheba Medical Center

1) Establish organoids for drug screening, discovery, repurposing and testing.
2) Personalized medicine: Utilize patient-derived stem cell-based organoids for disease modeling.
3) Establish patient-based disease-specific organoid biobanks.

4) Provide organoids for (re)generative medicine (repair of injured tissue and/or as an alternative to organs for transplantation).
5) Provide organoids for cancer research.

Altogether, the idea is to generate a national infrastructure for derivation, expansion, and manipulation of tissue stem cells for clinical use.

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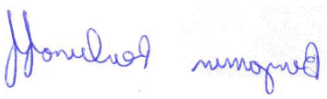
On behalf of all members of the Stem Cells Task Force



Prof. Benjamin Reubinoff
Hadassah Medical Center

Prof. Benjamin Dekel
Sheba Medical Center

Prof. Noam Shomron
Tel Aviv University

Prof. Ido Perlman
Technion-Israel Inst. Technol.

Prof. Nissim Benvenisty
Hebrew University at Jerusalem

Prof. Lior Gepstein
Rambam Health Care Campus

Prof. Mordechai Shani
Sheba Medical Center

Prof. Aaron Ciechanover
Technion-Israel Inst. Technol.