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Hellenic Foundation for Research & Innovation

Description of the funded research project

1st Call for H.F.R.I. Research Projects to Support Faculty Members & Researchers and Procure High-Value Research Equipment

Title of the research project:
“Ex vivo generation of innovative cellular immunotherapies by pharmacological epigenetic modulation”

Principal Investigator: **Spyridonidis Alexandros, MD, Professor of Hematology, Department of Medicine, University of Patras, Director of Bone Marrow Transplant Unit, University Hospital of Patras**

Scientific Area: **Life Sciences (medical & health sciences)**

Institution and Country: **Institute of Cellular Therapy, Greece**

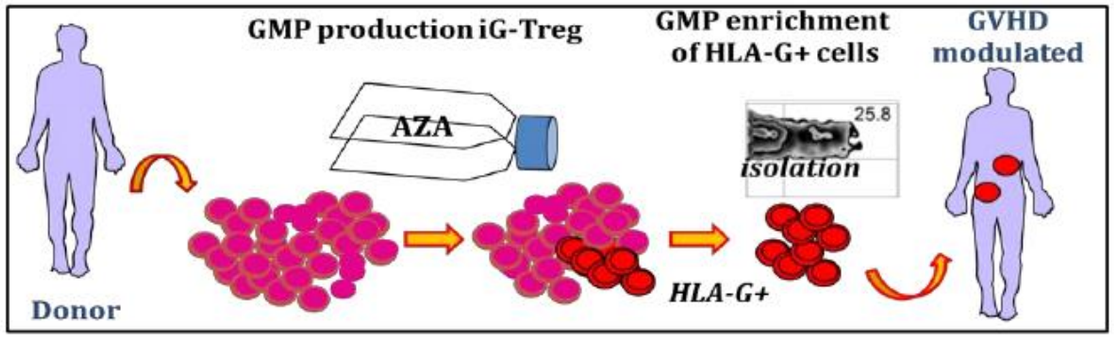
Host Institution: **University of Patras, School of Health Sciences, Department of Medicine**

Collaborating Institution(s):

1. **Gene and Cell Therapy Center, Dpt. of Hematology, Hematopoietic Cell Transplantation Unit, George Papanicolaou Hospital, Thessaloniki, Greece (Dr. Yannaki)**
2. **VUmc Cancer Center Amsterdam, Dpt. of Hematology, Amsterdam, Netherlands (Dr. Themeli)**

Budget: **185.451,11**

Duration: **36 months**



Alexandros Spyridonidis, MD, Professor of Hematology
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Research Project Synopsis

The present project aims to follow up the published work in Cytotherapy (2017;19:521–530) on the **“Simple in vitro generation of human leukocyte antigen-G–expressing Tregulatory cells through pharmacological hypomethylation for adoptive cellular immunotherapy against graft-versus-host disease**”. Briefly, in this study we endeavored the development of an effective immunotherapy against Graft-versus-Host Disease (GvHD), a frequent and life-threatening complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and for which current treatment approaches are far from satisfactory.

Our approach aspired to mimic the mechanism of the successful physiological immunotolerance during pregnancy where the HLA-G molecule expressed in placenta, a well-known immunoregulatory molecule, protects the “semi-allogeneic” fetus from maternal immune attack. Since the HLA-G gene is epigenetically repressed after prenatal life and the methylation status of HLA-G promoter regulates its transcriptional activity, we showed in small scale in vitro experiments that exposure of human peripheral T-cells to hypomethylating agents (HA) induces a de novo and stable expression of HLA-G and converts them to regulatory cells (Treg) with in vitro immunosuppresive functions. Though the suppressor activity of HA-treated Tregs is exclusively within the HLA-G^{pos} compartment, their suppressor function is dependent to a large extent, but not exclusively, on the HLA-G molecule and probably additional immunsuppresive molecules are upregulated through HAs and contribute to the regulatory function of the HLA-G^{pos} Tregs. HLA-G is not expressed in mice and therefore studies with the use of humanized mouse models are needed to demonstrate the in vivo suppressive abilities of these human HLA-G^{pos} Tregs. We propose the ex vivo generation of HLA-G-expressing T-cells through pharmacological hypomethylation as a simple, Good Manufacturing Practice (GMP)-compatible and efficient strategy to produce a stable Treg subset of a defined phenotype (HLA-G^{pos}), which can be easily purified for adoptive immunotherapy (iG-Tregs).

Project originality

There is convincing evidence in pre-clinical models that T-regulatory cell (Treg) immunotherapy can suppress superabundant immune system activation promoting immunologic tolerance. Tregs currently explored for use in clinical practice for the prevention and treatment of GVHD can be distinguished in two major subgroups including a) the thymus-derived $CD4^{+}CD25^{high}$ Tregs and b) the inducible Tregs (iTregs), which are induced in the peripheral lymphoid organs in vivo or can be generated in vitro under various tolerogenic conditions. Adoptive transfer of Tregs can rescue mice from graft- versus-host disease (GVHD) caused by overwhelming donor T-cell activation against host antigens after allogeneic hematopoietic stem cell transplantation (HSCT).

Although such an immunotherapeutic approach would be desirable, Treg immunotherapy protocols have several limitations. Firstly, the introduction of Tregs in clinical practice is tremendously restricted by their low circulating numbers. Secondly, there is not a specific cell surface marker for efficient isolation or purification after their ex vivo expansion. Furthermore, another limitation is the loss of expression of Treg signature molecules as well as the loss of suppressive function after in vitro stimulation or in a pro-inflammatory microenviroment.

Attempting to address these issues, this project suggest a novel and easily applied for clinical purposes protocol for the in vitro generation of a stable and purified Treg subset of a defined phenotype. More specifically, in vitro and in vivo studies have recognized HLA-G as an important mediator of immune tolerance exerting regulatory functions by engaging inhibitory receptors (e.g., ILT2R) on T cells. HLA-G is normally expressed during pregnancy at the fetal-maternal interface of human placenta protecting the “semi-allogeneic” fetus from maternal immune attack, which constitutes the perfect example of successful physiological immunotolerance of semi-allografts. More specifically, given that HLA-G expression is strongly regulated by methylation, we propose the epigenetic induction of the gene expression with clinical grade hypomethylating agents (HA), such as azacytidine (Aza) or Decitabine (Dec) on T cells. This protocol can yield pure Treg population in clinical scale under GMP conditions allowing the administration of the cells to allo-HSCT patients against of GvHD.

Expected results & Research Project Impact

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has become a standard treatment for patients with marrow failure syndromes and hematologic malignancies such as acute leukemia. Nearly 35.000 allo-HSCTs are performed every year in Europe with the numbers increasing.

One of the major complications of allo-HSCT is Graft-versus-Host Disease (GvHD), a chronically debilitating and life-threatening disease caused by donor T-cells reacting against host antigens. GvHD and its currently available immunosuppressive treatment itself is still the principal cause of post-transplant impairment of quality of life, morbidity (35-50%) and mortality (20%-30%) after allo-HSCT. Hence, GvHD remains a major health problem with untold physical, psychological and economic costs to the society as patients suffer or even die from a therapy-induced complication, despite having been cured from their hematological malignancy. Currently only a minority of patients have access to the new and promising cellular immunotherapies.

The findings of EPICELL could provide a realistic perspective of adoptive T-cell therapy for immunoregulation by delivering the manufacturing of a well-characterized, affordable and efficient cell product of a new subtype of Tregs. Cumulatively, EPICELL-based immunotherapy is expected to achieve a more favorable cost-effectiveness ratio than the standard conventional pharmacotherapy having the potential to be used as a “stand-alone” therapy post allogeneic hematopoietic stem cell transplantation or to extend its applicability for immunoregulation in solid organ transplantation and other T-cell mediated diseases. In the new era of disease treatment where the massive and standardized production of drugs has shifted to the personalized, “custom-made” production of living cell-drugs, such novel therapeutic frameworks could provide the ground for collaboration with R&D companies fostering entrepreneurship and attract many young researchers in biomedical sciences (haematology, immunology, biotechnology, immunogenetics), thus enabling the establishment of a conducive environment for multicentre innovative cell-based therapies.

Importance of this funding

The H.F.R.I funding enables the advancement and development of novel immunotherapeutic cell products (Advance Therapeutic Medical Products) in Greece and shall promote groundbreaking research at our university. This will in turn attract young researchers and investigators and establish a solid scientific team that strives for scientific merit. Moreover, impactful results would be published in peer reviewed journal as well as be presented at internationally respected scientific meetings leading to the spread of knowledge globally

. Lastly, the complete characterization of cell products and the development of GMP grade protocols for their production will lead to future clinical trials and hopefully change everyday clinical practice and ultimately improve patients' quality of life and overall survival.

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