1. Project Title (150 Character Limit including spaces and punctuation):

Environmental intervention decelerates aging among multi ethnic population of elderly cohort in Israel: epigenetic approach

2. Executive Summary (1,300 Character Limit including spaces and punctuation): \*

Aging can be defined as systematic deterioration of physiological functions and molecular degradation of cell components. We identified a distinct pattern of methylation that protects exceptional long lived Individuals(ELLI) from age-related diseases, and therefore affects healthy lifespan. Therefore, we ask; can environmental manipulation lead to decelerated aging processes and whether methylation can serve as a biomarker for such effect? We will address these questions by intervening with subject's environment and applying Hyperbaric oxygen therapy (HBOT) to create an artificial space that promotes aging deceleration (naturally occurs among ELLIs). We will examine the epigenome of elderlies treated with HBOT, and compare them with ELLIs epigenome to tease out the regulation mechanisms by which these ELLIs are protected from deleterious environmental and physiological processes. We will utilize HBOT in mice to gain a mechanistic view.

Such endeavors can lead to Identifying circulating biomarkers for aging deceleration and development of preventive strategies and disease-modifying treatments.

Achieving such a goal will put us closer to understanding aging mechanisms and their interaction with the environment, and will assist with closing the gap between life expectancy and healthy life expectancy.

3. Project Description (4,000 Character Limit including spaces and punctuation): \*

Please describe the work/activities you will undertake in your project.

4000 characters remaining

**Our main goal is** to create novel circulating biomarkers of decelerated aging process, and to explore their role as mediators of the link between lifestyle and healthy lifespan.

We will utilize state-of-the-art technologies (i.e. Infinium MethylationEPIC beadchip, (EPIC array) and a hyperbaric chamber oxygen treatment (HBOT)) to obtain significant information on epigenomic changes in Cytosine methylation in DNA from cd34+/Lin- cells isolated from whole blood. We will tie the epigenomic profiles obtained with data from a comprehensive cognitive assessment (NeuroTrax MindstreamsTM, NeroTrax Corp, NY), advanced brain imaging analysis (functional MRI+DTI+perfusion) and physiological examinations including cardiopulmonary exercise testing (CPET). We will include HBOT study of young and aged mice, to have a profound view of the mechanisms that trigger the deceleration of the aging process. Our proposed goals are:

**Goal 1: To establish the HBOT effect on the human epigenome.**

**1a.** Employing EPIC array to cross-sectionally monitor epigenomic changes in elderlies, that were admitted to HBOT (baseline, during treatment, following treatment) followed by Epigenome Wide Association Studies to identify candidate epiloci. We will correlate cognitive tests, functional MRI and CPET with our subject’s epigenome. We will validate candidate differential methylated loci by applying Sequenom's MassArray technology.

**1b.** To elaborate on the aging factor and the HBOT effect we will add a younger cohort (age 45-55YO) that will be treated similarly to the elderly group. Comparison between the three groups (centenarian, elderly and young) will be held in order to tease out the aging effect.

**Goal 2: To identify molecular networks and longitudinal trajectories predictive of transition to decelerated aging process following HBOT.** Integrate and investigate high dimensional data obtained in objective 1 to identify molecular networks and trajectories that result in recovery after HBOT. Integrative approaches will include model-based statistical analysis to score genes for their association with this healthy performance by considering not only their genetic association strengths, but also their associated phenotypes and, crucially, the relevant gene interactions that account for the polygenetic nature of a complex trait such as decelerated aging process.

**Goal 3: To establish candidate epiloci prioritization for further investigation.**

**3a.** This interplay of two fields of research (recovery therapy and centenarians as a model of successful aging) will further our understanding of the complex aspects of a healthy lifespan by identifying loci that, when altered epigenetically, may have important ramifications towards the transition between aging (elderly before HBOT) to healthy lifespan (loci with similar methylation status among both recoveries after HBOT and the centenarians).

**3b.** Embarking on our centenarian’s epigenome result. We will compare the treated and none treated subjects in objective 1a to our centenarians to identify epiloci that might affect the recovery or maintenance in both groups (centenarian and treated subjects).

**Goal 4: To establish a mechanistic view through the effect of HBOT on mice epigenome.**

Employing the EPIC array to mice at 4 and 12 month following HBOT treatment, we will further asses the methylation profile (baseline, during treatment, following treatment, 1 months after treatment and lifelong follow up). To decipher the mechanistic view of the treatment effects. We will sacrifice 20 animals at each 5 steps and study them physiologically, biologically and molecularly. We will look for association between the epigenomic profiling and these elements to understand the process of aging and recovery through this tool.

**The three-way comparison of Aging + HBOT, Animal model + HBOT and Centenarian will provide us with markers of decelerating aging process that can be used for further treatment to achieve slowing aging and aging maintenance.**

4. Statement of Significance (1,300 Character Limit including spaces and punctuation): \*

Describe the current conditions in the field(s) relevant to the project, identify the problems that the project will address, and articulate the specific opportunity that your project presents.

1300 characters remaining

Establishing mutual dependence between epigenetic modulation and decelerated aging process, in human and animal model on one hand, and cognitive function and physiological performance on the other, will be the main impact of this study. The influence of HBOT on the physiological and neurological status, down to the level of genetic expression, and the converse between the effects of the environment and genetic predisposition, manifested as epigenome performance have been recognized but not yet translated into a workable framework. The current study will attempt, perhaps as one of the pioneers, to link all these effects into one knot, to provide a truly holistic framework for psycho-physiological investigation and treatment, through using a combination of novel technologies of epigenetic analysis, cognitive and physiological assessment and HBOT treatment. By bringing together novel technologies (i.e. EPIC array and HBOT) and our cohort (45-85 year olds compared to centenarians) as well as animal model, we are ideally poised to discover the role of epigenetic changes across the life span in the elder, cognitively intact persons, and to decipher the effects of the environment on the healthy lifespan demonstrated by our centenarians.

5. Outputs (1,300 Character Limit including spaces and punctuation): \*

Outputs (sometimes called "deliverables") are important events and work products that your Project activities (described in #3, above) will lead to, and which are necessary in order for you to make progress towards your proposed Outcomes (#6 below). Please provide a list of the outputs you intend to produce.

1300 characters remaining

Our efforts within the four goals will results with substantial amount of data that will be thoroughly analyzed and will lead to the predicted outcomes (listed in the next paragraph). Accordingly, our outputs will be as follow.

1. Close to 870k epigenetic loci methylation level of 60 subjects in multiple stages (before, within and after the HBOT treatment).
2. A collection of multiple physiological and anthropometric determinants on each participant.
3. A collection of multiple brain imaging and function of each participant (before, within and after the HBOT treatment) using MRI+ perfusion +DTI and CPET.
4. Close to 870k epigenetic loci methylation level of 100 HBOT treated mice in various ages.
5. A collection of multiple mouse tissues across the study (HBOT) period of each age group.

6. Outcomes (1,300 Character Limit including spaces and punctuation): \*

Outcomes (sometimes called goals, results, or impacts) are the specific and identifiable changes that you expect your Outputs will bring about (or contribute to bringing about) within 5 years of your project's end date. These should describe what the success of your project would look like. Please provide a list of the outcomes you expect to come about as a result of your outputs.

1300 characters remaining

Our projection for the following outcomes is based on our outputs, preliminary results, prior knowledge and extensive literature datamining.

1. We will find candidate epi loci that can serve as biomarkers for decelerated aging as a result of HBOT treatment in any perspective, such as improved cognition, frailty, age associated disease maintenance and even recovery.

2. We will identify molecular networks that interact jointly and in parallel to delayed aging and age associated performance. This will set the ground for the understanding of the multilayer complexity of aging.

3. Our prioritization epiloci scheme and the animal study will zoom in on the genetic and epigenetic background of the response to the HBOT treatment. These studies will reveal the mechanism mediating this response and will provide us with a powerful tool to design similar environmental intervention that will support decelerating aging process and can be used for further treatment to achieve slowing aging and aging maintenance.

These biomarkers can be easily assessed repeatedly in any primary care clinic setting. These outcomes will progress us closer to understanding aging mechanisms and will assist with promote healthy life expectancy and aging with grace.

7. Capacity for Success (1,300 Character Limit including spaces and punctuation): \*

Explain why your team and/or organization is positioned to be successful in this project.

1300 characters remaining

Listed are the points that insure the capacity of success.

* Close interaction between the PI's, each representing a complementary filed of expertise, covering Epigenetic technologies and analysis (Prof. Atzmon), Enrolling elderlies to the study (Prof. Dwolatzky), all aspects of the HBOT (Prof. Efrati); Mice studies including HBOT (Prof. Asheri) computational and biostatistical analysis (Dr. Judith Somekh).
* Research infrastructure is already in place as a result of the ongoing collaborations among the PI’s through multiple programs in the University of Haifa, Rambam Health Care, Assaf-Harofeh Medical Center and Tel Aviv University.
* Use of state of the art high-throughput epigenetic, HBOT technologies and bioinformatics analyses.
* Use of state of the art brain function evaluation including computerized neurocognitive testing and brain functional MRI+ perfusion +DTI and CPET.
* Use of animal studies to explore the mechanistic view of the HBOT treatments.
* Excellent model to study successful, slow, healthy aging (centenarian).

This project is certain to lead to many future studies, which will help define the role of epigenetics on specific mechanisms related to healthy lifespan, and perhaps ultimately lead to the search for potential therapeutic options.

8. Relation to Sir John Templeton's Donor Intent (1,000 Character Limit including spaces and punctuation): \*

To learn more about the Foundation's Funding Areas please visit our Funding Areas page.

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In this project we aim to promote healthy aging by studying a remarkable out of the box human treatment that is already proved to decelerate aging i.e. HBOT. However, while most of the studies conducted within this field are physiology oriented, our goal is to explore the fundamental epigenetic mechanisms that regulate this phenomenon. We believe that such motivation is in line with the Foundation's Funding Areas which ties us to Sir John Templeton's Donor Intent. Such environmental exposure (HBOT) will causally impact the development and the onset of age associated diseases, and cognitive function. Further, the epigenetic biomarkers found in this project will assist with development of diagnostic platforms that may predict aging deterioration and the response for such treatment. We are harnessing new technologies to discover epigenetic mechanisms that may slow or even reverse aging. This research is poised to have an impact on seniors, their families and the health care community.