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STANFORD UNIVERSITY SCHOOL OF MEDICINE

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Independent Citizens' Oversight Committee (ICOC)
California Institute for Regenerative Medicine (CIRM)
1999 Harrison Street, Suite 1650
Oakland, CA 94612

Application: DISC2-11109

Title: Regenerative Thymic Tissues as Curative Cell Therapy for Patients with 22q11 Deletion Syndrome

Principal Investigator: Vittorio Sebastiano, PhD
Co-Principal Investigator: Katja Weinacht, MD, PhD

Dear Members of the ICOC,

22q11.2 Deletion Syndrome (22q11.2DS or DiGeorge Syndrome) is the most common genetic cause of thymic stromal defects leading to T-cell immunodeficiency. It is the result of abnormal patterning of the third and fourth pharyngeal arches during fetal development. The syndrome encompasses a wide range of morphogenic defects with varying clinical phenotypes. For patients with complete agenesis of thymus (complete DiGeorge) the prognosis is bleak. Patients present with a severe combined immunodeficiency (SCID)-like picture and succumb to fatal infections early in life. Unlike infants with SCID, immune reconstitution cannot be achieved by hematopoietic stem cell transplantation. Transplantation of allogeneic, HLA-unmatched thymic tissues has been the only therapeutic strategy employed to date to achieve immune reconstitution in infants with complete DiGeorge. Duke University School of Medicine has been the only

institution in the US to offer this life-saving procedure. Intractable autoimmunity remains a frequent and often debilitating complication. Unfortunately, the thymic transplant program at Duke University under the direction of Louise Markert, MD, PD, has suspended its services as of December 2017. While it is expected that the technology will be taken over by a private company, Enzyvant, the licensing is still pending; the date when allogeneic thymic tissue transplantation will become available again remains uncertain. Meanwhile, about 15 children in the US remain confined to the protective environment of pediatric and neonatal intensive care units, anxiously awaiting a thymic transplant at an undefined point in time in the future. Time, however, is not on their side as every infection can be fatal. Even when commercially processed allogenic thymic tissues become available again, the capacity of the technology is not expected to meet the current demand; the putative manufacturer has already closed the waiting list for patients in need of a thymic allograft. The medical, emotional and financial burden associated with the unavailability of transplantable thymic tissues represents a crisis for medical providers, patients/families and the healthcare system alike.

Our proposal (DISC2-11109) addresses this critically unmet medical need and aims to create a clinically superior, technically scalable, and more cost-effective therapeutic alternative to save the lives of over a dozen children in California and the United States. Our platform is designed to generate patient-specific or histocompatible (HLA-matched) transplantable thymic tissues from human induced pluripotent stem cells by recapitulating ontogeny. Our data demonstrate that we can effectively and reproducibly generate thymic epithelial precursor cells in vitro. In this proposal, we seek funding to I) further refine the differentiation platform, II) develop 3-dimensional organoids using engineered protein bio-matrices and III) demonstrate proof of concept in a mouse model.

We greatly appreciate the thoughtful and favorable reviewer comments, and are enthusiastic about their recommendation for funding. Three of the fifteen reviewers also raised a question or concern and we are grateful for the opportunity to address and clarify these points below:

First, "*the differentiation procedures are not been completely worked out in the preliminary research*". Over the past 18 months, we have developed an in vitro platform that efficiently and reproducibly yields putative thymic epithelial progenitor cells that are triple positive for the expression of FOXN1, Keratin 5, and Keratin 8, bona fide markers of thymic epithelial cells. Our strategy is based on the exact recapitulation of the developmental steps that occur during human thymic ontogeny, i.e. the formation of the definitive endoderm, the pharyngeal pouches and ultimately thymic epithelia.

Second, "*the mouse model seems quite complicated and is far from translational studies*". The athymic xenograft-permissible FOXN1$^{-/-}$ (nude) NSG mouse, indeed a complicated genetic construct, is the most precise and specific phenocopy of the human disease phenotype. It is considered the gold standard for modeling human thymic developmental defects in the mouse. NSG mice are immunocompromised, therefore amenable to transplantation of human tissues. In addition, the FOXN1$^{-/-}$ (nude) mutation confers complete lack of murine thymic development. Accordingly, all peripheral T cells must have matured on transplanted thymic epithelia. The

FOXN1$^{-/-}$ (nude) NSG mouse allows us to unequivocally prove efficacy of in vitro engineered thymic epithelia in vivo.

Lastly, "*the work seems too ambitious for the timeline*". The reviewer is correct; our timeline is ambitious, responding to the dire need for novel therapeutic strategies to treat an otherwise fatal childhood condition. Our goal is to provide an effective, scalable and affordable curative treatment for athymic infants with 22q11.2 DS in the foreseeable future. We believe that the proposed aims are all essential to achieving our goal. Upon completion of the aims put forth in this DISC proposal, the next step will be to generate pre-clinical safety data and filing of a pre-IND.

We are convinced that our complementary expertise combined with the unique infrastructure provided by the Stanford School of Medicine and by the Center for Definitive and Curative Medicine will allow us to successfully complete this proposal in the projected timeline. We are grateful for the opportunity to apply for DISC-funding through the California Institute of Regenerative Medicine. Thank you for your consideration.

Sincerely,

Vittorio Sebastiano

Katja G. Weinacht

CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE
ICOC Board
1999 Harrison Street, Suite 1650
Oakland, California 94612

RE: Disc 2-11109 Proposal: Regenerative Thymic Tissues as Curative Cell Therapy for Patients with 22q11 Deletion Syndrome

July 10, 2018

Dear Members of the Independent Citizens' Oversight and Application Review Committee:

My name is Katie Luckesen and I am writing in support of Dr. Katja G. Weinacht and Dr. Vittorio Sebastiano's proposal to make regenerative thymic tissues as curative cell therapy for patients with 22q11 Deletion Syndrome. I am advocating on behalf of my 7 months old son Charlie and the approximately 15 other children in the US waiting for a lifesaving functioning thymus gland.

Charlie was born with 22q11 Deletion Syndrome and has been diagnosed with complete agenesis of the thymus also known as complete DiGeorge Syndrome. He is completely athymic and has been hospitalized in the NICU at Rady Children's Hospital in San Diego, CA, for all but his first week of life. The staff at Rady's have been outstanding at keeping him free from infections, but without addressing his lack of a thymus, his prognosis is grim. As you know, any viral infection will most likely be fatal. As many children with 22q11 Deletion Syndrome, Charlie was also born with congenital heart disease. Because of his tenuous immune status, the doctors had to defer his cardiac surgery because the risk of an infection is too great.

There are more children like Charlie in California, the US and across the world who are desperately waiting for a lifesaving thymic transplant. As you know, Duke University has suspended its allogenic thymic transplantation program as of December 2017 and currently all thymic transplantation is the US is on hold. All patients without thymus are waiting in limbo at this time. Allogenic thymus transplantation uses HLA-unmatched thymic tissues which is not perfect but all we have for now. There is no other available alternative therapy for children like my son. Dr. Weinacht and Dr. Sebastiano are doing research to make HLA-matched thymic tissue for patients born without thymus. Because these regenerative thymic tissues would be derived from induced pluripotent stem cells, they could be made in sufficient quantity for all patients in need of a new thymus gland. In addition, because the proposed research seeks to make thymic tissues that are HLA-matched, patients would no longer suffer from autoimmune complications, a common side effect of allogenic thymus transplantation.

I would like to advocate in the strongest way I can and convey, that the kind of research that Dr. Weinacht and Dr. Sebastiano are requesting funding for, is vital and is one of the only ways my sweet little boy stands a chance at living past the first years of life. For most other conditions, there is some form of therapy, even if not perfect. For complete agenesis of the thymus, there is absolutely nothing. It is the most underserved clinical condition there is as medications can't replace a functional immune system. Because we have no alternative at all, I ask you to please consider this grant request for Charlie, and all the other young children with this condition. It is literally a matter of life and death.

Thank you for your consideration.

Katie Luckesen

Katie Luckesen
San Diego, CA.

The Children's Hospital of Philadelphia®

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California Institute of Regenerative Medicine (CIRM)
Independent Citizens' Oversight Committee Board
1999 Harrison Street, Suite 1650
Oakland, California 94612

Re: DISC Application 11109: Regenerative Thymic Tissues as Curative Cell Therapy for Patients with 22q11.2DS

July 18, 2018

Dear Members of the Independent Citizens' Oversight Committee Board:

I am writing to express my enthusiastic support and advocacy for the CIRM discovery grant application of Dr. Katja Weinacht and Dr. Vittorio Sebastiano from Stanford University who propose to derive regenerative thymic epithelia from induced pluripotent stem cells as therapy for patients with 22q11 Deletion Syndrome and agenesis of the thymus. As you know, complete thymic aplasia prohibits the development of any form of adaptive T cell immunity. Patients present with a Severe Combined Immunodeficiency (SCID)-like phenotype but the immune defect resulting from thymic aplasia cannot be overcome by hematopoietic stem cell transplantation.

I am the director of the 22q and You Center at the Children's Hospital of Philadelphia and have devoted my entire professional life to the study and treatment of patients with 22q11.2DS. Moreover, I am a founding member of the 22q11.2 Society, the professional organization supporting the pursuit of knowledge to ultimately benefit patients affected by chromosome 22q11.2 deletions. To that end, the therapy of complete thymic aplasia in patients with 22q11.2DS remains one of the most challenging and under-represented aspects in the management of this devastating childhood disease. Until recently, allogeneic thymus transplantation has been the only therapeutic option to address the T cell immunodeficiency. In the United States, this lifesaving procedure was solely available at Duke University Medical Center. Although immune reconstitution was achieved in the majority of patients, emergence of organ-specific autoimmunity post-transplant due to lack of histocompatibility was a frequent concern. Recently, the thymus transplantation program at Duke University was placed on hold after the graft processing procedure was licensed to a private company and is currently awaiting FDA review. When thymic transplantation will become available again, remains uncertain. What is certain, however, is that the production capacity of commercially prepared transplantable thymic tissues will not meet the current demand as the wait list to receive a thymic allograft has been suspended and no longer accepts new patient referrals. Meanwhile, about 15 patients with complete agenesis of the thymus who reside in the US are left in a state of total immunodeficiency, bereft of any therapeutic options. Without a thymic transplant, the disease is invariably fatal; patients must remain in complete isolation and usually don't survive beyond the first year of life.

In their application, Dr. Weinacht and Dr. Sebastiano propose to make regenerative thymic epithelia for therapeutic purposes from induced pluripotent stem cells. The advantage of this type of graft is that it is histocompatible and scalable to meet the current demand. I have recently heard Dr. Weinacht and Dr. Sebastiano speak at the 11th Biennial International 22q11.2 Symposium in Whistler, BC, and I was impressed how far their differentiation platform has been developed in the past 18 months. Stanford has recently demonstrated growing commitment to the study and care of various aspects of 22q11.2DS. In order for Dr. Weinacht and Dr. Sebastiano to continue their desperately needed work on regenerative thymic tissues, the support of CIRM is of critical importance. Due to the lack of alternative therapies in this condition, I strongly urge the Members of the Independent Citizens' Oversight Committee to favorably review and fund this application.

Sincerely,

Donna M. McDonald-McGinn, MS, LCGC
Clinical Professor of Pediatrics
Perelman School of Medicine at the University of Pennsylvania
Chief, Section of Genetic Counseling
Director, 22q and You Center
Associate Director, Clinical Genetics Center

The International 22q11.2 Foundation, Inc.
P.O. Box 532
Matawan, NJ, USA 07747
001-877-739-1849 www.22q.org

July 14, 2018

Dear Members of the Independent Citizens' Oversight Committee Board,

It is with enormous pleasure that I write this letter of support for the proposed CRIM discovery grant entitled, "DISC Application 11109: Regenerative Thymic Tissues as Curative Cell Therapy for Patients with 22q11.2DS", submitted by Dr. Katja Weinacht and Dr. Vittorio Sebastiano from Stanford University, as they strive to derive regenerative thymic epithelia from induced pluripotent stem cells as therapy for patients with 22q11.2 Deletion Syndrome, a condition associated with early death. As the mother of a child with 22q11.2DS, a leader of a worldwide movement to increase better awareness, detection, and ultimately improved treatment for this condition, and as an obstetrician gynecologist who understands better than most parents the need for basic science research – I applaud the efforts of Drs. Weinacht and Sebastiano. With this in mind, we sincerely hope that you will consider funding their most needed study and we wish them God speed in reaching these critical goals.

Sincerely,

Sheila P. Kambin, MD
Chairperson

California Institute of Regenerative Medicine (CIRM)
Independent Citizens' Oversight Committee Board
1999 Harrison Street, Suite 1650
Oakland, California 94612

RE: Disc 2-11109 Proposal: Regenerative Thymic Tissues as Curative Cell Therapy for Patients with 22q11 Deletion Syndrome

July 14, 2018

Dear Members of the Independent Citizens' Oversight and Application Review Committee:

We are a group of Pediatric Immunologists and Neonatologists from California writing to support Dr. Katja Weinacht and Dr. Vittorio Sebastiano's CIRM application proposing to derive regenerative thymic tissues from induced pluripotent stem cells as curative cell therapy for patients with 22q11 Deletion Syndrome. Collectively we represent physicians from the University of California campuses in San Francisco, Davis, Los Angeles, San Diego, Children's Hospital of Los Angeles and Stanford University. We feel impelled to write on behalf of this proposal because of the dire situation facing young patients born without a thymus who currently lack access to treatment for this condition.

The most common cause of athymia is Complete DiGeorge Syndrome, which is also associated with cardiac anomalies and other midline defects. Without thymus transplant, patients with Complete DiGeorge Syndrome and other causes of athymia succumb to infections within the first few years of life due to absence of functional T cells. Prior to 2017, Duke University offered allogeneic HLA-unmatched thymus transplantation, and though the wait was long, the overall immune reconstitution was good with autoimmunity being the major complication. The transplant program at Duke has been halted after a company has purchased the patent for the transplant procedure, and their application to the FDA is pending. Even after FDA approval has been obtained, we expect a long wait time for patients to receive a thymic graft as the anticipated production capacity is limited to one thymic transplant/month and the wait list for commercially available thymic grafts has already been closed. There is one patient currently admitted in isolation at Rady Children's Hospital in San Diego with Complete DiGeorge Syndrome, and several others in the state are waiting. The introduction of newborn screening for presence of T cells has led to early detection of these patients, and will continue to identify others, but there are currently no therapeutic options beyond supportive care. One patient evaluated at Stanford was successfully treated in the last 3 years prior to closure of the program at Duke. There is a pressing and ongoing need for treatments for this cohort of patients within our state and within our country.

Dr. Weinacht and Dr. Sebastiano now propose to make regenerative thymic tissues from induced pluripotent stem cells that are either patient specific (autologous) or HLA-matched. The advantage of this technology – once available- will be that regenerative thymic tissues can be cultured in virtually limitless quantities to meet to growing clinical demand. In addition, we expect that HLA-matching of the thymic grafts will prevent or decrease the intractable autoimmune complications of allogenic thymus transplants as tissue-restricted antigens will be presented in the appropriate HLA-matched context. We are hopeful about Dr. Weinacht and Sebastiano's proposal, because it represents a potentially better option for our patients than thymus transplant. Dr. Weinacht is a pediatric stem cell transplanter with clinical and research focus on immune defects and thymic development. Dr. Sebastiano is a developmental biologist with expertise in definitive endoderm development. Embedded in the academic and technologic infrastructure of the Center for Definitive and Curative Medicine at Stanford University, this proposal is poised to be successful.

At present, there is no therapy at all that we can offer to patients with 22q11 Deletion Syndrome and Complete DiGeorge and other causes of athymia. Patients remain in isolation until they suffer the fatal

consequences of their immunodeficiency. Due to the desperate prognosis of patients with athymia and complete lack of alternative treatment strategies, we urge to review committee to fund the lifesaving research proposed in this application.

Respectfully,

Cathleen Collins, MD PhD
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Rady Children's Hospital/UCSD

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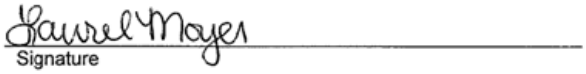

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