| | | |
|---|---|---|
| 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<br>(excludes [redacted]m F&A)<br>Year 1: | Animals: Y<br>Humans: N<br>Clinical Trial: N<br>Current HS Code: 10<br>HESC: N | New Investigator: N<br>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 |

**Always follow your funding opportunity's instructions for application format.** Although this application demonstrates good grantsmanship, time has passed since the grantee applied. The sample may not reflect the latest format or rules. NIAID posts new samples periodically: https://www.niaid.nih.gov/grants-contracts/sample-applications

**The text of the application is copyrighted.** You may use it only for nonprofit educational purposes provided the document remains unchanged and the PI, the grantee organization, and NIAID are credited.

**Note on Section 508 conformance and accessibility:** We have reformatted these samples to improve accessibility for people with disabilities and users of assistive technology. If you have trouble accessing the content, please contact the NIAID Office of Knowledge and Educational Resources at deaweb@niaid.nih.gov.

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

# APPLICATION FOR FEDERAL ASSISTANCE
# SF 424 (R&R)

**3. DATE RECEIVED BY STATE**

**State Application Identifier**

**1. * TYPE OF SUBMISSION**

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

**4. a. Federal Identifier**

**b. Agency Routing Identifier**

**2. DATE SUBMITTED**

**Applicant Identifier**

**5. APPLICANT INFORMATION**

*** Organizational DUNS:** [redacted]

* Legal Name: Cellerant Therapeutics, inc.

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

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:

**6. * EMPLOYER IDENTIFICATION** *(EIN) or (TIN):* [redacted]

**7. * TYPE OF APPLICANT:** R: Small Business

Other (Specify):

**Small Business Organization Type** ☐ Women Owned ☐ Socially and Economically Disadvantaged

**8. * TYPE OF APPLICATION:**

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

**9. * NAME OF FEDERAL AGENCY:** National Institutes of Health

**10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:**

TITLE:

**11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:**

Novel indication for myeloid progenitor use: Induction of tolerance

**12. PROPOSED PROJECT:**

* Start Date: 06/01/2013

* Ending Date: 05/31/2014

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**

CA-012

**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: Dr.

* First Name: Holger

Middle Name:

* Last Name: Karsunky

Suffix:

Position/Title: Director, Preclinical Development

* Organization Name: 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]

**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

# 424 R&R and PHS-398 Specific
# Table Of Contents


| | Page Numbers |
|---|---|
| **SF 424 R&R Face Page** | 1 |
| **Table of Contents** | 3 |
| **Performance Sites** | 4 |
| **Research & Related Other Project Information** | 5 |
| **Project Summary/Abstract (Description)** | 6 |
| **Public Health Relevance Statement (Narrative attachment)** | 7 |
| **Facilities & Other Resources** | 8 |
| **Equipment** | 11 |
| **Research & Related Senior/Key Person** | 12 |
| **Biographical Sketches for each listed Senior/Key Person** | 13 |
| **Research & Related Budget - Year 1** | 21 |
| **Budget Justification** | 24 |
| **Research & Related Budget - Cumulative Budget** | 26 |
| **Research & Related Budget - Consortium Budget (Subaward 1)** | 27 |
| **SBIR/STTR Information** | 33 |
| **PHS 398 Specific Cover Page Supplement** | 35 |
| **PHS 398 Specific Research Plan** | 37 |
| **Specific Aims** | 38 |
| **Research Strategy** | 39 |
| **Vertebrate Animals** | 45 |
| **Select Agent Research** | 48 |
| **Multiple PI Leadership Plan** | 49 |
| **Bibliography & References Cited** | 50 |
| **Consortium/Contractual** | 55 |
| **Resource Sharing Plan** | 57 |
| **PHS 398 Checklist** | 58 |

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



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

## 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 centrifuges with rotors, microcentrifuges, PCR machines, various microscopes, water baths and several electrophoresis units.

The vivarium contains numerous cage racks with single ventilated cages, one pass-through cage washer, one large autoclave, one water bottle washer, one water bottle filler. Every holding room is equipped with a cage changing station. One procedure room is equipped with a biosafety cabinet for surgical procedures including an isoflurane anesthesia machine. The radiation chamber houses one Faxitron CP-160 X-ray irradiator with an output of 72 cGy/min that can irradiate up to ten mice at a time.

Cellerant performs routine preventive maintenance and regular calibration on its critical equipment.

All occupied offices and cubicles are equipped with personal computers linked to a local network. Electronic data are archived daily and copies stored off-site.

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

Research laboratories are individually equipped with the basic equipment found in modern biomedical research laboratories including microscopes, refrigerated centrifuges, PCR machines, and very low temperature freezers. In addition a 8 parameter Veterinary blood cell counter (ScilVetABC) is available in the Domen 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.

Major equipment at CMH includes a Applied Biosciences 6-color Blue/Red laser Attune Flow Cytometer with plate reader located in the Domen Lab, Beckman UV-Visible spectrophotometer, Applied Biosystems Model 3100 Capillary electrophoresis-based separation and identification system, Agilent Bioanalyzer 2100, Molecular Dynamics STORM 860 imaging system, ABI Prism 7000 quantitative real-time PCR equipment, Beckman computer-controlled spectrophotometer, Savant Speed Vac vacuum centrifuge, Applied Biosystems automated nucleic acid purifier, and Amersham two laser imaging system for fluorescence and phosphorescence. A Paradigm ultrasound backscatter microscope is available to directly visualize *in utero* animal embryos that permits the introduction of agents to alter the course of organ formation.

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



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

## 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.
25. Staller P, Peukert K, Kiermaier A, Seoane J, Lukas J, **Karsunky H**, Möröy T, Bartek J, Massague J, Häne F, and Eilers M, Repression of p15INK4b expression by Myc through association with Miz-1. Nature Cell Biol. 3: 392-399, 2001.
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<br>Assistant Professor, University of Missouri Kansas City |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>[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)



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

## 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.)



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

## 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** | | |



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

## 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] |

| 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 - 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<br>☐ 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.<br>65 |
| ☐ Yes<br>☒ No | * 2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?<br>* If yes, insert the names of the Federal laboratories/agencies: |
| ☐ Yes<br>☒ 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<br>☐ No | * 4. Will all research and development on the project be performed in its entirety in the United States?<br>If no, provide an explanation in an attached file.<br>* Explanation: Add Attachment Delete Attachment View Attachment |
| ☐ Yes<br>☒ 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?<br>* If yes, insert the names of the other Federal agencies: |
| ☒ Yes<br>☐ 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.<br>* Attach File: Add Attachment Delete Attachment View Attachment |



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

# 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.***

| | |
|---|---|
| ☐ Yes<br>☐ No | * 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.<br>* Attach File: Add Attachment Delete Attachment View Attachment |
| ☐ Yes<br>☐ No | * 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.***

| | |
|---|---|
| ☒ Yes<br>☐ No | * 10. Please indicate whether the answer to BOTH of the following questions is TRUE:<br>(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<br>(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project? |
| ☒ Yes<br>☐ No | * 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? |



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

# 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



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

# 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

## SPECIFIC AIMS

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 are capable of tolerance induction, but widespread clinical application has been limited. Important limitations are the severity of the necessary preconditioning regimens, with its associated morbidity and mortality (including severe immuno-incompetence) and the availability of adequate bone marrow grafts. We have recently identified a population of myeloid progenitor (MP) cells that may allow the development of clinical protocols that will overcome these hurdles. Cellerant Therapeutics is a company that has developed methods to obtain and test mouse and clinical grade human MP [2]. Research by the academic partner, currently at Children's Mercy Hospital/University of Missouri Kansas City, has shown that injection of mouse MP cells and purified hematopoietic stem cells (HSCs) into irradiated hosts leads to long-term, MP donor-specific tolerance [3].

The Domen lab continues to expand on these promising observations in important ways. (1) We have demonstrated that MP cells purified from bone marrow and MP cells expanded in short-term cultures induce specific tolerance when infused into irradiated recipients. (2) MP cells function in the context of both allogeneic and autologous HSC transplantation. (3) Tolerance is induced when the graft is either placed at the time of MP infusion or at a later time. (4) Myeloid cells are sufficient for tolerance induction. MP cells from Rag2 $^{-/-}$ Il2rg $^{-/-}$ mice, incapable of producing functional lymphocytes because they lack the Rag2 recombinase and signaling through many Interleukin receptors, prove that the myeloid cells alone are sufficient. (5) A minor degree of mismatching between MP and graft seems to be acceptable. Based on these observations, Cellerant Therapeutics and Children's Mercy/Domen Lab propose to jointly investigate the following hypotheses:

(I) One or more, organ graft-matched, myeloid progenitor-derived cell populations are essential <u>and</u> sufficient for cellular tolerance induction in multiple transplant models, including lung transplant.

(II) MP cells can induce tolerance for both fully matched and partially matched allogeneic grafts.

*Specific aims*

1. *Aim*: Show that MPc can be used to induce tolerance for lung transplants in a mouse model using heterotopic tracheal allografts.
   *Milestone:* No luminal occlusion and maintenance of epithelium in allogeneic (MP-matched) tracheal transplants.

2. *Aim*: Determine the degree of mismatching allowed for tolerance induction by MP.
   *Milestone*: Determine transplant acceptance rates induced with haploidentical MP and MP matched for major but not minor MHC.

*Significance.* The long-term survival of lung transplant recipients (1,200 to 1,400 transplants per year) is low. As such this is a patient population that could benefit greatly from an efficient tolerance induction regimen. The high failure rate in these patients will limit both the size and time of the clinical trials needed to show efficacy. Our research has defined, in a preclinical model, a novel tolerance induction protocol. Our proposed research will focus on testing the efficacy of this approach in lung transplantation models. Human MP cells, CLT-008, are currently undergoing clinical trials in umbilical cord blood transplantation and high dose chemotherapy leukemia trials, both aimed at reducing infectious complications. Our efforts in this project will set the stage to translate this preclinical approach, tolerance induction by MP, into a testable and clinically relevant product. Our goal is to enable the prompt integration of MP cells into transplantation strategies, starting with organ systems that currently enjoy limited long-term success, such as lung transplantation.

*Innovation.* Few, if any, translational research protocols aimed at tolerance induction focus on the use of clinically available MP cells. This novel approach, specifically aimed at defining the components of a protocol that has the potential for broad clinical application, will lead to a clinical paradigm shift once translated into an approved and commercially available clinical option.

# RESEARCH STRATEGY

## (A) SIGNIFICANCE

Short-term survival following cardiopulmonary organ transplant is excellent but long-term survival remains limited. Only 20% of the transplanted lungs remain functional ten years after transplantation [4], even with continued immunosuppression. Daily life is complicated by the staggering morbidity associated with the diagnosis and treatment of frequent and debilitating rejection episodes. Long-term immunosuppression, essential to delay rejection, can itself be considered a complication, since the associated morbidities are numerous and debilitating (including neural and renal toxicity and higher risk for cancer and infections [5-8]).

Operational tolerance, the acceptance of the transplanted organ into an otherwise immunocompetent host, should greatly improve long-term outcome, improve patient quality of life and reduce the economic burden on society. However, tolerance induction remains an elusive goal [9]. Tolerance will especially benefit pediatric transplant recipients, as transplanted organs need to function for six or more decades to restore a normal lifespan in children. Many approaches to tolerance induction have been tested (reviewed in [10-20]). Table 1 below categorizes some of these treatment options and their limitations.

| | |
|---|---|
| Immunosuppressant (standard therapy, no tolerance induction) | Effective short term, less so long term. Significant side effects. |
| Costimulatory blockade, T cell depletion | Limited effectiveness alone, may be good in combination therapy. |
| Hematopoietic (stem) Cell Transplantation, BMT | Very effective, works at multiple levels, but severe co-morbidity. |
| Progenitor Cell Transplantation (Myeloid Progenitors) | This proposal. Effective. Can work through different cell types. |
| Other cellular therapies (eg Treg, macrophages or dendritic cells) | Promising but addresses fewer mechanisms (single cell type). |

*Table 1. Comparison of standard therapy and several tolerance inducing strategies.*

The success of hematopoietic cell transplantation (HCT) has been well established with various cell populations, including rigorously purified hematopoietic stem cells (HSCs) [21]. However, despite decades of work clinical translation has been limited to small-scale trials [12, 18, 19, 22-24], mainly due to HCT-associated complications. Although HCT-related outcomes continue to improve incrementally [25], increased acceptance and use of HCT for treatment of non-malignancies will require significant reductions in morbidity and mortality.

The power of myeloid cells in tolerance induction has been more fully appreciated recently. This proposal is centered on a population of cells called myeloid progenitor (MP) cells that give rise to all cells of the myeloid lineage. **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.** Our proposal focuses on obtaining the data needed to bring this work to commercial development and clinical availability in the near future.

Cellerant Therapeutics, Inc has worked on developing MP as an off the shelf clinical product (product name CLT-008) for the past 8 years, and is currently testing CLT-008 in two clinical trials. CLT-008 is being developed for two indications: (1) To prevent infectious complications in severely neutropenic patients and (2) to treat acute radiation syndrome (ARS). This proposal, based on laboratory work performed by the Domen lab at the Medical College of Wisconsin, Milwaukee, WI and Children's Mercy Hospital in Kansas City, MO, aims to add tolerance induction for solid organ transplantation as an indication for the use of MP. This will serve a currently unmet medical need. However, as tolerance induction requires matched MPs, the requirements for production will differ to some extend from those in the current clinical programs.

Development of CLT-008 as a marketable product is well underway, including ongoing clinical trials and scaling up of production, the latter aided by Contract HHSO 100201000051C with the Biomedical Advanced Research and Development Authority (BARDA) to develop and stockpile CLT-008 for the treatment of ARS. This should greatly aid in the eventual commercial development of CLT-008 for tolerance induction, even if modifications in the production process are found to be necessary for use as discussed in this proposal.

## (B) INNOVATION

Our proposal represents a major change to transplantation strategies. Our investigation centers on the unique properties of myeloid progenitor cells as inducers of tolerance. This approach includes a dramatic shift away from the currently explored cellular therapy strategies that require the use of HSC-containing populations, lymphocyte populations, or mature myeloid populations, most of which require the creation of stable chimeras. We have characterized MP cells and shown that they induce antigen-specific tolerance that permits animals to accept skin grafts without the need for sustained high level hematopoietic chimerism and its associated complications [3]. MP cells give rise to all cells of the myeloid lineage and recent data demonstrates the role of multiple myeloid populations in tolerance induction. By using progenitor cells that can differentiate into many potential effector populations, the potential of all of these populations will be harnessed. However, to translate this into clinical practice we will need to define a path. By combining the academic preclinical expertise with Cellerant's expertise in clinical use of MP we expect to be able to define a path such that clinical trials for this innovative approach will be feasible at the end of this proposal.

## (C) APPROACH

***Clinical Use of MP cells***. Cell surface marker combinations identifying MP were first characterized in the lab of Irving Weissman [26, 27]. Effectiveness of purified mouse MP against infections was first demonstrated in Dr. Janice Brown's lab at Stanford University [28, 29]. Subsequently, human MP were developed for clinical use by Cellerant Therapeutics [30]. MP are currently being tested in patients with hematologic malignancies receiving umbilical cord blood transplantation or chemotherapy to protect against infections. They can be cryopreserved, do not cause graft-versus-host disease, and can be used without HLA matching [31, 32]. ***MP give rise to a variety of cells that have been implicated in tolerance induction.*** MP comprise a set of progenitors that can generate all myelo-erythroid lineages [26, 27], including dendritic cells (DC) [33] and myeloid-derived suppressor cells (MDSC). Myeloid cells comprise a heterogeneous cell compartment that is far more complex and compartmentalized in pro-inflammatory and immunosuppressive cells than has been realized [34]. ***MDSC and Tolerance.*** MDSC have been mostly studied in the context of cancer, but accumulating evidence indicates an important role in tolerance induction [35-37]. Models studied include a rat kidney transplant model [38] in which MDSC are essential but not sufficient. In a heart transplantation model in mice suppressive monocytes ($CD11c^+$, $CD11b^+$, $CD115^+$) are essential for tolerance and their depletion prevented tolerance. Transfer of monocyte/dendritic cell (DC) precursors, but not DC precursors rescued the tolerance inducing phenotype [39]. Co-transplantation of MDSC, but not DC precursors, with islet allografts has been reported to results in allograft tolerance via B7-H1-mediated enhancement of Tregs [40, 41]. ***DC and Tolerance.*** It has long been known that DCs can induce antigen-specific tolerance [42-46]. Donor-derived DCs prolong graft acceptance [47], and when combined with co-stimulatory blockade or total lymphoid irradiation, induce lasting specific tolerance [48, 49]. In some cases, tolerance induced by donor DCs has been reported to involve delivery of donor antigen to recipient antigen presenting cells [50]. Donor-derived DCs induce specific tolerance when given simultaneously with the organ graft [51]. ***Tolerance and sublethal preconditioning protocols.*** Over the last decade sublethal preconditioning protocols for HCT and tolerance induction have been developed. The Boston group led by Sykes, Sachs, and Spitzer uses cyclophosphamide, thymic irradiation and lymphocyte depleting antibodies for preconditioning [22, 52]. Trials have been in effect for a decade. Though tolerance can be induced, the protocol has failed in sensitized individuals or donor/recipient pairing of more than haplo disparity. A protocol centered around total lymphoid irradiation and anti-thymocyte globulin (ATG), has been developed by Strober and colleagues [53]. Tolerance induction is limited by recipient/donor genetic disparity [54]. More recently, a trial has been conducted by Ildstad and Leventhal [24] using facilitator cells with which we also have experience [55]. Though this protocol is successful across significant HLA disparity, it has failed in sensitized patients and the implications of the high-level donor-derived reconstitution and resultant prolonged immunoincompetence are still unclear.

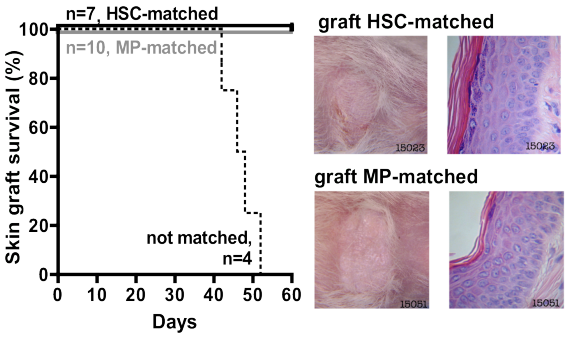


***Figure 1. Tolerance is achieved even when skin grafts are placed simultaneously with HCT.*** *BALB/c hosts, HSC donor AKR, FVB or C57BL/6 mice, MP donors FVB or C57BL/6 mice. Left overall survival of matched and unmatched grafts, right morphology (week 7) and histology (week 12) post transplant confirming the presence of tail skin at the graft site with its typical thick epidermis [3].*

**Skin graft survival in BALB/c mice reconstituted with 4,000 FVB HSC and 100,000 B10;B6-Rag2Il2rg MP**

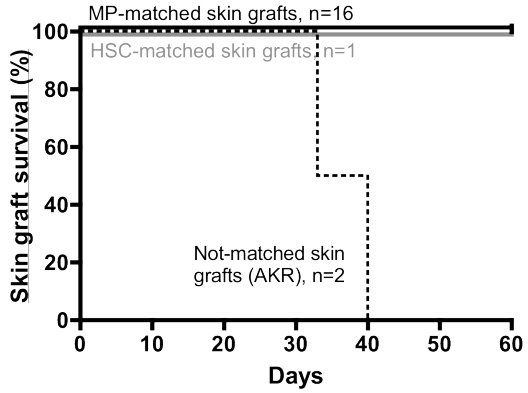


***Figure 2. Tolerance induction by MP does not require graft-matched lymphocytes.*** *Skin graft survival in BALB/c mice reconstituted with 4,000 FVB HSC and 100,000 B10; B6-*Rag2$^{-/-}$ Il2rg$^{-/-}$ *MP. Dotted line: AKR skin grafts, unmatched to host, HSC or MP donor. Solid lines, matched grafts: no graft failures in this data set. Skin grafts placed simultaneous with the transplantation of the hematopoietic cells. Grafts were followed up to 200 days.*

***Preliminary Studies***. Studies at Cellerant Therapeutics have established that MP, derived from *ex vivo* expanded progenitors, are fully functional with respect to their ability to reduce susceptibility to infections. The Domen Lab has recently published that MP can be used to induce specific tolerance for subsequently transplanted skin grafts in mouse [3]. The recipient animals were immunocompetent and rejected grafts unmatched to host, HSC donor, or MP donor. We have since significantly expanded these observations as detailed below.

✱ *Skin grafts can be placed at the time of MP administration.* 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). *(submitted for publication)*

✱ *Matched myeloid cells are the only essential cells.* We have demonstrated that tolerance induction only requires myeloid cells. B10;B6-*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ 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), (*submitted for publication)*.

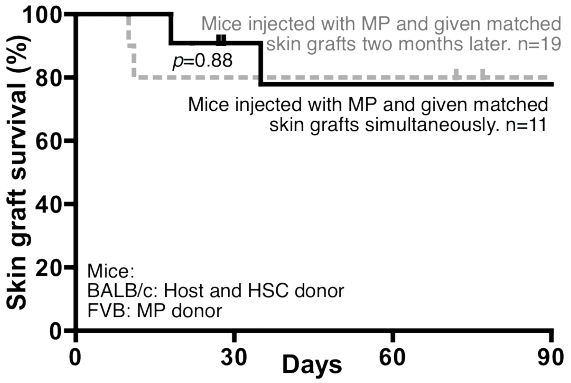


***Figure 3. Tolerance can be achieved with graft-matched MP and host-matched HSC.*** *BALB/c hosts were reconstituted with 250 BALB/c HSC and $10^5$ MP-directly sorted from BM or $10^6$ MPc-derived in ten day culture from HSC (the latter are more heterogeneous). Skin grafts were placed at the time of HCT (solid black line) or two months later (dashed grey): Skin graft survival does not differ (logrank).*

✱ *Allogeneic stem cell transplantation is not essential.* 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.

✱ *Expanded and cryopreserved MP (MPc) are fully functional in our tolerance induction model (Fig. 3).* While it is possible to sort sufficient quantities of MP from mouse bone marrow, this technique can not generate clinical quantities. Short-term expansion *in vitro* 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 *ex vivo* 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).

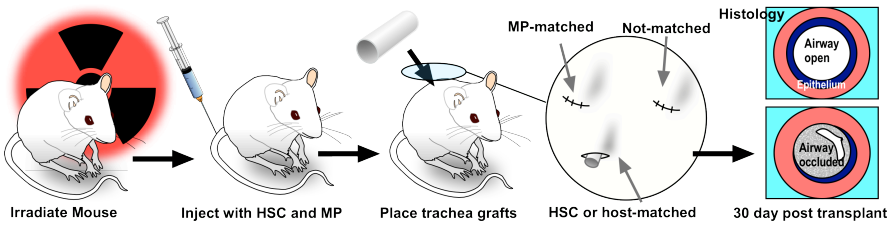


***Fig 4. Experimental design for Aim 1.*** *BALB/c mice will be lethally irradiated, injected with 4,000 FVB HSC and 500,000 C57BL/6 MPc, after which trachea grafts are placed (all on same day) from C57BL/6 (MP-matched), FVB (HSC-matched) and AKR (not-matched). Order of trachea grafts will vary randomly. Primary outcome will be airway occlusion, secondary loss of epithelium.*

***Division of the work***. Placement and monitoring of the trachea graft in mice will be performed by the academic partner. Sorting of HSC, *ex vivo* expansion of HSC into MPc and cryopreservation of the MPc will be performed by the corporate partner. All cryopreserved MPc will undergo lot release testing that (viability, composition, function, endotoxin levels, and bioburden) to ensure a product with consistent purity and potency.

***Milestone***: Preventing occlusion in mismatched allogeneic tracheal transplants. The goals of this aim will be considered met if we can demonstrate in a group of fifteen mice that allogeneic trachea's have significantly reduced luminal occlusion (<50% occlusion by surface area on all cross sections of H&E stained slides) beyond 30 days post transplant in mice that have been given MP matching the trachea graft prior to or simultaneous with the tracheal transplant. Control groups (on the same mice) will include (i) tracheas that do not match MP (or HSC or host), these controls should be occluded, and (ii) trachea's matched to HSC or host, these should not occlude. In addition to luminal occlusion we will score loss of epithelium as visible on H&E stained sections, as a secondary sign of rejection.

***Details of the model.*** We will use a variation of our established mouse model for the experiments in Aim 1. We will lethally irradiate (2x4.25 Gy, 4 hours apart using a RadSource 2000 irradiator at 160 kV, 25 mA, calibrated using chemical dosimetry in collaboration with J.D. van Horn, Dept of Chemistry, UMKC) host mice (typically BALB/c; other strains require a higher radiation dose). The mice will be injected on the day of irradiation with defined combinations of fluorescence-activated cell sorting (FACS)-purified HSCs [3, 55, 65]. Depending on the strain of origin (Table 2), one of two phenotypes of HSC will be sorted from BM: for CD90.1 strains such as FVB we will use 4,000 cells of phenotype CD117$^{+}$, CD90.1$^{lo}$, Lin$^{-}$, Sca-1$^{+}$ while from CD90.2 strains (such as C57BL/6 or BALB/c) we will use $10^4$ cells of phenotype CD117$^{+}$, Lin$^{-}$, Sca-1$^{+}$. Prior to injection, the HSCs will be mixed with either $10^5$ FACS-purified bone marrow derived MP cells (Lin$^{neg/low}$, CD117$^{+}$, Sca-1$^{-}$) or $10^6$ MPc cells expanded from FACS-purified HSCs in a short-term culture (8-10 days in Xvivo15 medium supplemented with recombinant mouse SCF, Flt3L, and Tpo) [2]. On the day of injection tracheas are placed using published procedures [58, 66]. Donor mice are euthanized and the heart lung block is excised. The trachea and main bronchi are removed and implanted in the host mouse in a subcutaneous pocket on the back. Typically a mouse will be transplanted with both an MP-matched trachea, an HSC or host-matched trachea and an unmatched trachea, placed on either side of the midline below the shoulder bone. Trachea's will be harvested from euthanized recipients on days 7, 14 and 30 following transplantation and analyzed by (immuno) histochemistry and flow cytometry for donor derived cells and status of the trachea graft. The most important analysis will be at day 30, as defined in milestones. Flow cytometry will focus on detection of lymphocytes, either in the graft itself, or systemically in the hematopoietic tissues of the host. This analysis will include mixed lymphocyte reactions (MLR) aimed at testing activation/proliferation of Celltrace Far Red labeled lymphocytes by various activators (irradiated host-, donor- or third party lymphocytes) to test *in vitro* for tolerance.

| Strain | MHC | CD90 | CD45 |
|---|---|---|---|
| AKR | k | 1 | 2 |
| BALB/c | d | 2 | 2 |
| CBA | k | 2 | 2 |
| C57BL/6 | b | 2 | 2 |
| FVB | q | 1 | 1 |
| SJL | s | 2 | 1 |
| F1(B6x129) | b | 2 | 2 |
| F1 (B6xBALB/c) | b/d | 2 | 2 |
| F1 (B6xCBA) | b/k | 2 | 2 |
| B10;B6-*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ | b | 2 | 2 |

*Table 2. Mouse strain characteristics*

***Potential Problems/Alternative Approaches.*** The tracheal transplant model has been used successfully in a large number of studies. The simplicity of surgical techniques and reproducibility of outcome should greatly facilitate the generation of data necessary to move this project forward. More sophisticated and complex models in the form of orthotopic transplantation of trachea [64], or orthotopic lung transplantation [67, 68], will be considered for the second phase of this project if we find questions that the heterotopic trachea transplant model can not answer. More important will be adaptations of this model to test human MPc [69]. If acceptance of the graft with HSC but not with culture derived MPc is seen, we are prepared to either increase the dose of MPc, change their culture conditions, or resort to using freshly purified MP from the bone marrow.

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

Our data, published as well as unpublished, has clearly established that myeloid cells in the MP preparations have the potential to induce tolerance for simultaneously or subsequently placed solid organ grafts that are fully matched to the MP's. However, it has not yet been determined whether matching needs to be absolute (clinically that means the cells have to be derived from the organ donor) or whether it is sufficient to use MP from a donor matching only major histocompatibility antigens. The latter would translate clinically into a scenario where MP with known combinations of transplantation antigens can be stockpiled. When organs

become available for transplant a matching batch of MP could be selected and be available at the time of organ transplant. If the MP have to be derived from the organ donor itself the logistics become more complicated. Bone marrow cells would have to be harvested with the other organs from the donor and used either immediately following the transplant, after enrichment of progenitor and stem cells, or a week or two later after expansion of the MP at a central facility. Our preliminary data clearly indicates that full mismatching (major and minor transplantation antigens) leads to rejection: Tolerance induction by MP is specific. However, matching may not need to be absolute. BALB/c mice reconstituted with 4,000 FVB HSC and $10^6$ C57BL/6 MPc can accept skin grafts from B10;B6 mice (4/4 accepted) while rejecting an AKR skin graft (0/1 accepted). While the degree of mismatching in this particular experiment is not clearly defined [70] it does indicate that there may be some leeway in the amount of matching required. We propose to determine the degree of mismatching tolerated in MP induced tolerance (Fig. 5).

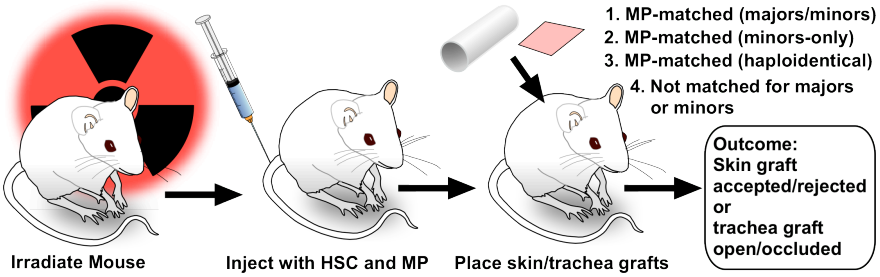


***Fig. 5. Experimental design for aim 2****. BALB/c mice will be lethally irradiated, injected with 4,000 FVB HSC and 500,000 MPc (table 2), after which skin grafts or trachea grafts are placed (all on same day) from donors matched in varying degrees to the MP (table2). Primary outcome is skin/trachea graft acceptance or rejection.*

***Milestone***: Determine the ability of standard doses of MP ($10^6$ cells obtained after expansion) to protect grafts that are either fully matched to the MP (same inbred strain), matched in major MHC only (F1 to parent or HLA-matched strains) or are haploidentical to the MP. As acceptance rates may vary, and will not necessarily be 100%, we will test fifteen mice per group (see data analysis section). If we fail to observe accepted grafts in mice receiving MP (ten or more rejected grafts) we will repeat that group using trachea grafts (if successful in aim 1) and an increased dose ($5x10^6$ per mouse) of expanded MP cells. The group size will again be fifteen mice to give sufficient power (as detailed in the Data Analysis Section below).

***Division of the work****.* The academic partner will inject MP and place and monitor skin grafts. Sorting of HSC, *ex vivo* expansion of HSC into MPc and cryopreservation of the MPc will be performed by the corporate partner. All cryopreserved MPc will undergo lot release testing that includes viability, composition, function, endotoxin levels, and bioburden to ensure a product with consistent purity and potency.

| Model | Host | HSC donor | MP donor | Skin Graft 1 | Skin Graft 2 |
|---|---|---|---|---|---|
| Fully matched | BALB/c | FVB | C57BL/6 | C57BL/6 | AKR |
| Majors | BALB/c | FVB | AKR | CBA | AKR |
| Majors | BALB/c | FVB | F1(B6x129) | C57BL/6 | AKR or 129 |
| Haplo | BALB/c | FVB | F1(B6xBALB/c) | F1(B6xCBA) | SJL or 129 |

*Table 3. Strain distribution for Aim 2 experiments. Skin graft 1 and 2 represent the two skin grafts that can be placed simultaneously. They are accepted or rejected independently (submitted). Actual position (front or backward) will be assigned randomly. All strains (including F1's) are directly available from approved vendors. To confirm that the results apply to lung transplants key experiments will be repeated using trachea grafts. Skin grafts are used initially because it is the most stringent mouse transplant model.*

***Details of the model.*** HSC and MP sorting and expansion will be as detailed under Aim 1, except that the mouse strains will be different (Table 3). On the day of injection, skin grafts will be placed that either are MP-matched, have matched major MHC antigens to the MP donor, are haploidentical to the MP-donor, or are fully mismatched. Skin grafts will be fully mismatched to host and HSC donors. The graft will either be a single skin graft (tail to belly) using our published protocol [3] or a double skin graft (two tail skin grafts to belly). Placement of the skin grafts on the same day as the HSC administration allows us to match the skin donor to the MP donor or HSC donor, which can be important when using mutant strains that may lack a fully inbred background. The fate of the tail skin will be monitored with digital photography, holding the non-anesthetized mouse at a fixed position over an upward facing camera with macro lens. In addition to skin grafts we will also test the model using trachea-grafts as detailed under Aim 1. Hematopoietic cells originating from host, HSC donor and MP donor will be identified with 6-color flow cytometry using MHC class I or congenic markers (CD45) to determine origin.

***Potential Problems/Alternative Approaches.*** We may find that the level of tolerance achieved (defined as skin grafts not rejected) may not only depend on the degree of mismatching, but also on the number of MPs used. While that would not be a primary milestone, this may provide an opportunity to define the effects of increasing MP cell dose. In addition we are prepared to change MP culture conditions or to resort to using freshly purified MP from the bone marrow if we find it difficult to achieve tolerance with other than skin donor-derived MP. We may also test the ability of MP using less easily rejected transplant models such as ear-heart transplantation, in which neonatal heart is transplanted into the pinna of the hosts ear [21]. While most experiments will be performed using MP from wild-type mice, some of the crucial data will be confirmed where appropriate using MP from B10;B6-*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice to confirm that only myeloid cells are essential.

***Data Analysis.*** 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 0.6, assuming the grafts have a correlation of 0.2 or less. Although every effort will be made to avoid missing data, it is possible that some measurements will be unavailable. Whenever data are missing, the causes will be examined. Mice that die before analysis due to hematopoietic failure (<30 days post reconstitution) or infection (death <10 days post reconstitution) will be censored, as these experiments are not designed to study HCT-associated morbidity. In our experience MP administration does not increase mortality, and clinical studies are ongoing with MP to address this. All possible data will be included in the analysis by using appropriate methods to deal with missing data. Multiple imputations for missing items will be explored if the data are missing at random. These assays typically result in either clear rejection or acceptance of hypotheses for a given set of conditions. We will therefore take advantage of a two-step approach. Data will be analyzed using SPSS, Cytel StatXact, LogXact, and SAS version 9.01. For survival data we will use LogXact and Kaplan-Meier curves with a Wilcoxon logrank test

***Timeline.*** Initial efforts will focus on Aim 1, starting with *ex vivo* expansion of MP. As these assays will be relatively rapid (with a read out typically at one month, though some of the mice may be followed longer) we expect to be able to analyze at least four iterations in six months, enabling us to meet the milestones defined for Aim 1. The assays for Aim 2 will take approx. two months to obtain results, and if more than a few iterations are needed will become be main focus of the second half of this Phase I project. We expect to meet the milestones described for Aim 2 9 to 12 months after initiation of the project.

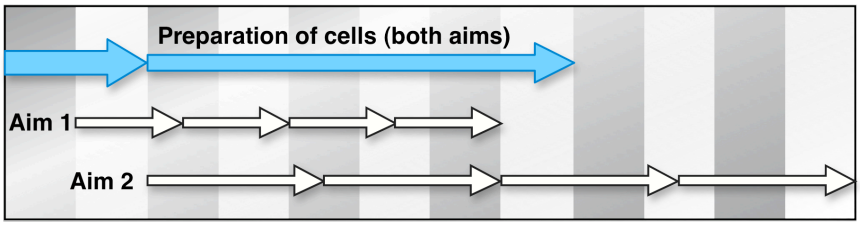

*Fig 6. Proposed timeline for Phase I covering a 12 month period.*

***Goals for Phase II.*** Once the milestones for Phase I have been met we plan to submit a proposal for Phase II funding with the focus in Phase II on two major aims. (1). The first aim would be to test human, clinical grade, MP for their ability to induce tolerance for human skin grafts in an immunodeficient mouse model. Specifically, we aim to test whether graft matched clinical grade human MP can prevent rejection of human skin transplanted to immunodeficient mice, by third party peripheral blood lymphocytes. (2) The second area of focus would be to define reduced intensity preconditioning regimens that allow induction of transplantation tolerance with MPc. The fact that MP induce tolerance for matched skin grafts when given with autologous, rather than allogeneic HSC indicates that sub-lethal preconditioning, with targeted destruction of host immune cells will be feasible. The information obtained in these aims, combined with the data from the proposed Phase I experiments, should allow us to design and implement trials to test this approach in patients. Clinical trials will be an obvious and essential step in commercializing MP for tolerance induction.

# 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 animal is fully recovered from anesthesia, moving around comfortably, and is able to drink easily. If recovery is prolonged a heat lamp, placed at least 15 inches away from the animals, will be used to prevent hypothermia. Buprenorphine is administered by s.c. injection at a dose of 0.05mg/kg twice daily starting immediately following recovery from anesthesia. Three injections are given routinely, more if the need is indicated by the animal. Limited discomfort is expected with collection of **bloodsamples** through nicking the tailvein. The limited discomfort does not justify the additional stress and risks associated with anesthesia. **Trachea Transplant Model**. Tracheas will be removed from transplant donors and kept in sterile ice-cold HBSS or PBS. A small piece on the back of recipient mice is shaved, prepped sterile and a subcutaneous pocket is generated. The trachea is placed and the skin closed with sutures. Up to four tracheas can be placed simultaneously on the same donor. The mice are euthanized, and the transplanted trachea removed for histological analysis, at different timepoints ranging from 7 days to 3 months post transplant. Implants in syngeneic mice or tolerant mice will retain their epithelial lining, while allogeneic transplants in animals that have not been tolerized will loose this. The airway will become fibrotic and close. This process is usually completed by 30 days, but may take longer in the context of hematopoietic reconstitution.

5). Methods of euthanasia: Mice will be sacrificed to obtain tissues for staining, FACS sorting, to harvest skingrafts and for terminal analysis. Consistent with the guidelines, the mice will first either be anesthetized by intraperitoneal injection using ketamine (200mg/kg) plus xylazine (10mg/kg) or by $CO_2$ asphyxiation. The depth of anesthesia will be assessed by the withdrawal reflex. If the reflex persists, additional anesthetic will be administered. All mice then undergo cervical dislocation. The mouse will be exsanguinated by cardiac puncture only if peripheral blood is wanted.

## 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:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

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: Yes ☐ No ☐

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

* Previously Reported: Yes ☐ No ☐



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

**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



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