PI: Fong, Timothy C	Title: Novel indication for myeloid progenitor use: Induction of tolerance	
Received: 12/04/2012	FOA: PA12-089	Council: 05/2013
Competition ID: ADOBE-FORMS-B2	FOA Title: PHS 2012-02 OMNIBUS SOLICITATION OF THE NIH FOR SMALL BUSINESS TECHNOLOGY TRANSFER GRANT APPLICATIONS (PARENT STTR [R41/R42])	
1 R41 AI108016-01	Dual: CA,HL,NR	Accession Number: 3546121
IPF: 10000154	Organization: CELLERANT THERAPEUTICS, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 IMM-G (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1:	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	Organization:	Role Category:
Holger Karsunky	Cellerant Therapeutics	PD/PI
Adrianus Domen	The Children's Mercy Hospital, Kansas City, MO	MPI

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15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	[redacted]
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	0.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Dr. * First Name: Ram Middle Name:

* Last Name: Mandalam Suffix:

* Position/Title: President & CEO

* Organization: Cellerant Therapeutics

Department: Division:

* Street1: 1561 Industrial Road

Street2:

* City: San Carlos County / Parish:

* State: CA: California Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 94070-4111

* Phone Number: [redacted] Fax Number: [redacted]

* Email: [redacted]

*** Signature of Authorized Representative**

Holger Karsunky

*** Date Signed**

12/04/2012

20. Pre-application

Add Attachment | Delete Attachment | View Attachment

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OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Cellerant Therapeutics, Inc.

DUNS Number: [redacted]

* Street1: 1561 Industrial Road

Street2:

* City: San Carlos County: San Mateo

* State: CA: California

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 94070-4111 * Project/ Performance Site Congressional District: CA-012

Project/Performance Site Location a ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Children's Mercy Hospital, Kansas City, MO

DUNS Number: [redacted]

* Street1: 2401 Gillham Road

Street2:

* City: Kansas City County: Jackson

* State: MO: Missouri

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 64108-4619 * Project/ Performance Site Congressional District: MO-005

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. * Is proprietary/privileged information included in the application? ☒ Yes ☐ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-ProjectSummary Final.pdf Add Attachment | Delete Attachment | View Attachment

8. * Project Narrative 1235-ProjectNarrative.pdf Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited 1236-ReferencesV17HK.pdf Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources 1237-Combined FacilitiesAndOtherResou Add Attachment | Delete Attachment | View Attachment

11. Equipment 1238-Combined Equipment FINAL.pdf Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

PROJECT SUMMARY

State of the art techniques result in 10-year solid organ graft loss of up to 80% in cardiopulmonary organ transplantation, and re-transplantation is often not possible. Establishment of donor-specific immunological tolerance (DSIT), a condition in which a recipient accepts a transplant without immunosuppression, while retaining the ability to fight infections, would reduce graft loss. The only identified method of inducing robust tolerance involves Hematopoietic Cell Transplantation (HCT), usually in the form of bone marrow transplantation (BMT). Though long recognized experimentally as a means of inducing DSIT, clinical translation has been limited due to the associated complications. A major problem has been the treatment necessary to prepare a recipient for a blood cell transplant. White blood cell counts fall precipitously, resulting in neutropenia and increased susceptibility to infections. To reduce infections associated with neutropenia in HCT recipients, unrelated myeloid progenitors (MP) can be injected together with the HCT. This therapy is effective in the laboratory setting in reducing deaths caused by bacterial and fungal infections, and several trials testing a clinical MP product developed by Cellerant Therapeutics (CLT-008) MP in humans are ongoing. We have discovered that injection of MP under these conditions results in MP-specific tolerance, even though there may be only very low-level MP engraftment after the first month. Important, MP from B10;B6-Rag2$^{-/-}$Il2rg$^{-/-}$ mice, which are incapable of producing functional B, T or NK cells, induce tolerance and clearly show that organ graft-matched lymphoid cells are not essential under these conditions. **Uniquely, MP cells induce antigen-specific tolerance in our experimental model system, are clinically available, and have been associated with minimal to no side effects in current clinical trials. MP constitute an ideal and innovative approach in tolerance induction protocols, preferred over efforts aimed at achieving high level donor chimerism.**

The proposed research in phase I will focus on (Aim 1) testing whether MP can prevent specific lung transplant rejection symptoms, important because lung transplants may be a good patient population for initial trials and (Aim 2) on testing the degree of mismatch allowed between MP and organ graft, as this will affect the production of clinical grade MP for clinical tolerance trials. The design of these trials using a Cellerant produced clinical MP product would be the next stage following this STTR project.

PROJECT NARRATIVE

The proposal aims to develop methods that can be used to improve the long-term outcome of clinical organ transplantations. State of the art technology results in up to 80% organ graft loss over ten years mostly due to rejection, and re-transplantation is often not possible. The academic partner has developed preclinical tolerance models and has shown that myeloid cells can induce specific tolerance. The small business partner is currently studying an innovative product (CTL-008, human Myeloid Progenitor Cells) in clinical trials for the prevention of infections. Together we are aiming to test if a commercial Myeloid Progenitor Cell product for the induction of tolerance in solid organ transplantation is feasible and to transfer this technology to the small business partner for further clinical development and application such that it can improve the long-term outcome for patients undergoing transplantation for end-stage organ failure.

FACILITIES AND OTHER RESOURCES

1) At Cellerant Therapeutics (Laboratory of Dr. Karsunky)

Facilities: Cellerant has ample of space and adequate facilities to accommodate the proposed work. Cellerant has approximately 42,000 sq. ft. total of laboratory, animal vivarium, office, and common area space in San Carlos, CA. The facility consists of twenty two laboratories and a vivarium.
Laboratory: Ten laboratories are dedicated tissue culture suites for mouse, human or NHP tissue culture. Each of them is equipped with 1-3 biological safety cabinets, 2-6 incubators, centrifuge, microscope and other tissue culture related equipment. All rooms are supplied with HEPA filtered air. Seven laboratories are multipurpose labs with multiple wet benches for molecular biology, PCR, protein work, histology, immunochemistry, QC and other techniques. Two laboratories are dedicated to process and assay development work for cell culture processes and contain bioreactors, gas and metabolic analyzers, etc. One laboratory contains equipment for flow cytometry and cell sorting. This laboratory also contains three biological safety cabinets and multiple incubators dedicated to non-human tissue culture and processing. Two are dedicated microscope rooms.

In addition, there are four freezer rooms that house the liquid nitrogen cryogenic freezers, -150°c ultra low mechanical freezers and controlled rate freezers for cell cryopreservation and stability studies. One dedicated glass wash room with autoclave and glassware washer and one 4°c walk-in cold room. Incubators, refrigerator and freezers are monitored. Critical equipment undergoes regularly scheduled preventive maintenance and calibration or certification. The facilities are back up with emergency generators.

Animal: Cellerant has approximately 3,700 sq. ft. animal facility with a separate entry from the laboratories for the housing and maintenance of mouse colonies. The vivarium consists of four housing rooms, two procedure rooms, one quarantine room, a dirty and clean wash/preparation room, including an autoclave, cage rack washer, bottle filler and an X-ray irradiation room for whole body irradiation of mice. The facility includes a changing room, protective gowning room, toilet and shower. This facility can house approximately 5,000 mice and its environment is controlled and monitored for temperature, humidity and light cycles. Entry into the building and vivarium is controlled by electronic key card.

Office: The remaining space houses offices, cubicles and workstations, conference, computer server room, electronic key card documentation room and break rooms. All staff have their own computer work station that are connected network. All computers are equipped with MS Office and other work relevant software.

2) At Children's Mercy Hospital/University of Missouri, Kansas City (Laboratory of Dr. Domen)

Facilities: Part of the proposed research will be performed at Children's Mercy Hospital and Clinics in Kansas City where the lab has 600 sq ft of wet lab space and 100sq ft of office space for the PI. In addition there is access to the animal facilities at UMKC to allow with shared access to a mouse room as well as two procedure rooms. The animal space is located in the school of Pharmacy across the road from Children's Mercy Hospital.

The Ward Family Center for Congenital Heart Disease has a uniquely developed research and informatics section. The academic and research section has six full time employees and one part time employee dedicated strictly to the administration of research projects. This group currently administers 25 cardiac IRB protocols, as well as 6 IACUC protocols, and 12 IBC protocols. Two of the cardiovascular operating room suites within Children's Mercy Hospitals and Clinics are equipped with a GMP grade clean room connected directly between the suites. This state-of-the-art facility was specifically designed to handle bio-engineered tissue, and allow for the implantation of this tissue in the clinical setting.

Laboratory: The 600sq ft research laboratory is equipped with the basic equipment found in modern biomedical research laboratories including microscopes, refrigerated centrifuges, PCR machines, and -20 and

-80 freezers. In addition a 8 parameter Veterinary blood cell counter (ScilVetABC) is available in the lab Full capabilities for tissue culture exist. Major equipment is shared among the laboratories. Major equipment includes a CMH-owned RadSource 2000 irradiator in the mouse facility at the UMKC medical school where the mouse facilities used for this project are located and a 6 color Blue/Red laser Attune Flow Cytometer located in the Domen lab.

Clinical: The clinical laboratory is a full service laboratory operated by the Department of Pathology which occupies 8,000 ft^2 on the second floor of the hospital and provides laboratory services to the hospital and ambulatory clinics 24 hours/day, 7 days per week. Areas of clinical service provided include routine and special chemistry, endocrinology, flow cytometry, coagulation, hematology, histology, diagnostic immunology, microbiology, transfusion services, toxicology/TDM, in vitro nuclear medicine, cytogenetics, biochemical genetics and urinalysis. The laboratory is fully accredited by CAP #19365-01 and licensed by CLIA #26DO443323. It is capable of providing clinical laboratory testing required for clinical research protocols.

Animal: Animal facilities are available through a cooperative agreement with the University of Missouri-Kansas City. Facilities (AAALAC certified) are provided for small and large animals with the animal facility directed by a veterinarian. Equipment essential to the proposed experiments, most notably a RadSource 2000 mouse x-ray irradiator, are present. The animal facility is located across the road from Children's Mercy.

Computer: All hospital staff are connected to a Children's Mercy Hospital client/server LAN with word processing, spreadsheet, database, electronic mail, presentation, and graphical software. In addition, Research and Grants Administration has a color scanner with OCR and graphics software, a HP 1000C color printer and laser printers. A full range of scientific graphical (Sigma Plot v 4.0) and statistical packages (SAS, SPSS, S+, StatXact, and DBMS-Copy) are available. On-line library/literature search software, including Knowledge Server for NLM access, End Note bibliographic software, and Current Contents is available on the network. A fractional DS-3 line provides high speed, firewall-protected access to the Internet.

Users logging onto the system have access to a personal directory on a server that is backed up daily. The network is maintained by the Hospital Information Services.

A public site as well as an internal Research and Grants Tools Portal website with links to internal CMH and external websites (http://www.childrensmercy.org/MedicalResearch.aspx) is maintained to provide assistance to researchers.

Members of the lab are equipped with iMac computers running system 10.6 or 10.5.

Office: A 100 sq ft office (3730.08) equipped with high speed internet, a 27inch iMac computer (System 10.6) with appropriate software (Microsoft Office, iWorks, Canvas, Filemaker, Endnote, Adobe Acrobat, Graphpad Prism, OmniGraffle Pro) and a color laser printer and scanner is available for the PI, located next to the wet lab space.

Interactions: Members of the laboratory participate in the regular meetings and interactions of the larger Immunology community that brings together researchers at the University of Kansas Medical Center, the University of Kansas Lawrence Campus and Children's Mercy Hospital (http://www.kumc.edu/immunology.html), bringing together researchers addressing a wide range of immunological questions.

Other: Library: The CMH library is located on the ground floor of the hospital. Three hundred and twenty-five current periodical subscriptions and 3,900 bound volumes are maintained. Library staff provides online access to the NLM (MEDLINE) and BRS. Interlibrary loan is readily available. Adjacent to the hospital, the University of Missouri-Kansas City (UMKC) School of Medicine Library maintains 722 periodical subscriptions, 71,666 bound volumes and faculty support services.

The UMKC library system contains 1,005,724 volumes, 8,868 current serial subscriptions, 1,876,258 microfilms, and 797,602 government documents. The libraries have major access agreements with CRL, NLM, UM OVID data bases, UM SiteSearch databases, OCLC FirstSearch Basic, RLIN/BIB Eureka, ICPSR, and MIRACL.

UMKC medical and health sciences resources are supplemented by the nearby libraries of the University of Kansas Medical Center Archie Dykes Library.
Biomedical: Children's Mercy Hospital provides a machine, electrical and biomedical shop to service technical equipment.

EQUIPMENT

1) At Cellerant Therapeutics (Laboratory of Dr. Karsunky)

Cellerant has already on-site all major laboratory equipment needed to complete the proposed work.

For tissue culture work a total of 19 biosafety cabinets and 30 humidified CO_2 incubators are currently installed and operational. Two of the incubators include triple gas controls for low oxygen conditions. The flow cytometry lab is equipped with two Becton Dickinson FACSAria II flow cytometer cell sorters, one Becton Dickinson FACSCalibur flow cytometer, one Beckman Coulter Gallios and one Dako Cyan flow cytometer. For magnetic cell isolation one Miltenyi AutoMACS and two AutoMACSpro as well as two Isolex 300i cell isolators and a Miltenyi CliniMACS cell isolator are at disposal. For storage the labs are equipped with multiple refrigerators and freezers for reagent storage (2-8°, -20° and -80°C), six liquid nitrogen tanks for cryogenic cell storage, and one control rate freezer. Other equipment includes one Coulter hematology analyzer for human blood, a Hemavet 950S hematology analyzer for animal blood, one microtome for sectioning, two multi-well plate readers with automated plate washer, two UV Vis spectrophotometers, one NucleoCounter and one Vi-Cell cell counter, and an Alpha Innotech ChemiImager. The process development lab houses a Wave bioreactor, Micro24 mini bioreactor system, a Haemonetics PCS2 cell collection system, an YSI bionalyzer and a StatProfile pHOX gas analyzer. Additional bench top laboratory equipment includes several bench top

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Holger Middle Name:
* Last Name: Karsunky Suffix:
Position/Title: Director, Preclinical Development Department:
Organization Name: Cellerant Therapeutics Division:
* Street1: 1561 Industrial Road
Street2:
* City: San Carlos County/ Parish:
* State: CA: California Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 94070-4111
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PhD
Degree Year: 2000

***Attach Biographical Sketch** 1244-biosketch Karsunky 2012 Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix: Prof. * First Name: Adrianus Middle Name: G.W.
* Last Name: Domen Suffix:
Position/Title: Assistant Professor Department: Pediatrics
Organization Name: The Children's Mercy Hospital, Kansas City, MO Division:
* Street1: 2401 Gillham Road
Street2:
* City: Kansas City County/ Parish: Jackson
* State: MO: Missouri Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 64108-4619
* Phone Number: [redacted] Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PhD
Degree Year: 1993

***Attach Biographical Sketch** 1245-BiosketchJD_2012DomenSTT Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Holger Karsunky	Director, Preclinical Development
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Marburg, Germany	M.Sc.	1991-1996	Molecular Biology
University of Essen Medical School, Germany	Ph.D.	1996-2000	Immunology
University of Essen Medical School, Germany	Postdoctoral Fellow	2000-2001	Hematopoiesis and T cell development
Stanford University, California	Postdoctoral Fellow	2001-2005	Hematopoietic stem cells and progenitors

A. Personal Statement

For the last 10+ years I have been working on the biology of myeloid progenitor cells and their potential clinical application. Since joining Cellerant the focus of my work has been the development of CLT-008, human Myeloid Progenitor Cells, which is in advanced clinical development. The use of myeloid progenitor cells offers the opportunity to have a novel off-the-shelf cell therapeutic that can be stored long term and used without recipient HLA matching. CLT-008 is currently developed for three indications: 1) to enhance hematopoietic recovery in patients receiving umbilical cord blood transplantation after myeloablative conditioning; 2) to prevent infection in leukemia patients receiving high dose chemotherapy; and 3) as a medical countermeasure for acute radiation syndrome. Through the combination of safety studies in animal xenograft models and proof-of-concept efficacy studies using a comparable animal product my group was able to demonstrate its safety and efficacy in animal models. Cellerant subsequently filed an investigational new drug (IND) application and has since then initiated two currently ongoing clinical trials. I have extensive experience in the field of human and mouse hematopoietic stem and progenitor cell biology. As a postdoctoral fellow at Stanford I worked on the characterization, lineage relationship and developmental potential of myeloid and lymphoid progenitor populations. I am very familiar with the regulatory requirements for cell based therapies and have led as the responsible PI or Program Director similar and significantly larger research projects. In response to Program Announcement PA-12-089 together with my colleagues Dr. Adrianus (Jos) Domen we are proposing to conduct proof-of-concept preclinical work to test the hypothesis if Cellerant's Myeloid Progenitor technology can be used to induce tolerance in patients receiving allogeneic solid organ transplants. Based on the initial work performed in Dr. Domen's lab I believe it is feasible to develop a modified version of CLT-008 specific for the induction of tolerance which could have a significant impact on the clinical outcome of many transplants.

B. Positions and Honors

Positions and Employment

2011-current	Director, Preclinical Development, Cellerant Therapeutics, San Carlos, CA
2009-2011	Associate Director, Development, Cellerant Therapeutics, San Carlos, CA
2008-2009	Group Leader, Development, Cellerant Therapeutics, San Carlos, CA
2007-2008	Senior Staff Scientist, Cellerant Therapeutics, San Carlos, CA
2005-2006	Research Staff Scientist, Cellerant Therapeutics, San Carlos, CA
2001-2005	Post-doctoral fellow, Stanford University School of Medicine, Department of Pathology (PI, Dr. Irving L. Weissman)
2000-2001	Post-doctoral fellow, University of Essen School of Medicine, Institute for Cell Biology and Cancer Research (PI, Dr. Tarik Möröy)
1996-2000	Graduate student, University of Essen School of Medicine, Institute for Cell Biology and Cancer Research (PI, Dr. Tarik Möröy)
1995-1996	Graduate student, University of Marburg (Dr. Tarik Möröy)

Other Experience and Professional Memberships
2009- Member International Society of Stem Cell Research
2010- Member Radiation Research Society

Honors
2001 – Postdoctoral Fellowship of the Ernst Schering Research Foundation
2000 – Earned doctoral degree with *summa cum laude* from the University of Essen
1996 – Doctoral Fellowship of the German Research Foundation (DFG)

C. Selected Peer-reviewed Publications (Selected from 37 peer-reviewed publications)

1. Singh, VK, Christensen J, Fatanmi OO, Gille D, Ducey EJ, Wise SY, **Karsunky H**, and Sedello AK. Myeloid progenitors: a radiation countermeasure that is effective when initiated days after irradiation Radiat Res, 177: 781-791, 2012.
2. Fathman. J, Bhattacharya D, Inlay M, Seita J, **Karsunky H**, and Weissman IL. Identification of the earliest transplantable Natural Killer cell committed progenitor in murine bone marrow. Blood 118: 5439-5447, 2011.
3. Ji H, Ehrlich L, Seita J, Murakami P, Doi A, Lindau P, Lee H, Aryee M, Irizarry R, Kim K, Rossi D, Inlay M, Serwold T, **Karsunky H**, Ho L, Daley G, Weissman IL and Feinberg, AP. A comprehensive methylome map of lineage commitment from hematopoietic progenitors. Nature 467: 338-342, 2010.
4. Papathanasiou P, Attema JL, **Karsunky H**, Hosen N, Sontani Y, Hoyne GF, Smale ST, and Weissman IL. Self-renewal of the long-term reconstituting subset of hematopoietic stem cells is regulated by Ikaros. Stem Cells 27, 3082-3092, 2009.
5. Inlay MA, Bhattacharya D, Sahoo D, Serwold T, Seita J, **Karsunky H**, Plevritis SK, Dill DL, and Weissman IL. Ly6d marks the earliest stage of B cell specification and identifies the branchpoint between B cell and T cell development. Genes & Development 23: 2376-81, 2009.
6. Papathanasiou P, Attema JL, **Karsunky H**, Xu J, Smale ST, and Weissman IL. Evaluation of the Long-Term Reconstituting Subset of Hematopoietic Stem Cells with CD150. Stem Cells 27: 2498-2508, 2009.
7. Ooi AG, **Karsunky H**, Majeti R, Butz S, Vestweber D, Ishida T, Quertermous T, Weissman IL, and Forsberg EC. The Adhesion Molecule ESAM1 is A Novel Hematopoietic Stem Cell Marker. Stem Cells 27: 653-661, 2008.
8. Cao Y, Wagers AJ, **Karsunky H**, Zhao H, Reeves R, Wong RJ, Stevenson DK, Weissman IL, and Contag CH. Heme Oxygenase 1 Deficiency Compromises Stress Responses of Hematopoietic Stem Cells. Blood 112: 4494-4502, 2008.
9. **Karsunky H**, Inlay, MA, Serwold TF, Bhattacharya D, and Weissman IL. Flk2^{+} common lymphoid progenitors possess equivalent differentiation potential for the B and T lineages. Blood 111: 5562-5570, 2008.
10. Frontelo MP, Manwani D, Galdass M, **Karsunky H**, Gallagher PG, and Bieker JJ. Novel role for EKLF in megakaryocyte-erythroid lineage differential commitment. Blood 110: 3871-3880, 2007.
11. Fernandez I, Zeiser R, **Karsunky H**, Kambham N, Soderstrom K, Negrin RS and Engleman EG. CD101 surface expression discriminates potency among murine FoxP3^{+} regulatory T cells. J. Immunol. 179: 2808-2814, 2007.
12. Sanyal M, Tung JW**, Karsunky H**, Zeng H, Selleri L, Weissman IL, Herzenberg LA, and Cleary ML. B cell development fails in the absence of the Pbx1 proto-oncogene. Blood 109: 4191-4199, 2007.
13. Mende I, **Karsunky H**, Weissman IL, Engleman EG, and Merad M. Flk2^{+} myeloid progenitors are the main source of Langerhans cells during inflammatory conditions. Blood 107: 1383-1390, 2006.

14. **Karsunky H**, Merad M, Mende I, Manz MG, Engleman EG, and Weissman IL. Ontogeny of Interferon alpha Producing Dendritic Cells. Exp. Hematol. 33: 173-181, 2005.

15. So CW, **Karsunky H**, Wong P, Weissman IL, and Cleary M. Leukemic transformation of hematopoietic progenitors by MLL-GAS7 in the absence of Hoxa7 or Hoxa9. Blood 103: 3192-3199, 2004.

16. Cozzio A, Passegue E, Ayton PM, **Karsunky H**, Cleary ML, and Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes & Develop.17: 3029-3035, 2003.

17. Adhikary S, Peukert K, **Karsunky H**, Lutz W, Elsässer HP, Möröy T, and Eilers M. Miz1 is required for early embryonic development during gastrulation. Mol. Cell Biol. 23: 7648-7657, 2003

18. **Karsunky H**, Merad M, Cozzio A, Weissman IL, and Manz MG. Flt3 ligand regulates dendritic cell development from Flt3-positive lymphoid and myeloid committed progenitors to Flt3-positive dendritic cells in vivo. J. Exp. Med., 198: 305-313, 2003.

19. Yücel R, **Karsunky H**, Klein-Hitpass L, and Tarik Möröy. The transcriptional repressor Gfi1 affects development of early, uncommitted c-Kit+ T-cell progenitors and CD4/CD8 lineage decision in the thymus. J. Exp. Med., 197: 831-844, 2003.

20. So CW, **Karsunky H**, Passegue E, Cozzio A, Weissman IL, and Cleary ML. MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. Cancer Cell, 3: 161-71, 2003.

21. Geisen C, **Karsunky H**, Yücel R, and Möröy T. T-cell lymphoma in CD2-cyclin E transgenic mice that are deficient for p27Kip1. Oncogene 22: 1724-1729, 2003.

22. Merad M, Manz MG, **Karsunky H**, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, and Engleman EG. Langerhans cells renew in the skin throughout life under steady-state conditions. Nat. Immunol. 3:1135-41, 2002

23. **Karsunky H** , Zeng H, Schmidt T, Zevnik B, Kluge R, Schmid KW, Dührsen U, and Möröy T. Inflammatory reactions and severe neutropenia in mice lacking the transcriptional repressor Gfi1. Nature Genetics 30: 295-300, 2002.

24. **Karsunky H**, Mende I, Schmidt T, and Möröy T. High levels of the onco-protein Gfi-1 accelerate T-cell proliferation and inhibit activation induced T-cell death in Jurkat T-cells. Oncogene 21: 1571-1579, 2002.

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26. Rödel B, Tavassoli K, **Karsunky H**, Schmidt T, Schaper F, Heinrich P, Shuai K, Elsässer HP, and Möröy T. The zinc finger protein Gfi-1 can enhance STAT3 signaling by interacting with the STAT3 inhibitor PIAS3. EMBO J. 19: 5845-5855, 2000.

27. Beier R, Burgin A, Kiermaier A, Fero M, **Karsunky H**, Saffrich R, Möröy T, Ansorge W, Roberts J, and Eilers M. Induction of cyclin E-cdk2 kinase activity, E2F-dependent transcription and cell growth by Myc are genetically separable events. EMBO J. 19: 5813-5823, 2000.

28. Napirei M, **Karsunky H**, Zevnik B, Stephan H, Mannherz HG, and Möröy T. Systemic Lupus Erythematosus (SLE) in Dnase 1 deficient mice. Nature Genetics 25: 177-181, 2000.

29. **Karsunky H**, Geisen C, Schmidt T, Zevnik B, Gau E, and Möröy T. Oncogenic potential of cyclin E in Tcell lymphomagenesis in transgenic mice: Evidence for cooperation between cyclin E and Ras but not Myc. Oncogene 18: 7816-7825, 1999.

30. Schmidt T, **Karsunky H**, Zevnik B, Elsässer HP, and Möröy T. Zinc fingerprotein Gfi-1 has low oncogenic potential but cooperates strongly with Pim and Myc genes in T-cell lymphomagenesis. Oncogene 17: 2661-2668, 1998.

31. Schmidt T, **Karsunky H**, Rödel B, Zevnik B, Elsässer HP, and Möröy T. Evidence implicating Gfi-1 and Pim-1 in pre T-cell differentiation steps associated with beta-selection. EMBO J. 17: 5349-5359, 1998.
32. Haas K, Johannes C, Geisen C, Schmidt T, **Karsunky H**, Blass-Kampmann S, Obe G, and Möröy T. Malignant transformation by cyclin E and Ha-ras correlates with resistance against cell death but requires functional Myc and CDK4. Oncogene 15: 2615-2624 1997.
33. Zörnig M, Schmidt T, **Karsunky H**, Grzeschiczek A, and Möröy T. Zinc finger protein GFI-1 cooperates with myc and pim-1 in T-cell lymphomagenesis by reducing the requirements for IL-2. Oncogene 12: 1789-801, 1996.

D. Research Support.

Ongoing Research Support

HHSN261200100076C Karsunky (PI) 09/20/12 – 06/19/13
Development of a Process for Generating Human MKP.
Role: PI
Time effort: 5%

HHSO100201000051C Mandalam (PI) 09/01/10 – 08/31/13
Advanced Therapeutics for Treating Neutropenia Resulting from Acute Exposure to Ionizing Radiation
Role: Program Director
Time effort 90%

Completed Research Support

1 RC1AI080314-01S1 Karsunky (PI) 09/17/09 - 08/31/11
Development of ex vivo Expanded Megakaryocyte Progenitors for Platelet Recovery
Role: PI
Time effort: 10%

HHSO 100200800063C Mandalam (PI) 09/15/08 – 08/31/11
Development of human Myeloid Progenitor Cells, CLT-008, as a countermeasure for the treatment of Acute Radiation Induced Hematopoietic Syndrome
Role: Leading Investigator
Time effort 40%

1 RC1AI080314-01 Karsunky (PI) 07/01/08 - 12/31/09
Development of ex vivo Expanded Megakaryocyte Progenitors for Platelet Recovery
Role: PI
Time effort: 50%

1 R43 AI064156-01 Karsunky (PI) 06/15/05 - 06/14/06
Application of Expanded Progenitors against Infection
Role: PI

1 R43 AI061856-01 Karsunky (PI) 07/01/2004 – 12/31/2006
Expansion of HSC for rescue in biodefense applications
Role: PI

BIOSKETCHES for the Principal Investigator and all Co-Investigators

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Adrianus (Jos) G.W. Domen	Lab manager, Cardiac Transplant Lab, CMH Assistant Professor, University of Missouri Kansas City
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Nijmegen	M.Sc.	1978-1985	Biology
Netherlands Cancer Institute, University of Amsterdam	Ph.D.	1985-1993	Oncology
Stanford University	Postdoctoral	1993-1999	Immunology/Stem Cell Biology

A. Personal Statement

The aim of the proposed research is to use myeloid progenitors as a cellular therapeutic for tolerance induction. The proposed research describes preclinical studies necessary to device an approach that can be tested in the clinical realm, and I have the experience and capabilities to bring this project to a successful completion. My background in research using mouse models is extensive. I obtained expertise in mouse hematopoietic system studies in multiple environments including the Netherlands, the UK (Manchester) and the USA (Stanford, Duke and in Biotechnology). This experience dates as far back as my thesis work at the Netherlands Cancer Institute during which I studied lymphomagenesis and performed analysis of oncogene-null mutant mice and transgenic mice. I extended this as a postdoctoral fellow at Stanford and faculty member at Duke to more basic Hematopoietic Stem Cell biology. While at Cellerant Therapeutics, I began focusing on Myeloid Progenitor cells in work that was supported by an NIH SBIR grant, specifically on obtaining clinically useful amounts of cells using short term cultures of mouse and human hematopoietic stem cells and testing efficacy of the expanded in mouse infection models. The program that I initiated has since garnered major government support and has progressed to successful clinical trials. In a longstanding collaboration with my wife, Kimberly Gandy, many of our lab efforts have become focused on the use of HCT to induce tolerance. Tolerance is a longstanding area of interest to us, uniquely suiting her dual background (Cardiac and lung transplant surgeon with a PhD in immunology/stem cell biology).

Due to the training/job requirements of my wife the lab has been relocated four times in a period of a little over six years (2004-2010), significantly reducing productivity. However, during the three years in Milwaukee, supported by outside funding (including a 2 year grant from Advancing Healthier Wisconsin), the foundation was laid for the current work in Kansas City. The myeloid progenitor/tolerance induction work has been published in 2011. In addition, one manuscript is currently submitted and another is in the final stages of preparation. We are confident that in direct collaboration with Cellerant Therapeutics we can help accelerate this work to the clinical test stage, necessary for translation to the clinic.

B. Positions and Honors

Positions and Employment

2000–2004 Assistant Research Professor of Medicine, Dept. of Medicine, Duke University, Durham NC
2001–2004 Assistant Research Professor of Immunology, Dept. of Immunology, Duke University
2004–2005 Staff Scientist, Cellerant Therapeutics Inc., Palo Alto, CA
2005–2007 Research Assistant Professor of Surgery, Dept. of Surgery, University of Arizona, Tucson
2007–2010 Assistant Professor of Surgery, Dept. of Surgery, Medical College of Wisconsin, Milwaukee
2008–2010 Assistant Professor of Surgery and Cell Biology, Neurobiology & Anatomy, MCW
2011-present Lab manager, Cardiac Transplant Research Lab, Children's Mercy Hospital
2011-present Assistant Professor, Dept of Pediatrics, University of Missouri Kansas City

Other Experience and Professional Memberships

1995–present American Association for the Advancement of Science
1998–present American Society of Hematology
1998–present American Association of Immunologists
2000–present International Society for Stem Cell Research
2003–present American Society for Bone Marrow Transplantation

Patents:

Methods and compositions for modulating lifespan of hematolymphoid cells.
Weissman, I.L., Lagasse, E. and Domen, A.G.W. US Patent 5,614,397
Methods of expanding Myeloid Cell Populations and uses thereof.
Fong, T., Domen, A.G.W. and Christensen, J.L. US Patent App: 20060134783.

Honors

1989 Short-Term EMBO Fellowship
1993–1994 Dutch Cancer Foundation Fellowship
1992 Invited lecture: Modern Trends in Human leukemia, Wilsede X. Leukemia 7:s108-s112(1993)
2001 Kimmel Scholar Award

C. Selected Peer-reviewed Publications (Selected from 44 published)

Most relevant to the current application

- **Domen, J.**, Sun, L., Trapp, K., Maghami, N., Inagaki, E., Li, Y., Simpson, P. and Gandy, K.L. Tolerance induction by hematopoietic cell transplantation: combined use of progenitor and stem cells. J. Heart Lung Transplant, 30:507-514, 2011. PMID: 21256050
- **Domen, J.**, Gandy, K.L. and Dalal, J. Emerging uses for pediatric hematopoietic stem cells. *Pediatric Res* 71:411-417 (2012). PMID: 22278186
- **Domen J.**, Gandy K.L., Weissman I.L. Systemic overexpression of *BCL*-2 in the hematopoietic system protects transgenic mice from the consequences of lethal irradiation. *Blood* 91:2272–2282, 1998. PMID: 9516125.
- Gandy K.L., **Domen, J.**, Aguila L. and Weissman I.L. $CD8^{+}TCR^{+}$ and $CD8^{+}TCR^{-}$ cells in whole bone marrow facilitate the engraftment of HSC across allogeneic barriers. *Immunity* 11:579–590, 1999. PMID: 10591183.
- **Domen J.**, Cheshier S.H., and Weissman, I.L. The role of apoptosis in the regulation of hematopoietic stem cells; overexpression of *BCL*-2 increases both their number and repopulation potential. *J Exp Med* 191:253–263, 2000. PCMID: PMC2195763.

Additional recent publications of importance to the field (in chronological order)

- Saris C.J., **Domen J.**, Berns A. The *pim*-1 oncogene encodes two related protein-serine/threonine kinases by alternative initiation at AUG and CUG. *Embo J* 10:655–664, 1991. PMCID: PMC452698.
- **Domen J.**, van der Lugt N.M., Laird P.W., Saris C.J., Clarke A.R., Hooper M.L., Berns A. Impaired interleukin-3 response in *Pim*-1-deficient bone marrow-derived mast cells. *Blood* 82:1445–1452, 1993. PMID: 7689870.
- **Domen J.**, van der Lugt N.M., Acton D., Laird P.W., Linders K., Berns A. *Pim*-1 levels determine the size of early B lymphoid compartments in bone marrow. *J Exp Med* 178:1665–1673, 1993. PMCID: PMC2191259.
- van der Lugt N.M., **Domen J.**, Linders K., van Roon M., Robanus-Maandag E., te Riele H., van der Valk M., Deschamps J., Sofroniew M., van Lohuizen M., Berns A. Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the *bmi*-1 proto-oncogene. *Genes Dev* 8:757–769, 1994. PMID: 7926765.
- Kondo M., Akashi K., **Domen J.**, Sugamura K., Weissman I.L. *Bcl*-2 rescues T lymphopoiesis, but not B or NK cell development, in common-© chain-deficient mice. *Immunity* 7:155–162, 1997. PMID: 9252128.
- De Vivo I., Cui X., **Domen J.**, Cleary M.L. Growth stimulation of primary B cell precursors by the anti-phosphatase *Sbf1*. *Proc Natl Acad Sci U S A* 95:9471–9476, 1998. PMCID: PMC21362.
- **Domen J.** and Weissman I.L. Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, Kitl/c-Kit signaling the other. *J Exp Med* 192:1707–1718, 2000. PMCID: PMC2213494.

- **Domen J.** and Weissman I.L. HSC and other hematopoietic cells show broad resistance to chemotherapeutic agents *in vivo* when overexpressing BCL-2. *Exp Hematol* 31:631–639, 2003. PMID: 12842708.
- Reya T., Duncan A.W., Ailles L., **Domen J.**, Scherer D., Willert K., Hintz L., Nusse R. and Weissman I.L. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 423:409–414, 2003. PMID: 12717450.
- Chen B.J., Cui X., Sempowski G.D., **Domen J.**, Chao N.J. Hematopoietic stem cell dose correlates with the speed of immune reconstitution after stem cell transplantation. *Blood* 103:4344–4352, 2004. PMID: 14976038.
- Dalal, J., Gandy, K.L. and **Domen, J.** Role of mesenchymal stem cell therapy in Crohn's disease. *Pediatric Res.* 71:445-451(2012). PMID: 22430380.

D. Research Support

Ongoing Research Support

Katherine B Richardson Endowment Award 7/2011-6/2013
Tolerance induction using myeloid progenitors: Are lymphoid cells essential.
The aim of this proposal is to test whether tolerance induction by myeloid progenitor cells is dependent on the presence of lymphoid cells derived from HSC and multi-potent progenitors present in the MP preparation
Role: Principal Investigator

CMH Cancer Research Grant Shreve (PI) 7/2012-6/2014
Incidence ofVitamin D deficiency in pediatric hematopoietic cell transplant patients.
The aim of this proposal is to determine to what extend pediatric transplant patients are vitamin D deficient before and following transplant, and if that correlates with infection rate and immune function.
Role: co-investigator

Completed Research Support

Thoracic Surgery Foundation for Research and Education Gandy (PI) 7/2008–6/2011
Nina Braunwald Career Advancement Award
The use of autologous HSC transplantation for tolerance induction.
The aim of this proposal is the investigate whether resetting the immune system through autologous HCT is a viable option to induce tolerance to a transplanted solid organ.
Role: Co-Investigator

Advancing a Healthier Wisconsin 7/2008–6/2010
The use of Myeloid Progenitors to improve immune competence after HSC transplantation for tolerance induction.
The aim of this proposal is to investigate the use of third-party myeloid progenitors as an adjuvant therapy to HCT as used for tolerance induction for solid organ transplantation. Myeloid Progenitors have the potential to reduce the consequences of treatment related neutropenia.
Role: Principal Investigator

NIH Small Business Biodefense Programs grant
1 R43 AI 61856 -01 Fong (PI) 8/2004–7/2006
Expansion of HSC for rescue in biodefense applications.
The aim of this proposal is to produce a pre-clinical system based on Cellerant's proprietary HSC expansion technology. This technology entails the transient genetic modification of purified HSC by transduction to express genes that result in their ex vivo expansion.
Role: Co-Investigator

NIH SBIR grant
1 R43 AI64156-01 6/2005–6/2006
Application of expanded progenitors against infections.
The aim is to characterize and define human myeloid progenitors in various source tissues, and establish parameters under which murine myeloid progenitors, generated from highly purified HSC in culture, can be used to protect neutropenic mice from potentially lethal infections, e.g. by fungus. The end goal is to develop human myeloid progenitors as an off-the-shelf product that can be used to treat neutropenic humans.
Role: Principal Investigator

Medical College of Wisconsin Institutional Research Grant Gandy (PI) 1/2008–1/2009
The use of autologous HSC transplantation for tolerance induction.
The aim of this proposal is the investigate whether resetting the immune system through autologous HCT is a viable option to induce tolerance to a transplanted solid organ.
Role: Co-Investigator

Children's Research Institute Pilot Innovative Research Award 3/2008–2/2009
The use of bone marrow to reduce damage due to cardiomyopathy.
The aim of this proposal is to rigorously test for engraftment by bone marrow derived cells in the myocardium of a mouse model of congenital cardiomyopathy. In this model i.v. administration of bone marrow improves heart function without readily apparent engraftment.
Role: Principal Investigator

CTSI-Medical College of Wisconsin Gandy (PI) 8/2008–10/2009
The immune competence of children after congenital heart surgery.
The aim of this proposal is to evaluate the immune function of children in the first year after congenital heart surgery to determine if there are detectable immune deficits which changes in practice may improve.
Role: Co-Investigator

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Cellerant Therapeutics, inc.

Delete Entry *** Start Date:** 06/01/2013 *** End Date:** 05/31/2014 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Holger		Karsunky		PD/PI	[redacted]	12.00			[redacted]	[redacted]	[redacted]
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	12.00			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Cellerant Therapeutics, inc.

Delete Entry *** Start Date:** 06/01/2013 *** End Date:** 05/31/2014 **Budget Period 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Cellerant Therapeutics, inc.

Delete Entry *** Start Date:** 06/01/2013 *** End Date:** 05/31/2014 **Budget Period 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. General and Aministrative	[redacted]	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency HHS/ASPR/ACMG Ethan Mueller [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-Budget Justification Cellerant.pd Add Attachment Delete Attachment View Attachment

(Only attach one file.)

BUDGET JUSTIFICATION

For Cellerant Therapeutics (Laboratory of Dr. Karsunky)

Cellerant will use its laboratory resources in San Carlos, CA to accomplish the aims of this grant application. The assumptions for the budget have been prepared based on previous experience and prior purchases.

Section A – Senior/Key Person: Dr. Holger Karsunky (PD/PI) will lead and be responsible for the scientific management of the project. Dr. Karsunky will contribute his expertise in all aspects of myeloid progenitor biology, hematopoietic stem cell biology, in vivo animal models and cell therapeutic drug development.

Name	Project Role	Proposed % effort	% effort on existing grants for the proposed budget period
Holger Karsunky	PD/PI	10%	90%

Section B – Other Personnel: We budgeted for 50% time effort for a Research Associate who will contribute to the project by harvesting tissues, isolating HSC from mice, ex vivo generation of mouse MPC, flow cytometric analysis and sorting, colony formation assays, and cryopreservation.

Name	Project Role	Proposed % effort	% effort on existing grants for the proposed budget period
Greg Boucher	RA	50%	50%

Fringe benefits, which are 38.94% of salary requested, cover payroll taxes, employee fringe benefits, and workers compensation insurance.

Section C – Equipment: This proposal includes no equipment budget. All equipment needed is currently operational at Cellerant.

Section D – Travel: We are requesting $[redacted] for two trips to the academic partner in Kansas City, MO at the beginning of the project and after 6-9 months. Costs are based on an average non-refundable economy class ticket, two nights per trip at the GSA lodging rate and GSA per diems rates.

Section F.1 – Materials and Supplies: We are requesting a total of $[redacted] for materials and supplies. The major cost factors is the purchase of tissue culture reagents and consumables in particular recombinant growth factors but also media, media supplements, and culture vessels for the ex vivo expansion of mouse myeloid progenitor cells. A second major cost factor are the various fluorochrome labeled antibodies needed for flow cytometry to sort the starting material and characterize the end product for its compositions. Also included are the costs of procuring up to 540 mice form various mouse strains that are required to isolate the starting material for the mouse MP. Cellerant has three qualified mouse vendors (JAX Mice Services, Charles River Laboratories and Taconic) and will also use in-house bred animals. The remaining funds are for general laboratory supply.

Section F.3 – Consultant: No consulting costs are requested as part of this proposal.

Section F.5 – Subawards/Consortium: The budget for the academic partner is $[redacted] ($[redacted] direct costs plus $[redacted] indirect costs). Their budget justification (see below) and their detailed budget are provided.

Section H – Indirect Costs: The G&A rate, currently at [redacted]%, is used to support all of the administrative, operational and contracting matters for Cellerant. Indirect support services include vivarium operations, lab infrastructure and facilities, IT support, facilities support, accounting, procurement of materials and human resources. The current rate is based upon a bilateral rate agreement date Nov 6, 2012 between Cellerant Therapeutics, Inc and HHS/ASPR/ACMG. The indirect rates are reviewed annually by an auditor from HHS/ASPR/ACMG and the Peninsula Branch office is the Cognizant DCAA office.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Children's Mercy Hospital and Clinics

*** Start Date:** 06-01-2013 *** End Date:** 05-31-2014 **Budget Period: 1**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Jos		Domen		PD/PI	[redacted]	12.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	12.00			[redacted]	[redacted]	[redacted]
1	Research Technician	12.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT11279532

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Children's Mercy Hospital and Clinics

*** Start Date:** 06-01-2013 *** End Date:** 05-31-2014 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Children's Mercy Hospital and Clinics

*** Start Date:** 06-01-2013 *** End Date:** 05-31-2014 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Mouse Costs Aim 1	[redacted]
9. Mouse Costs Aim 2	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. General and Administrative	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: 1243-Budget Justification Domen.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	2
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	[redacted]
1. Domestic	[redacted]
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	[redacted]
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

BUDGET JUSTIFICATION

For Subawardee - Children's Mercy Hospital/University of Missouri, Kansas City (Laboratory of Dr. Domen)

Personnel.
The personnel listed in this proposal is in place and is experienced in performing the assays and techniques described in this proposal.

Jos Domen (10% effort) will function as PI for the efforts at Children's Mercy Hospital and will be mainly involved in designing and interpreting experiments performed by the academic partner. He will also be involved in other aspect of the experiments as needed.

Lei Sun (25% effort) and Yongwu Li (25% effort) both have worked in the lab for years. Both are experienced in obtaining bone marrow, staining it with antibodies, sorting HSC and MP, expand HSC in culture into MP, placing skin grafts and flow cytometric analysis of mice. Yongwu Li will focus on the animal experiments such as placements and follow-up of skin grafts. Lei Sun will focus on cell sorting and analysis, HSC to MP cultures and the *in vitro* assays as described in Aim 1. In addition she will be responsible for tasks such as ordering supplies.

Equipment. N/A

Supplies.
Both the FACS sorts as well as the flow cytometric analysis require fluorochrome labeled antibodies. A typical Hematopoietic Stem Cell sort requires a total of 11 different antibodies in 4 colors, while a typical analysis uses 5 to 6 different labeled antibodies. While we have complete sets of antibodies we will have to replace regularly as tubes are emptied. We are budgeting for 12-15 replacements per year (costs varies with fluorochrome).

ScilVet Bloodcell counter. In flow cytometric analysis of blood we typically include a blood cell count to determine absolute cell numbers. The lab has a ScilVet ABC veterinary blood cell counter for this purpose. The requested consumables should allow us to do approx 900 cell counts, each requiring 12µl of blood. The machine will also be used for cell counts when harvesting and analyzing organs.

Flow cytometry will be used extensively for analysis purposes. The six-color Attune is available for this purpose and doesn't require an hourly fee. However, we have budgeted for consumables such as focusing buffer, wash and shutdown solution and test beads, all of which are essential in using the instrument.

Sort time. We have budgeted for sixteen 1 hour sorts at the KU Medical Center Flow Core facility. This should allow us to sort the HSC needed in these experiments. MP will be provided by Cellerant Therapeutics. In addition to HSC we may sort MP subpopulations for the MLR assays in Aim 1.

Histology. The outcome of skin graft experiments typically is very clear by gross morphology, but trachea grafts need to be evaluated microscopically. We have budgeted for histological analysis of 150 samples, using the core facility at Children's Mercy Hospital. The procedures include embedding, sectioning, H&E stain as well as two immune stains. We aim to use immunohistochemistry to test for graft infiltrating lymphocytes or MP-derived cells in tissues.

Other consumables. In addition to these major categories there will be need for a variety of consumables, including plasticware (FACS tubes, tissue culture plates), media and buffers, IgG for blocking, surgical supplies such as sutures and anesthesia.

Travel. Travel costs will be used for a meeting with the corporate partner in San Carlos, CA. This in addition to the meetings listed under the Cellerant budget justification section. In addition, travel funds may be used to present the data in a meeting such as the annual meeting of the American Society of Hematology or the International Society for Heart and Lung Transplantation.

Patient Care. N/A

Other.
Mouse purchasing. Typical experiments require 50 mice (15 hosts, plus 5 HSC-donors, and upto 30 trachea or skin graft donors). We expect to use approx. 700 mice, at least 6 groups for aim 1, and 8 groups for aim 2. Mouse purchase prices average $20 for young mice of these standard strains.

Mouse per diems. Host mice, 3 cages per group, will be kept one to six months to evaluate hematopoietic transplantation and trachea or skin graft. Trachea grafts are harvested early (one month) while skin grafts are followed longer. Donor mice will be kept for an average less than one month before tissue harvest. We expect that mice, on average, will be kept for 2 months. Cage cost is $0.54 per day and we expect approximately 188 cages for the experiments described.

SBIR/STTR Information

OMB Number: 4040-0001
Expiration date: 06/30/2011

*** Program Type (select only one)**

☐ SBIR ☒ STTR

☐ Both *(See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)*

*** SBIR/STTR Type (select only one)**

☒ Phase I ☐ Phase II

☐ Fast-Track *(See agency-specific instructions to determine whether a particular agency participates in Fast-Track)*

Questions 1-7 must be completed by all SBIR and STTR Applicants:

☒ Yes ☐ No	* 1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?
	* 1b. Anticipated Number of personnel to be employed at your organization at the time of award. 65
☐ Yes ☒ No	* 2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies? * If yes, insert the names of the Federal laboratories/agencies:
☐ Yes ☒ No	* 3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov
☒ Yes ☐ No	* 4. Will all research and development on the project be performed in its entirety in the United States? If no, provide an explanation in an attached file. * Explanation: Add Attachment Delete Attachment View Attachment
☐ Yes ☒ No	* 5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work? * If yes, insert the names of the other Federal agencies:
☒ Yes ☐ No	* 6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?
	* 7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions. * Attach File: Add Attachment Delete Attachment View Attachment

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

	* 8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment. * Attach File:
	* 9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

	* 10. Please indicate whether the answer to BOTH of the following questions is TRUE: (1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND (2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?
	* 11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Holger
Middle Name:
* Last Name: Karsunky
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Dr.
* First Name: Holger
Middle Name:
* Last Name: Karsunky
Suffix:

* Phone Number: [redacted]
Fax Number: [redacted]
Email:

* Title: Director, Preclinical Development

* Street1: 1561 Industrial Road
Street2:
* City: San Carlos
County/Parish:
* State: CA: California
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 94070-4111

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Tracking Number:GRANT11279532 Funding Opportunity Number:PA-12-089 Received Date:2012-12-04T19:14:17-04:00

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1241-SpecificAimsSTTR2012v2	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1242-ResProposalSTTR2012v20	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1246-Combined Vertebrate An	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research	1247-11 SelectAgentResearch	Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan	1248-12 MultiplePDPI_FINAL.p	Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	1249-STTR Letter verifying e	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support		Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1250-15 ResourceDataSharing	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

A

Despite many advances in the field of transplantation, 10-year graft survival rates are still low: 20% for lung transplantation and just over 50% for heart transplantation. The latter has one of the highest long-term success rates. Rejection is thought to cause the majority of graft loss. Operational tolerance, the ability of an otherwise immunocompetent host to accept a transplanted organ without immunosuppression, would likely improve long-term organ function and offer significant benefits for recipient's quality of life and longevity. Specifically for lung transplantation, the most important cause of late mortality is obliterative bronchiolitis, characterized by specific lesions and a decrease in lung function [1]. Despite decades of research, no widely applicable clinical protocol has emerged that can achieve consistent operational tolerance. Certain bone marrow transplantation regimens

RESEARCH STRATEGY

(A) SIGNIFICANCE

S - e a ca d a a a a e ce e b - e a e a ed. O 20% e a a ed e a c a e ea a e a a a [4], e e c ed e . Da e c ca ed b e a e b d a ca ed e d a a d ea e e e a d deb a e ec e de . L - e e , e e a de a e ec , ca e be c de ed a c ca , ce e a ca ed b d e a e e a d deb a (c d e a a d e a c a d e ca ce a d ec [5-8]).

O e a a e a ce, e acce a ce e a a ed a a e e c e e , d ea e - e c e, e a e a e a d ed ce e ec c b de c e . H e e , e a ce d c e a a e e a [9]. T e a ce e ec a be e ed a c a a ec e , a a a ed a eed c e decade e e a a e a c d e . Ma a ac e e a ce d c a e bee e ed (e e ed [10-20]). Tab e 1 be ca e e e e e ea e a d e a .

I e a (a da d e a , e a ce d c)	E ec e e , e e . S ca de e ec .
C a b c ade, T ce de e	L ed e ec e e a e, a be d c b a e a .
He a e c (e) Ce T a a a , BMT	Ve e ec e, a e e e , b e e e c - b d .
P e Ce T a a a (M e d P e)	T a . E ec e. Ca d e e ce e .
O e ce a e a e (e T e , ac a e de d c ce)	P b add e e e e ec a (e ce e).

Tab e 1. C a f a da d he a a d e e a e a ce d c g a eg e .

T e cce e a e c ce a a a (HCT) a bee e e ab ed a ce a , c d ed e a e c e ce (HSC) [21]. H e e , de e decade c ca a a a bee ed a - ca e a [12, 18, 19, 22-24], a d e HCT-a c a ed c ca . A HCT- e a ed c e c e e c e e a [25], c ea ed acce a ce a d e HCT ea e - a a c e e e ca ed c b d a d a . T e e e d ce e a ce d c a bee e a ec a ed ece . T a ce e ed a a ce ca ed e d e (MP) ce a e e a ce e e d ea e. **Key characteristics of myeloid progenitors include: clinically availability; minimal to no side effects in ongoing clinical trials and importantly; specific tolerance induction potential in our experimental model system.** O a c e b a e da a eeded b c e c a de e e a d c ca a a ab e ea e.

Ce e a T e a e c , I c a ed de e MP a a e e c ca d c (d c a e CLT-008) e a 8 ea , a d c e e CLT-008 c ca a . CLT-008 be de e ed d ca : (1) T e e ec c ca e e e e c a e a d (2) ea ac e ad a d e (ARS). T a , ba ed ab a e ed b e D e ab a e Med ca C e e W c , M a ee, WI a d C d e Me c H a Ka a C , MO, a add e a ce d c d a a a a a a d ca e e MP. T e e a c e e ed ca eed. H e e , a e a ce d c e e a c ed MP , e e e e d c d e e e e d e e c e c ca a .

De e e CLT-008 a a a e abe d c e de a , c d c ca a a d ca d c , e a e a ded b C ac HHSO 100201000051C e B ed ca Ad a ced Re ea c a d De e e A (BARDA) de e a d c e CLT-008 e ea e ARS. T d ea a d e e e a c e ca de e e CLT-008 e a ce d c , e e d ca e d c ce a e d be ece a e a d c ed a .

(B) INNOVATION

O a e e e a a c a e a a a a e e . O e a ce e e e e e e d e ce a d ce e a ce. T a ac c de a d a a c a a e c e e ed ce a e a a e e a e e e e HSC-c a a , c e a , a e e d a , c e e e c ea ab e c e a . We a e c a a c e e d MP ce a d a e d ce a e - ec c e a ce a e a a acce a e eed a ed e e e a e c c e a d a ca ed c ca [3]. MP ce e e a ce e e d ea e a d ece da a de a e e e e d a e a ce d c . B e ce a ca d e e a e a e a e ec a , e e a a e e a be a e ed. H e e , a a e c ca ac ce e eed de e a a . B c b e acade c ec ca e e e Ce e a e e e c ca e MP e e ec be ab e de e a a c a c ca a a e a ac be ea b e a e e d a .

(C) APPROACH

*

Our original model of performing the skin grafts two months after HCT confines clinical translation to transplants from living donors. We have determined that specific tolerance can be obtained when the skin graft is placed at the same time as the MP graft, broadening the applicability of this strategy to deceased donor organs (Figs.1-2).

*

We have demonstrated that tolerance induction only requires myeloid cells. B10;B6- mice lack both the Rag-2 recombinase and the gamma chain, essential for signal transduction, common to the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. As a consequence, they have no T-cells, B cells or NK cells [56]. MP cells from these mice induce tolerance for matched skin grafts (Fig. 2), (

.

*

Allogeneic MP cells induce tolerance when given simultaneously with host-autologous, rather than allogeneic, HSCs (Fig. 3). Importantly, this indicates that the full replacement of the hematopoietic system through an allogeneic HSC transplant is not necessary and that it should be possible to develop sublethal regimens for the temporary engraftment of MP.

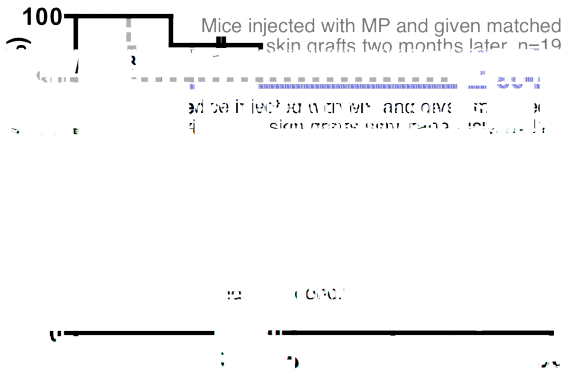

Figure 3. Tolerance can be achieved with graft-matched MP and host-matched HSC. *BALB/c h e ec ed h 250 BALB/c HSC a d 10^5 MP-d ec ed f BM 10^6 MPc-de ed e da c e f HSC (he a e a e e he e ge e). S g af e e aced a he e f HCT (d b ac e) h a e (da hed g e): S g af a d e d ffe (g a).*

*

While it is possible to sort sufficient quantities of MP from mouse bone marrow, this technique can not generate clinical quantities. Short-term expansion under defined conditions [2, 30, 32] is used for generating clinical MP preparations [57]. E.g. in the group with skin grafts placed after two month 10 mice received MP sorted from BM, 16 mice MPc expanded from HSC (shown as single group (dashed grey line) in Fig. 3). Skin graft survival does not differ (90 vs 81%, p=0.58, logrank) between sorted or expanded and cryopreserved MP.

Aim 1: Show that MPc can be used to induce tolerance for lung transplants in a mouse model using tracheal allografts. Lung transplant, with its low long-term graft survival rate, is a primary candidate to test novel approaches aimed at prolonging the useful lifespan of transplanted organs for several reasons. There is the potential to show significant improvements faster and with a more limited set of patients than otherwise necessary. In addition, it would help patients whose only alternative currently is a re-transplant, which is not always available. The main cause of late failure in lung transplant is bronchiolitis obliterans syndrome (BOS), clinically evident as decreased lung function, and morphologically characterized by obliterative bronchiolitis (OB). Airway epithelium becomes a target of the alloimmune response, resulting in loss of airway patency. Several models have been developed to study OB. In the Phase I part we propose to utilize a tracheal transplant, a model developed twenty years ago [58] which is still used extensively to study OB [59-63]. In this assay allografts lose their epithelium and airway patency following transplantation, while isografts retain it. Major advantages of this model are the ease of use and the reproducibility of the assay [64]. We will use this model to test whether addition of graft matched MP can prevent occlusion and loss of airway epithelium in transplanted trachea (Fig. 4). The readout should be clear and with paired analysis (three grafts will be placed per mouse) we calculate that groups of 15 grafts will give us sufficient power (as described in the data analysis).

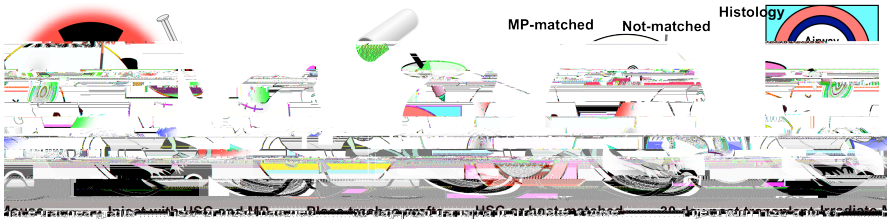

Fig 4. Experimental design for Aim 1. *BALB/c ce be e ha ad a ed, ec ed h 4,000 FVB HSC a d 500,000 C57BL/6 MPc, af e h ch achea g af a e aced (a a e da) f C57BL/6 (MP- a ched), FVB (HSC- a ched) a d AKR (- a ched). O de f achea g af a a d . P a c e be a a cc , ec da f e he .*

Division of the work. P ace e a d e ac ea a ce be e ed b e acade c a e . S HSC, *ex vivo* e a HSC MPc a d c e e a e MPc be e ed b e c a e a e . A c e e ed MPc de e ea e e a (ab , c , c , e d e e , a d b b de) e e a d c c e a d e c .

Milestone: P e e cc a c ed a e e c ac ea a a . T e a a be c de ed e e ca de a e a ee ce a a e e c ac ea a e ca ed ced a cc (<50% cc b ace a ea a c ec H&E a ed de) be d 30 da a a ce a a e bee e MP a c e ac ea a a e e ac ea a a . C (e a e ce) c de ( ) ac ea a d a c MP (HSC), e e c d be cc ded, a d ( ) ac ea a c ed HSC , e e d cc de. I add a cc e c e e a b e H&E a ed ec , a a ec da e ec .

Details of the model. We e a a a e ab ed e de e e e e A 1. We e a ad a e (2 4.25 G , 4 a a a RadS ce 2000 ad a a 160 V, 25 A, ca b a ed c e ca d e c ab a J.D. a H , De C e , UMKC) ce (ca BALB/c; e a e e a e ad a d e). T e ce be ec ed e da ad a de ed c b a e ce ce-ac a ed ce (FACS)- ed HSC [3, 55, 65]. De e d e a (Tab e 2), e e e HSC be ed BM: CD90.1 a c a FVB e e 4,000 ce e e CD117^{+}, CD90.1 , L $^{-}$, Sca-1^{+} e CD90.2 a (c a C57BL/6 BALB/c) e e 10^{4} ce e e CD117^{+}, L $^{-}$, Sca-1^{+}. P ec , e HSC be ed e e 10^{5} FACS- ed b e a de ed MP ce (L $^{e/}$, CD117^{+}, Sca-1^{-}) 10^{6} MPc ce e a ded FACS- ed HSC a - e c e (8-10 da X 15 ed e e ed ec b a e SCF, F 3L, a d T) [2]. O e da ec ac ea a e aced b ed ced e [58, 66]. D ce a e e a ed a d e ea b c e c ed. T e ac ea a d a b c a e e ed a d a ed e e a bc a e c e e bac . T ca a e be a a ed b a MP- a c ed ac ea, a HSC - a c ed ac ea a d a a c ed ac ea, aced e e de e d e be e de b e. T ac ea be a e ed e a ed ec e da 7, 14 a d 30 a a a a d a a ed b ( ) c e a d c e d de ed ce a d a e ac ea a . T e a a a be a da 30, a de ed e e . F c e c de ec c e , e e e a e , e ca e e a e c e e . T a a c de ed c e eac (MLR) a ed a e ac a / e a Ce ace Fa Red abe ed c e b a ac a (ad a ed -, d - d a c e) e *in vitro* e a ce.

Strain	MHC	CD90	CD45
AKR		1	2
BALB/c	d	2	2
CBA		2	2
C57BL/6	b	2	2
FVB		1	1
SJL		2	1
F1(B6 129)	b	2	2
F1 (B6 BALB/c)	b/d	2	2
F1 (B6 CBA)	b/	2	2
B10;B6-*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$	b	2	2

Tab e 2. M e a cha ac e c

Potential Problems/Alternative Approaches. T e ac ea a a de a bee ed cce a a e be d e . T e c ca ec e a d e d c b c e d ea ac a e e e e a da a ece a e ec a d. M e ca ed a d c e de e c a a a ac ea [64], c a a a [67, 68], be c de ed e ec d a e ec e d e a e e e c ac ea a a de ca a e . M e a be ada a de e a MPc [69]. I acce a ce e a HSC b c e de ed MPc ee , e a e e a ed e e c ea e e d e MPc, c a e e c e c d , e e ed MP e b e a .

Aim 2: Determine the degree of mis-matching allowed for tolerance induction by MP.

O da a, b ed a e a b ed, a c ea e ab ed a e d ce e MP e a a a e e e a d ce e a ce a e b e e aced d a a a a e a c ed e MP . H e e , a e bee de e ed e e a c eed be ab e (c ca a ea e ce a e be de ed e a d) e e c e e MP a d a c a c a b a e . T e a e d a a e c ca a ce a e e MP c b a a a a a e ca be c ed. W e a

bec e a a ab e a a a
a c ba c MP c d be e ec ed
a d be a a ab e a e e a
a a . l e MP a e be de ed
e a d e e c
bec e e c ca ed. B e a
ce d a e be a e ed e
e a e d a d ed
e e ed a e e
a a , a e e c e e
a d e ce , a ee a e
a e e a e MP a a ce a
ac . O e a da a c ea
d ca e a a c (a a d
a a a a e) ead
e ec : T e a ce d c b MP ec c. H e e , a c a eed be ab e. BALB/c ce
ec ed 4,000 FVB HSC a d 10^6

Potential Problems/Alternative Approaches. We a d a e e e e a ce ac e ed (de ed a a e ec ed) a de e d e de ee a c , b a e be MP ed. W e a d be a a e e, a de a de e e e ec c ea MP ce d e. I add e a e e a ed c a e MP c e c d e e ed MP e b e a e d d c ac e e e a ce e a d - de ed MP. We a a e e ab MP e ea e ec ed a a de c a ea - ea a a a , c e a a ea a a ed e a e ea [21]. W e e e e be e ed MP d- e ce, e e c c a da a be c ed e e a a e MP B10;B6- mice to confirm that only myeloid cells are essential.

Data Analysis. T e a a ab e A 1 e e a a e a ce/ab e ce cc ba ed a d A 2 a acce a ce e ec ba ed , c ed b . Sec da a ab e a e e be , , a d e ce e e ec ed ce a a e , c be e e ed a ab e ce be (ca) e ce ce a d e e ac ea (e e ed a e ce c c e e ce e e). W e e ece a a a e c a , a a e a a e da a be e ed a d ed. I ece a , a a e c e c a e W c a e be ed. We c a e a a a ed - e . P e : W 15 eac a a a a 0.05 e a e a ea 80% e de ec a d e e ce e ce a e a a e a c e 0.6, a e a a e a c e a 0.2 e . A e e e be ade a d da a, b e a e ea e e be a a ab e. W e e e da a a e , e ca e be e a ed. M ce a d e be e a a d e e a e c a e (<30 da ec) ec (dea <10 da ec) be ce ed, a e e e e e a e de ed d HCT-a c a ed b d . I e e e ce MP ad a d e c ea e a , a d c ca d e a e MP add e . A b e da a be c ded e a a b a a e e d dea da a. M e a e be e ed e da a a e a a d . T e e a a ca e e e c ea e ec acce a ce e e a e e c d . We e e e a e ad a a e a - e a ac . Da a a 12 (c)1 e(a 12 (c)1 (e)6 (a c)1 (e)(e)23)6 [( )6 ( )-23 ( )17 (e)236 6 ( )29d ( )K e a 35 ( )6 ( )-2

VERTEBRATE ANIMALS

1) For Cellerant Therapeutics (Laboratory of Dr. Karsunky)

Cellerant's Animal Assurance Number: [redacted]
IACUC approved protocols for all animal work and procedures proposed under this proposal are already approved and in place at Cellerant. Prior to award Cellerant will amend those protocols to include the strains and number of animals needed for the work proposed here.

1. Description of proposed use

This protocol involves the use of mice in order to study the therapeutic potential of human myeloid progenitor cells (MPC) for the induction of operational tolerance to enable stable long term solid organ engraftment. We will use C57Bl/6, Balb/c, AKR, FVB, F1 (B/6x129), F1 (B/6xBalb/c) and B10;B6-Rag2-/-IL2rg-/- to isolate hematopoietic stem cells as the starting material for MPC *ex vivo* expansion cultures. All mice will be males of 6-10 weeks of age at tissue harvest.

Strain	**Number of animals estimated**
C57Bl/6	100
Balb/c	100
AKR	100
FVB	100
F1 (B/6x129)	50
F1 (B/6xBalb/c)	50
B10;B6-Rag2-/-IL2rg-/-	40
Total	**540**

2. Justify the use of animal, choice of species and numbers used

For the isolation of hematopoietic stem cells (HSC) we depend on Thy1.1 expressing mouse strains. This is a less common allele but it is expressed in certain Bl/6 substrains, AKR and FVB mice. Therefore, these three were chosen as the main strains for HSC isolation as the starting material for MKP cultures. One of the key scientific questions will be to test for immunogeneic barriers for MPC to engraft and induce tolerance. For experiments proposed under Aim 1 we will use *ex vivo* derived MPC generated at Cellerant from FVB, C57Bl/6 and AKR mice which are either syngeneic (matched) to the organ graft, matched to the HSC or unmatched to both graft sources. To the actual degree of matching that is required between MPC and the graft we will generate MPC from C57Bl/6, AKR, F1 (B/6x129), and F1 (B/6xBalb/c) mice. B10;B6-Rag2-/-IL2rg-/- mice will be used to confirm that the tolerance induction seen from ex vivo expanded MPC is truly due to myeloid cells only without any contribution from lymphoid cells. We expect that the majority of our proposed experiments will be done using C57Bl/6, Balb/c, AKR, and FVB mouse strains, which is reflected in the higher mouse numbers for these strains.

The species mouse (Mus musculus) is clearly the best and probably only laboratory species where immunogenic barriers can readily be experimentally addressed. Mice are also a well established system for *in vivo* studies of the potential of hematopoietic stem and progenitor cells. The use of a standard laboratory species such as the mouse also helps to ensure optimal care of the animals because of the availability of well-established facilities and extensive veterinary expertise. The number of mice required reflects the need to generate and maintain the mice and to generate sufficient MPC for the planned engraftment studies. Experience with these models has shown that this is the number of mice necessary for study of each experimental parameter in order to convincingly answer the questions posed.

3. Veterinary care

Cellerant Therapeutics has its own animal care facility. The facility complies with all applicable provisions of the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The animal care staff are trained laboratory animal technologists and the investigators working on the experiments proposed in this application receive regular training on the proper handling and treatment of mice.

4. Procedures for ensuring that discomfort is minimized

Animals used for the harvesting of bone marrow will be between 6-10 weeks of age. Mice will be housed as per Cellerant's standard procedures. The mice will be euthanized through CO_2 asphyxiation followed by cervical dislocation. The femoral, coxae and tibiae bones will be removed and used as a source of bone marrow cells for HSC sorting. The carcasses will be disposed of as pathological material by approved methods of disposal.

5. Methods of euthanasia

Mice will be sacrificed to obtain tissues e.g. for tissue analysis by flow cytometry and other assays. Mice will usually be euthanized by CO_2 asphyxiation. In some instances mice may be anesthetized by isoflurane and the depth of anesthesia assessed by the withdrawal reflex, followed by cervical dislocation. If the reflex persists, additional anesthetic will be administered.

2) For Children's Mercy Hospital/University of Missouri, Kansas City (Laboratory of Dr. Domen)

1). Description of proposed use: This protocol involves the use of mice in order to study the tolerance induction/maintenance abilities of hematopoietic stem and progenitor cells. The strains used will include wild-type mice such as BALB/c, AKR, FVB, C57BL/6 (purchased from JAX) as well as mutant mice such as B10;B6-Rag2$^{-/-}$Il2rg$^{-/-}$ mice (purchased from Taconics) and other strains as listed and available from JAX. The ages will range from 5 weeks to 1-year-old adult mice. These mice will be used as sources for the purification of cells (HSC and MP) and as a source of tissue for skin transplants. In addition, mice will be used as recipients of both cells and skin transplants. All these experiments are aimed at developing conditions for inducing tolerance for skin transplantations.

2). Justify the use of animal, choice of species and numbers used: The experiments proposed in this proposal addresses the complex interactions between many different types of effector cells and molecules that occurs after transplantation of a non-matched solid organ. There are no protocols to mimic this in culture. This makes it impossible to predict the outcome of transplantations based on *in vitro* assays, despite the tremendous clinical value that such an assay would have. The mouse is the best model for the proposed studies. There are multiple well-characterized mouse strains available, wild-type and with defined mutations. Their hematopoietic stem and progenitor cells have been well characterized and can be sorted to near homogeneity using commercially available antibodies. Both congenic (same genetic makeup, typically except for a marker gene) and allogeneic (genetically different) models are available that allow the tracking of host and donor cells. In addition their response to radiation as a preconditioning regimen has been well documented, reducing the number of animals needed to set up the experiments. Solid organ transplant models, including ear-heart transplantation and skin graft transplantations, have been established. Most importantly, the results obtained in mice have historically translated well to clinical practice. No lower animal model has all of these features.

The primary variable in aim 1 is epithelial maintenance/absence of occlusion based on histology and for aim 2 skin graft acceptance or rejection based on gross morphology, confirmed by histology. Secondary variables are the number, origin, and types of cells present in reconstituted mice at various times, which will be expressed as absolute cell numbers (typical) or percent of cells and loss of epithelium in tracheas (expressed as percent of circumference without epithelium). Where necessary for parametric assumptions, appropriate transformations like log data will be employed and justified. If necessary, nonparametric tests such as the Wilcoxon rank sum test will be used. We will compare grafts using a paired t-test . Power: With 15 in each group at an alpha of 0.05 we will have at least 80% power to detect a difference in percentage of animals with the primary outcome of .6, assuming the grafts have a correlation of .2 or less. Ultimately we are interested in pair wise comparisons. We will not adjust for multiple comparisons. However, we will report the actual P values and repeat significant experiments for validation.

With each group we expect to use approx 50 mice (15 hosts, 5 HSC donors, 30 trachea donors). We expect to use approx. 700 mice, 6 groups for aim 1, and 8 groups for aim 2. Transplanted mice will be followed for long periods of time (2 to 6 months) and will be analyzed (eg by flow cytometry of blood) regularly.

3). Veterinary care: The animal care facility of the University of Missouri Kansas City Medical School is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and complies with all applicable provisions of the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The animal care facility employs a veterinarian. The investigators working on the experiments proposed in this application receive regular training on the proper handling and treatment of mice. Animals which will be irradiated will be kept on antibiotic water prior to and after irradiation.

Animal welfare assurance number: [redacted] The techniques in this proposal have been IACUC reviewed and are currently approved as protocol 1112-Domen. Last approval date 11-01-2012.

4). Procedures for ensuring that discomfort is minimized: **Total body irradiation** used in the competitive repopulation assays will be performed in standard allentown cages in a RadSource 2000 X-ray machine. The mice will be exposed to a irradiation dose of 8-10 Gy total, depending on the mouse strain. All animals will be monitored for signs of distress (hunching, failure to groom, weight loss) and euthanized when moribund. **Skin Transplantation Procedure**. The surgeon's hands will be washed with hibiclens scrub (chlorhexidine) prior to putting on sterile gloves. Instruments will be prepared by immersion in clidox or sporocidin according to the manufacturers recommendations. A mouse will receive preoperative anesthetic/sedation by IP injection of xylazine/ketamine at 10/100mg/kg. The abdomen is prepped in a sterile fashion by shaving the hair using an Oster Animal Clipper. The mouse is then placed supine an on operating board and secured followed by prepping several times with hibiclens scrub. Eyes will be protected using antibiotic eye ointment. The skin is patted dry with sterile gauze prior to the incision. Sterile gauze or a sterile paper drape will be used to isolate the surgical field. A 1.5 X 1.5 cm segment of skin is removed, the subcutaneous tissues being left in place. Hemostasis is assured. The skin graft is then placed over the prepared recipient site, the size discrepancy between donor site and recipient site allowing the graft to be slightly stretched. Mouse grafts are placed such that the hair will grow in a direction incongruous with the surrounding hair. The skin is first secured at each of the four corners with 6-0 Maxon or Biosyn sutures. A 6-0 Maxon or Biosyn is then used in an interrupted fashion to secure the graft to the surrounding skin. All fluid is decompressed from the graft site. A 2 X 2 piece of gauze is then sewn over the graft at its four corners with 4-0 Sofsilk. The procedure is expected to last less then 20 min. The animal is closely observed until determined to be recovered from anesthesia. The respiratory rate and behavior of the animal will be monitored until the

Select Agent Research

Not applicable.

MULTIPLE PD/PI LEADERSHIP PLAN

Rationale. This STTR application has two project sites. The small business project site is at Cellerant Therapeutics in San Carlos, CA. Holger Karsunky, PhD, will be the Principal Investigator at Cellerant Therapeutics. The academic project site is at Children's Mercy Hospital and Clinics/University of Missouri Kansas City in Kansas City, MO, and Jos Domen PhD will be the on-site Principal Investigator and direct the efforts

Communication Plan. The two project leaders have talked and met regularly over the last 7 years. In the context of this project these contacts would be intensified and scheduled on a regular basis to include a standing tele conference at least every two weeks to discuss the progress towards the project's milestones. It is also planned to have reciprocal visits to further strengthen the collaboration and hold one face-to-face kick-off meeting at the beginning of the performance period as well as one in the middle of the project to discuss progress and the strategy to apply for continued funding.

Role. Dr. Karsunky will lead in coordinating the efforts related to this project at Cellerant Therapeutics, and in determining how this project will fit into the commercial development of Cellerant Therapeutics. Dr. Domen will lead the efforts at Children's Mercy Hospital and Clinics/University of Missouri Kansas City, and is responsible for the in vivo preclinical tests that are necessary to determine the parameters under which this novel indication for myeloid progenitor use can be tested and used clinically.

Conflict Resolution. Conflicts will be resolved in consultation between the two PI's. Decisions on the commercial use and development of this technology, the eventual aim of this project, by Cellerant Therapeutics will rest with Dr. Ram Mandalam the CEO of Cellerant Therapeutics. According to SF424 guidelines the small business concern will be the primary party that will exercise management direction and control of the performance of the project.

Budget allocation. Half of the budget is currently allocated to each of the project sites, conforming to the requirement that at least 40% of the work is performed at the business site and at east 30% at the academic site. This will allow both groups to contribute significantly in this first phase of the transfer of this technology from the Domen Lab to Cellerant Therapeutics.

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RESOURCE/DATA SHARING PLAN

Data Sharing Plan
Based on the SF424 (R&R) SBIR/STTR Application Guide for NIH and Other PHS Agencies a formal Data Sharing does not need to be provided since the requested direct costs are under $[redacted] Data generated by this project will be shared via publication in peer reviewed journals. Additionally, study team members may present the findings in lectures or posters at conferences and seminars.

Sharing Model Organisms
The proposed work does not include the development of new model organisms. Only mouse strains that are readily available from commercial sources will be used for the proposed work.

Policy for Genome-Wide Association Studies
No genome-wide association studies (GWAS) to identify common genetic factors are planned as part of this proposal.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents:

If the answer is "Yes" then please answer the following:

* Previously Reported:

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No